Reducing the Genetic Risk of Age-Related Macular Degeneration With Dietary Antioxidants, Zinc, and ω-3 Fatty Acids

The Rotterdam Study

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Objective: To investigate whether dietary nutrients can reduce the genetic risk of early age-related macular degeneration (AMD) conferred by the genetic variants *CFH* Y402H and *LOC387715* A69S in a nested case-control study.

Methods: For 2167 individuals (≥55 years) from the population-based Rotterdam Study at risk of AMD, dietary intake was assessed at baseline using a semiquantitative food frequency questionnaire and genetic variants were determined using TaqMan assay. Incident early AMD was determined on fundus photographs at 3 follow-up visits (median follow-up, 8.6 years). The synergy index was used to evaluate biological interaction between risk factors; hazard ratios were calculated to estimate risk of early AMD in strata of nutrient intake and genotypes.

Results: Five hundred seventeen participants developed early AMD. Significant synergy indices supported the possibility of biological interaction between *CFH* Y402H and zinc, β-carotene, lutein/zeaxanthin, and eicosapentaenoic/docosahexaenoic acid (EPA/DHA) and between *LOC387715* A69S and zinc and EPA/DHA (all $P<.05$). Homozygotes of *CFH* Y402H with dietary intake of zinc in the highest tertile reduced their hazard ratio of early AMD from 2.25 to 1.27. For intakes of β-carotene, lutein/zeaxanthin, and EPA/DHA, these risk reductions were from 2.54 to 1.47, 2.63 to 1.72, and 1.97 to 1.30, respectively. Carriers of *LOC387715* A69S with the highest intake of zinc and EPA/DHA reduced their risk from 1.70 to 1.17 and 1.59 to 0.95, respectively (all *P* trends <.05).

Conclusions: High dietary intake of nutrients with antioxidant properties reduces the risk of early AMD in those at high genetic risk. Therefore, clinicians should provide dietary advice to young susceptible individuals to postpone or prevent the vision-disabling consequences of AMD.

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Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries, accounting for 50% of blindness.[1] Approximately 2.5 million elderly individuals are affected by late AMD in Europe[2] and 21 million worldwide.[3] The short-term visual outcome of neovascular AMD has been improved by antiangiogenic pharmacotherapy; however, the long-term prognosis of this type of late AMD is still poor.[4,5] For geographic atrophy, there are no options to improve vision. These poor visual outcomes ask for interventions that can be applied earlier in the disease process.

The etiology of AMD is complex with both genetic and environmental factors contributing to pathogenesis.[6] Inflammation and oxidative stress are known to be fundamental pathways.[7-14] Complement factor H (*CFH*) and *LOC387715/HTRA1* have been identified as the most prominent susceptibility genes.[15-24] Carriers of the risk alleles of these genes have a significantly higher risk of AMD: the *CFH* Y402H variant increases the risk of AMD up to 11 times and the *LOC387715* A69S variant, up to 15 times. Together, these variants contribute to late AMD in more than 80% of cases.[25,26] To reduce the burden of this disease, it is therefore essential to find means to counteract these major gene effects.

The only protective factors for AMD known to date are nutrients. The Age-Related Eye Disease Study (AREDS), a randomized clinical trial, showed that a combination of zinc, β-carotene, vitamin C, and vitamin E reduced the risk of progression from intermediate to advanced AMD by

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25%.[27] In our population-based Rotterdam Study, we found that an above-median intake of these nutrients was associated with a 35% lower risk of incident AMD.[28] Similarly, several studies have shown that higher dietary intake of ω-3 fatty acids reduced progression of AMD by 30% to 59%.[29-31] Studies investigating interaction between nutrients and genetic risk have been relatively small and their results, inconsistent. Thus, whether the protection offered by these nutrients is sufficient to counteract the genetic risk is unclear.[32,33]

Within the framework of the large population-based Rotterdam Study, we explored the relationship between a healthy diet, genetic risk, and early AMD. We assessed the intake of antioxidants, zinc, and ω-3 fatty acids in daily foods, diagnosed the onset of early AMD during a lengthy follow-up, and investigated the risk-reducing effect of these nutrients in the various genotypes of *CFH* Y402H and *LOC387715* A69S.

METHODS

STUDY POPULATION

This study was nested in the prospective, population-based Rotterdam Study investigating chronic diseases in subjects 55 years and older. Details of the Rotterdam Study have been provided elsewhere.[34] In short, 6780 participants (78% of those eligible) underwent an extensive physical assessment including ophthalmic examination during 1990 to 1993 (baseline). This was followed by 3 reexaminations during 1993 to 1994, 1997 to 1999, and 2000 to 2004. The Erasmus Medical Center Ethics Committee approved the study, which complies with the Declaration of Helsinki. All participants gave written informed consent.

Eligible for the current study were participants who either had no AMD during the entire study period (n = 2079) or who developed early AMD during follow-up (n = 689). Subjects were included when they were successfully genotyped for *CFH* Y402H and/or for *LOC387715* A69S, lived independently, and had normal cognition, reliable dietary assessment, and gradable fundus photographs from at least 1 follow-up examination. This resulted in 2167 individuals available for analysis. These subjects did not differ in dietary intake of nutrients, eg, vitamin C ($P = .63$) or lutein/zeaxanthin ($P = .69$), from eligible subjects who did not fit the inclusion criteria.

DIAGNOSIS OF AMD

Fundus photographs covering a 35° field centered on the macula were taken at each visit (Topcon TRV-50VT fundus camera; Topcon Optical Co, Tokyo, Japan) after pharmacologic mydriasis. All signs of AMD were graded according to the modified international classification and grading system for AMD.[28,35] Grading procedures, definitions, and well-trained graders were identical at baseline and follow-up.[28] For this study, we considered the outcomes no AMD and incident early AMD. No AMD consisted of no or only small hard drusen and early AMD, of either soft distinct drusen with pigmentary irregularities or soft indistinct drusen without pigmentary irregularities, or of soft indistinct drusen with pigmentary irregularities. Incident early AMD was defined as no sign of AMD in both eyes at baseline and the appearance of signs of early AMD in at least 1 eye at follow-up.

DIETARY ASSESSMENT

Dietary assessment was performed in 2 stages at baseline. First, participants completed a checklist at home, which queried foods and drinks they had consumed at least twice a month during the preceding year, as well as dietary habits, use of supplements, and prescribed diets. Subsequently, a trained dietitian interviewed the participants at the research center using a 170-item, validated semiquantitative food frequency questionnaire.[36,37] Using the computerized Dutch Food Composition Table, these dietary data were converted to total energy intake and nutrient intake per day.[37,38] Intake of specific fatty acids was based on a food composition database derived from the TRANSFAIR Study.[39,40] For this database, the 100 food items that contribute most to fat intake in the Dutch dietary pattern were sampled and analyzed as methyl esters of fatty acids present in food. For the current study, we used data on the nutrients iron; zinc; vitamins A, C, and E; β-carotene; lutein/zeaxanthin; and eicosapentaenoic/docosahexaenoic acid (EPA/DHA).

GENOTYPING

Genomic DNA was extracted from peripheral blood leukocytes. Participants were genotyped for the *CFH* Y402H (rs1061170) and *LOC387715* A69S (rs10490924) polymorphisms using the TaqMan assay (Applied Biosystems, Foster City, California).

ASSESSMENT OF CONFOUNDERS

Information on potential confounders was collected at baseline. Smoking status was categorized as current, former, or never. Serum total cholesterol level was measured in nonfasting blood samples using an automated enzymatic procedure. Blood pressure was defined as the mean of 2 measurements taken in a sitting position at the right brachial artery with a random-zero sphygmomanometer. Carotid intima thickness and atherosclerotic plaques were assessed ultrasonographically and aortic calcifications, on lateral radiographic films of the lumbar spine. A subclinical atherosclerosis composite score (range, 1-4) was constructed by summing points for the population-based deciles of carotid wall thickness and ankle-arm index, with points added for the presence of carotid plaques and aortic calcifications.[41]

DATA ANALYSIS

Characteristics of participants with and without incident early AMD were compared using analysis of covariance for continuous variables and logistic regression analysis for discrete variables, adjusting for age and sex. Hardy-Weinberg equilibrium of genotype distributions was tested using a Fisher exact test.

We adjusted the dietary intake of nutrients for the total energy intake by means of the residual method described by Willett.[42] We defined tertiles of antioxidant intake on the basis of the distribution within the study sample. Participants were classified as noncarriers or heterozygous or homozygous carriers of the *CFH* Y402H risk variant and noncarriers or carriers of the *LOC387715* A69S risk variant. In the interaction analyses, participants with the lowest tertile of nutrient intake and homozygous nonrisk genotype were used as the common reference group, as described by Botto and Khoury.[43] The risk of AMD associated with each antioxidant-genotype group was estimated by entering a dummy variable representing 1 of the 8 other groups in the table in a Cox proportional hazards regression model (SPSS version 15.0; SPSS Inc, Chicago, Illinois). We adjusted for age, sex, smoking status, and composite atherosclerosis score. Associations were presented as hazard ratios (HRs) with 95% confidence intervals (CIs). Biological interaction with *CFH* or *LOC387715* was assessed by calculating the synergy index.[44] This measures deviation from additivity of 2 factors and is based on the ratio of the combined effect to the

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Table 1. Baseline Characteristics of the 2167 Individuals in the Total Study Sample[a]

Characteristic	Mean (SD)		*P* Valuetbl[b]
	Incident Early AMD (n = 517)	No AMD at Follow-up (n = 1650)	
Age, y	68.1 (7.0)	65.9 (7.2)	<.001
Female, No./total No. (%)	292/517 (56.5)	936/1650 (56.7)	.57
Body mass index[c]	26.2 (3.4)	26.5 (3.7)	.90
Smoking status, No./total No. (%)			
Never	165/515 (32.0)	521/1643 (31.7)	.91
Former	231/515 (44.9)	720/1643 (43.8)	
Current	119/515 (23.1)	402/1643 (24.5)	
Blood pressure, mm Hg			
Systolic	138.5 (20.1)	137.1 (21.2)	.99
Diastolic	73.5 (10.5)	73.9 (10.6)	.42
Total cholesterol level, mg/dL	254.83 (46.33)	258.69 (46.33)	.50
HDL cholesterol level, mg/dL	54.05 (15.44)	50.19 (15.44)	.25
Atherosclerosis composite score, No./total No. (%), quartile[d]			
1	83/454 (18.3)	348/1456 (23.9)	.63
2	123/454 (27.1)	387/1456 (26.6)	
3	127/454 (28.0)	379/1456 (26.0)	
4	121/454 (26.7)	342/1456 (23.5)	
Diabetes mellitus, No./total No. (%)	33/493 (6.7)	157/1558 (10.1)	.003
Dietary intake			
Total energy, kcal/d	2009.0 (521.8)	1982.3 (514.8)	.89
Alcohol, g/d	11.2 (15.8)	10.5 (14.9)	.87
Total milk, mL/d	407.8 (271.1)	382.1 (251.9)	.69
Total meat, g/d	108.1 (46.1)	110.6 (47.9)	.55
Total fish, g/d	14.8 (17.0)	15.5 (18.4)	.34
Total fruit, g/d	227.1 (130.2)	223.1 (127.8)	.26
Total vegetable, g/d	354.3 (120.5)	349.0 (132.3)	.15
CFH Y402H, No./total No. (%)			
Noncarrier TT genotype	196/515 (38.1)	733/1642 (44.6)	<.001
Heterozygous CT genotype	234/515 (45.4)	748/1642 (45.6)	
Homozygous CC genotype	85/515 (16.5)	161/1642 (9.8)	
LOC387715 A69S, No./total No. (%)			
Noncarrier AA genotype	300/509 (58.9)	1100/1630 (67.5)	.002
Heterozygous AG genotype	188/509 (36.9)	478/1630 (29.3)	
Homozygous GG genotype	21/509 (4.1)	52/1630 (3.2)	

Abbreviations: AMD, age-related macular degeneration; HDL, high-density lipoprotein.
SI conversions: To convert total and HDL cholesterol to millimoles per liter, multiply by 0.0259.
[a] Because of missing data, numbers do not sum to the heading totals.
[b] *P* values were calculated using analysis of covariance for continuous variables and logistic regression for discrete variables, adjusted for age and sex.
[c] Body mass index was calculated as weight in kilograms divided by height in meters squared.
[d] Information on the atherosclerosis composite score is presented in the "Methods" section of the text.

sum of the separate effects. We defined the highest risk category of each factor as the reference category. Therefore, a synergy index less than 1.00 suggests that the protective effect of both factors together is greater than the sum of the effect of the separate factors. To distinguish between the effect of nutrients from food and from supplements, all analyses were repeated after supplement users were excluded at baseline.

RESULTS

During follow-up (median, 8.6 years), 1650 participants remained free of AMD, whereas 517 developed early AMD. Baseline characteristics of participants with and without incident early AMD are presented in **Table 1**. Participants with early AMD were slightly older (mean age, 68.1 years for early AMD vs 65.9 years for no AMD; $P<.001$), and fewer were diabetic (6.7% vs 10.1%; $P=.003$). They also had a higher frequency of *CFH* Y402H (61.9% vs 55.4%) and *LOC387715* A69S (41.0% vs 32.5%) carriership. Other baseline characteristics were not significantly different in the 2 groups. First, we investigated the relationship for nutrients and early AMD in the entire population. In line with our previous report, we found zinc, β-carotene, and vitamins C and E to be significantly associated with a reduced risk of AMD.[28] Then, we formed subgroups based on level of nutrient intake by genotypes of *CFH* Y402H and *LOC387715* A69S. The number of cases, the total number of participants, and the amount of nutrient intake per day are provided for each stratum in **Table 2**. Per tertile, nutrient intake was equally distributed over the genotypes.

The synergy indices of interaction for dietary nutrients and genetic variants are presented in **Table 3**. A significant interaction was found between the *CFH* Y402H genotype and zinc, β-carotene, lutein/zeaxanthin, and EPA/DHA. For *LOC387715* A69S, a significant interaction was found with zinc and EPA/DHA. There was no

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Table 2. Mean Dietary Nutrient Intake by Tertile and *CFH Y402H* and *LOC387715* A69S Genotype in the Study Population Included in the Incidence Analyses

	CFH Y402H (n = 1891)[a]			*LOC387715* A69S (n = 1877)[a]	
Antioxidant	Noncarrier	Heterozygous	Homozygous	Noncarrier	Carrier
Zinc (RDA, male: 10 mg/d, female: 9 mg/d)					
1st tertile					
Mean (SD), mg/d	7.47 (0.98)	7.57 (0.87)	7.49 (1.00)	7.53 (0.94)	7.49 (0.91)
Range in total study sample, mg/d	3.60-8.69	3.92-8.71	3.78-8.67	3.60-8.71	4.59-8.71
No. of outcomes/No. at risk (%)	65/291 (22.3)	72/288 (25.0)	27/63 (42.9)	86/404 (21.3)	78/234 (33.3)
2nd tertile					
Mean (SD), mg/d	9.54 (0.46)	9.56 (0.47)	9.50 (0.48)	9.53 (0.48)	9.55 (0.45)
Range in total study sample, mg/d	8.71-10.34	8.73-10.34	8.71-10.33	8.71-10.34	8.71-10.33
No. of outcomes/No. at risk (%)	52/264 (19.7)	71/290 (24.5)	26/72 (36.1)	90/414 (21.7)	55/203 (27.1)
3rd tertile					
Mean (SD), mg/d	11.74 (1.24)	11.78 (1.30)	11.99 (1.63)	11.76 (1.27)	11.85 (1.41)
Range in total study sample, mg/d	10.34-16.77	10.35-20.46	10.36-17.39	10.34-20.46	10.34-18.98
No. of outcomes/No. at risk (%)	56/265 (21.1)	64/280 (22.9)	17/78 (21.8)	82/411 (20.0)	54/211 (25.6)
β-Carotene (RDA, not registered)					
1st tertile					
Mean (SD), mg/d	2.39 (0.61)	2.34 (0.64)	2.56 (0.52)	2.41 (0.60)	2.36 (0.63)
Range in total study sample, mg/d	0.15-3.15	0.25-3.16	1.11-3.15	0.25-3.16	0.15-3.16
No. of outcomes/No. at risk (%)	51/259 (19.7)	70/290 (24.1)	29/74 (39.2)	83/396 (21.0)	67/226 (29.6)
2nd tertile					
Mean (SD), mg/d	3.74 (0.32)	3.77 (0.33)	3.71 (0.30)	3.72 (0.32)	3.79 (0.32)
Range in total study sample, mg/d	3.17-4.35	3.17-4.35	3.17-4.32	3.17-4.35	3.17-4.35
No. of outcomes/No. at risk (%)	58/279 (20.8)	73/277 (26.4)	26/75 (34.7)	85/409 (20.8)	69/214 (32.2)
3rd tertile					
Mean (SD), mg/d	5.85 (2.71)	5.74 (1.50)	5.45 (0.98)	5.71 (1.56)	5.83 (2.91)
Range in total study sample, mg/d	4.36-43.5	4.36-20.4	4.37-9.42	4.36-20.4	4.36-43.51
No. of outcomes/No. at risk (%)	64/282 (22.7)	64/291 (22.0)	15/64 (23.4)	90/424 (21.2)	51/208 (24.5)
Lutein/zeaxanthin (RDA, not registered)					
1st tertile					
Mean (SD), mg/d	1.47 (0.32)	1.46 (0.34)	1.50 (0.25)	1.48 (0.31)	1.45 (0.34)
Range in total study sample, mg/d	0.08-1.90	0.04-1.91	0.97-1.91	0.04-1.90	0.08-1.91
No. of outcomes/No. at risk (%)	50/269 (18.6)	69/284 (24.3)	23/65 (35.4)	79/403 (19.6)	63/215 (29.3)
2nd tertile					
Mean (SD), mg/d	2.26 (0.20)	2.24 (0.19)	2.25 (0.21)	2.24 (0.20)	2.25 (0.20)
Range in total study sample, mg/d	1.91-2.61	1.91-2.61	1.91-2.61	1.91-2.61	1.91-2.61
No. of outcomes/No. at risk (%)	63/290 (21.7)	71/272 (26.1)	31/85 (36.5)	89/410 (21.7)	72/226 (31.9)
3rd tertile					
Mean (SD), mg/d	3.38 (1.17)	3.30 (0.6)	3.23 (0.46)	3.29 (0.71)	3.39 (1.19)
Range in total study sample, mg/d	2.62-17.69	2.62-6.67	2.66-4.94	2.62-10.72	2.63-17.69
No. of outcomes/No. at risk (%)	60/261 (23.0)	67/302 (22.2)	16/63 (25.4)	90/416 (21.6)	52/207 (25.1)
EPA/DHA (RDA, 450 mg/d)					
1st tertile					
Mean (SD), mg/d	23.40 (5.80)	21.85 (5.36)	22.83 (5.51)	23.40 (5.83)	21.31 (5.29)
Range in total study sample, mg/d	0.07-51.56	0.07-51.58	0.03-51.44	0.07-51.58	0.06-51.44
No. of outcomes/No. at risk (%)	65/274 (23.7)	69/283 (24.4)	27/69 (39.1)	91/413 (22.0)	69/207 (33.3)
2nd tertile					
Mean (SD), mg/d	93.88 (28.94)	93.68 (28.18)	92.24 (25.46)	93.50 (28.21)	93.58 (28.18)
Range in total study sample, mg/d	51.65-146.71	51.71-146.72	51.90-145.49	51.65-146.71	51.74-146.72
No. of outcomes/No. at risk (%)	52/269 (19.3)	70/293 (23.9)	22/70 (31.4)	76/420 (18.1)	67/208 (32.2)
3rd tertile					
Mean (SD), mg/d	305.00 (260.52)	295.52 (234.79)	268.80 (136.12)	290.18 (209.36)	307.97 (282.21)
Range in total study sample, mg/d	146.82-3038.83	146.80-2308.95	147.34-962.90	146.80-1718.66	148.65-3038.83
No. of outcomes/No. at risk (%)	56/277 (20.2)	68/282 (24.1)	21/74 (28.4)	91/396 (23.0)	51/233 (21.9)

Abbreviations: EPA/DHA, eicosapentaenoic/docosahexaenoic acid; RDA, recommended dietary allowance.
[a]Heading totals correspond to the actual number of participants included in the respective Cox proportional hazards regression analyses, adjusted for age, sex, smoking status, and atherosclerosis. Because of missing data, numbers do not sum to the heading totals of Table 1.

significant interaction between the genetic variants and any of the vitamins.

Those interactions that were significant are further specified in **Table 4** and the **Figure**. Regarding *CFH* Y402H and zinc (Figure, A), homozygotes in the lowest tertile had the highest risk of early AMD (HR, 2.25; 95% CI, 1.43-3.53 vs noncarriers in this tertile). Higher zinc intake reduced this risk to an HR of 1.27 (95% CI, 0.74-2.18) for the highest tertile. This level of intake also reduced risks for the other genotypes, but the effects were

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Table 3. SI of Dietary Antioxidant Intake and *CFH* Y402H and *LOC387715* A69S Genotype

	Dietary Intake, mg/d, Mean (SD)	SI (95% CI)	
		CFH Y402H[a]	*LOC387715* A69S[b]
Trace elements			
Zinc	9.67 (2.01)	0.54 (0.44-0.66)	0.76 (0.60-0.98)
Vitamins			
Vitamin A (retinol equivalents)	0.82 (0.35)	0.73 (0.41-1.31)	2.10 (0.45-9.85)
Vitamin C	120.20 (52.49)	0.76 (0.32-1.79)	0.31 (0.06-1.58)
Vitamin E	13.42 (5.19)	0.98 (0.25-3.75)	1.19 (0.37-3.79)
Carotenoids			
β-Carotene	3.84 (2.23)	0.49 (0.35-0.70)	0.71 (0.34-1.47)
Lutein/zeaxanthin	2.37 (1.08)	0.53 (0.31-0.90)	0.56 (0.29-1.08)
ω-3 Fatty acid, mg/d, median (IQR)			
EPA/DHA	90.35 (146)	0.65 (0.42-0.99)	0.31 (0.13-0.76)

Abbreviations: CI, confidence interval; EPA/DHA, eicosapentaenoic/docosahexaenoic acid; IQR, interquartile range; SI, synergy index.
[a]The SI for dietary antioxidant intake and *CFH* Y402H genotype, adjusted for age, sex, smoking, and atherosclerosis.
[b]The SI for dietary antioxidant intake and *LOC387715* A69S genotype, according to dominant model, adjusted for age, sex, smoking, and atherosclerosis.

Table 4. Joint Effects of Dietary Nutrient Intake and *CFH* Y402H or *LOC388716* A69s Genotype on the Risk of Early AMD[a]

	Tertile of Nutrient Intake						
	1		2		3		
	No. of Outcomes/ No. at Risk	HR (95% CI)	No. of Outcomes/ No. at Risk	HR (95% CI)	No. of Outcomes/ No. at Risk	HR (95% CI)	*P* Value for Trend
***CFH* Genotype (n = 1891)**							
Zinc							
Noncarrier	65/291	1 [Reference]	52/264	0.89 (0.62-1.29)	56/265	0.98 (0.68-1.40)	.74
Heterozygous	72/288	1.26 (0.90-1.77)	71/290	1.18 (0.84-1.66)	64/280	1.07 (0.76-1.51)	.41
Homozygous	27/63	2.25 (1.43-3.53)	26/72	1.86 (1.18-2.94)	17/78	1.27 (0.74-2.18)	.02
β-Carotene							
Noncarrier	51/259	1 [Reference]	58/279	1.10 (0.75-1.60)	64/282	1.25 (0.86-1.81)	.31
Heterozygous	70/290	1.40 (0.97-2.01)	73/277	1.42 (0.99-2.03)	64/291	1.27 (0.88-1.83)	.51
Homozygous	29/74	2.54 (1.61-4.02)	26/75	2.04 (1.27-3.25)	15/64	1.47 (0.83-2.63)	.05
EPA/DHA							
Noncarrier	65/274	1 [Reference]	52/269	0.82 (0.57-1.18)	56/277	0.79 (0.55-1.12)	.21
Heterozygous	69/283	1.04 (0.74-1.47)	70/293	1.07 (0.76-1.49)	68/282	1.07 (0.76-1.50)	.87
Homozygous	27/69	1.97 (1.26-3.09)	22/70	1.62 (0.99-2.63)	21/74	1.30 (0.79-2.13)	.03
Lutein/zeaxanthin							
Noncarrier	50/269	1 [Reference]	63/290	1.30 (0.89-1.88)	60/261	1.39 (0.96-2.03)	.13
Heterozygous	69/284	1.54 (1.07-2.21)	71/272	1.63 (1.13-2.34)	67/302	1.33 (0.92-1.93)	.37
Homozygous	23/65	2.63 (1.60-4.32)	31/85	2.15 (1.38-3.42)	16/63	1.72 (0.97-3.03)	.05
***LOC387715* Genotype (n = 1877)**							
Zinc							
Noncarrier	86/404	1 [Reference]	90/414	1.04 (0.77-1.40)	82/411	0.97 (0.72-1.32)	.86
Carrier	78/234	1.70 (1.25-2.31)	55/203	1.29 (0.92-1.81)	54/211	1.17 (0.83-1.64)	.03
EPA/DHA							
Noncarrier	91/413	1 [Reference]	76/420	0.85 (0.63-1.15)	91/396	1.03 (0.77-1.37)	.86
Carrier	69/207	1.59 (1.16-2.17)	67/208	1.50 (1.09-2.06)	51/233	0.95 (0.68-1.34)	.01

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; EPA/DHA, eicosapentaenoic/docosahexaenoic acid; HR, hazard ratio.
[a]The HRs are estimates of the relative risk of early AMD, and represent the risk of disease (early AMD vs no AMD) in the various genetic-environmental risk groups divided by the risk of disease (early AMD vs no AMD) in the common reference group (noncarriers: first tertile of nutrient intake). The HRs are estimated with Cox regression analyses and include age, sex, smoking status, and atherosclerosis.

smaller. In the highest tertile, we found no increased risk for carriers of *CFH* Y402H.

Similar effects were observed for *CFH* Y402H and β-carotene (Figure, B). Homozygous participants in the lowest tertile had an HR of 2.54 (95% CI, 1.61-4.02). Higher intake reduced this risk to an HR of 2.04 (95% CI, 1.27-3.25) for the second tertile and to an HR of 1.47 (95% CI, 0.83-2.63) for the third tertile. As with zinc, the risk reduction with increasing intake of β-carotene was smaller for the heterozygous persons. At higher intakes, the risk showed a slight, but nonsignificant increase in noncarriers (*P* trend = .31). The point esti-

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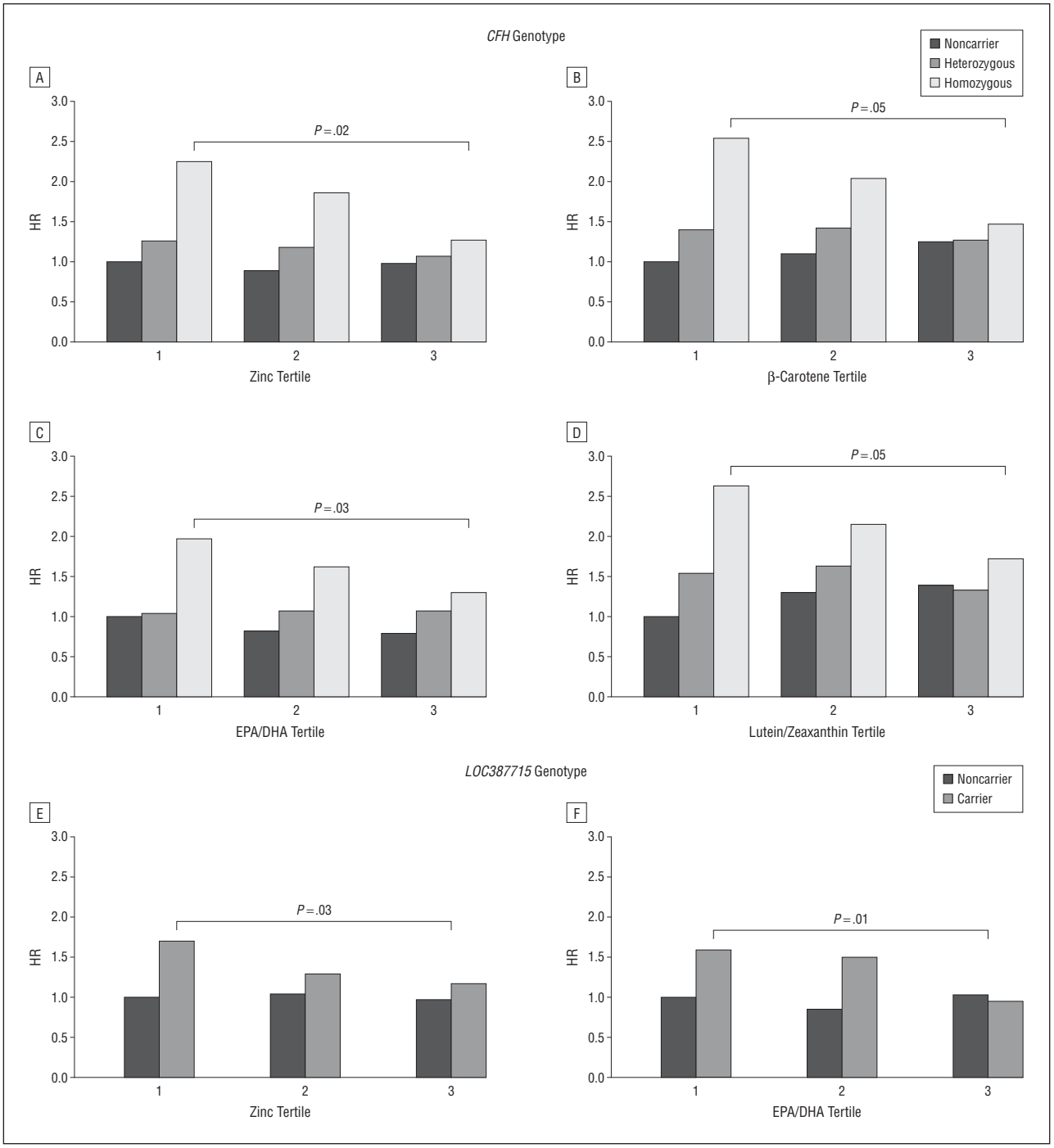

Figure. Joint effect of dietary nutrient intake and *CFH* Y402H and *LOC387715* A69S genotypes on the risk of early age-related macular degeneration). A-D, *CFH* Y402H genotype. E and F, *LOC387715* A69S genotype. Additional information is given in Table 4. EPA/DHA indicates eicosapentaenoic/docosahexaenoic acid; HR, hazard ratio.

mates were similar across genotypes in the highest tertile, indicating a limited genetic influence in this stratum.

Risk of early AMD classified by *CFH* Y402H genotype and dietary EPA/DHA intake are given in the Figure, C. Homozygotes in the lowest EPA/DHA tertile had an HR of 1.97 (95% CI, 1.26-3.09). Higher EPA/DHA intake reduced this risk to an HR of 1.62 (95% CI, 0.99-2.63) for the second tertile of EPA/DHA intake and to an HR of 1.30 (95% CI, 0.79-2.13) for the third tertile. In heterozygous individuals and noncarriers, risks did not differ significantly across the tertiles of dietary intake (P trend $>$ .21).

The interaction between *CFH* Y402H and lutein/zeaxanthin intake is presented in the Figure, D. Homozygotes in the lowest tertile had an HR of 2.63 (95% CI, 1.60-4.32). A higher intake reduced this risk to an HR of 1.72 (95% CI, 0.97-3.03) in the highest tertile. Heterozygotes and noncarriers showed nonsignificant trends with higher intake (P trend = .37 and P trend = .13, respectively).

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Significant interactions with *LOC387715* A69S are demonstrated in the Figure, E and F. Since the frequency of *LOC387715* A69S was lower than that of *CFH* Y402H, the number of participants in each stratum was low; we therefore grouped all carriers of this variant to increase power to detect significant interaction. For zinc, carriers in the lowest tertile had the highest risk (HR, 1.70; 95% CI, 1.25-2.31), and higher intake reduced this to an HR of 1.17 (95% CI, 0.83-1.64) in the highest tertile. The risk in noncarriers with higher intakes did not change. For EPA/DHA, carriers in the lowest tertile had an HR of 1.59 (95% CI, 1.16-2.17). Higher intake of EPA/DHA reduced this risk only in the highest tertile (HR, 0.95; 95% CI, 0.68-1.34).

Exclusion of participants (n=559) who used antioxidant supplements at baseline did not substantially alter risk estimates (data not shown).

COMMENT

Modifying environmental factors is currently the only approach to reduce the genetic risk of AMD. Our study showed that higher dietary intake of zinc, ω-3 fatty acids, β-carotene, and lutein/zeaxanthin can attenuate the incidence of early AMD in those carrying important genetic risk variants. To achieve this benefit, it does not appear necessary to consume excessive amounts of these nutrients; the recommended dietary allowance will suffice.

Strengths of this study include the prospective design in a large population-based cohort, the lengthy duration of follow-up, the detailed and validated food frequency questionnaire, and the comprehensive grading of all AMD features at baseline and follow-up by the same experienced graders. The relatively low number of cases in the stratified analyses is a limitation. Nevertheless, despite the relatively large CIs, we were able to identify clear trends of risk reduction. Misclassification may have occurred in the stratification of nutrient intakes. We do not think that this caused false-positive findings because stratification procedures proved to be highly reproducible according to earlier investigations of the Rotterdam Study.[37] This misclassification, if present, is random and would have caused underestimation. Another issue is our inability to disentangle a healthy diet from other potentially protective factors that profile a healthy lifestyle. In particular, nutrients from foods are not consumed as single items but are always accompanied by other nutrients. Therefore, we cannot be sure that the observed association can be attributed solely to 1 factor as it may reflect a biological relationship with other food components.

Two other studies have examined interaction between genetic risk variants and nutrients in the development of AMD. In the AREDS antioxidant supplementation trial, Klein et al[33] calculated the risk of progression to late AMD for the *CFH* Y402H and *LOC387715* A69S genotypes in the various treatment arms. A high zinc dosage was most protective against AMD in noncarriers of the risk variant of *CFH* but produced the greatest, albeit nonsignificant, risk reduction in those carrying the risk variant of *LOC387715*. In the Blue Mountains Eye Study, a high fish intake resulted in a higher risk reduction in homozygous carriers of *CFH* Y402H than in noncarriers. However, this was not apparent for early AMD.[32] What could explain the discrepancies between our studies? The design of the studies was very different. The Age-Related Eye Disease Study was clinic based and the study population consisted of AMD cases with intermediate or unilateral late AMD, whereas ours consisted of participants without any sign of AMD. The outcome event was therefore different; for AREDS, this was progression from early to late AMD or from unilateral to bilateral late AMD, while we studied incident early AMD. The Age-Related Eye Disease Study was enriched for risk genotypes, leading to a much smaller number of noncarriers than in our study (97 in AREDS vs 820 in the Rotterdam Study). In the Blue Mountains Eye Study, the number of events was much lower (early AMD, 185 in the Blue Mountains Eye Study vs 517 in the Rotterdam Study). The lower numbers may have affected the estimation of the progression rate. We think that our study was most empowered to compare the risk of AMD in all these strata. Larger data sets with even more subjects per subgroup will be necessary to provide conclusive answers.

It is now well established that complement activation and inflammation play an important role in the pathogenesis of AMD.[7-10] *CFH* is a key regulator of complement by inhibiting the alternative pathway and amplification phase of the cascade. The risk allele Y402H appears to impair this regulatory function of *CFH*,[45-47] leading to complement overactivation, thereby increasing the risk of AMD.[15-19] The reason diet has an antagonistic effect in *CFH* Y402H carriers can be explained by several biological mechanisms: (1) Oxidative damage can activate the complement cascade by oxidation of fatty acids in retinal cell membranes.[12] Nutrients with antioxidant properties will counteract this process by reducing the production of reactive oxygen species in the outer retina.[12] (2) Even after activation of the complement cascade, zinc may reduce terminal cell lysis by prohibiting binding of C9 to C5-8[48] and by preventing the formation of the membrane-attack complex. (3) Zinc can bind C3b and C4b directly and thereby reduce further complement activation.[49] (4) ω-3 Fatty acids have been described to lower acute-phase proteins (including complement C4, IgM, haptoglobin, C-reactive protein [CRP], and fibrinogen) and to function as an anti-inflammatory agent in the retina.[50-52] (5) *CFH* Y402H impairs CRP-CFH binding, resulting in decreased inhibitory function of CFH, especially at high CRP levels.[46] β-Carotene is inversely related to CRP levels.[53,54] (6) Oxidative stress activates nuclear factor κB and thereby increases CRP levels. β-Carotene inhibits nuclear factor κB, thereby lowering CRP and decreasing complement activation, particularly in carriers.[55,56]

With respect to the *LOC387715* gene, its increased risk of AMD conferred by the A69S variant is evident.[20-24] Nevertheless, the precise mechanism has not been elucidated. The gene product was localized to mitochondrial outer membranes, in particular of rods and cones.[23,57] Earlier findings of disorganized mitochondrial membranes, as well as a decreased number of mitochondria, in reti-

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nal pigment epithelium cells of AMD donors have provided evidence of mitochondrial dysfunction in AMD.[58,59] Taken together, this suggests that the A69S variant may jeopardize mitochondrial function and consequently lead to the formation of reactive oxygen species, apoptosis, and AMD.[57-62] Nutrients may neutralize these oxygen radicals and reduce these devastating effects.[63-66] Since zinc and EPA/DHA have multiple biological functions besides antioxidant (ie, anti-inflammatory and antiatherogenic), it is also possible that the interaction acts via 1 or more of these other mechanisms.[49,63,64] For instance, dysfunctional mitochondria may increase complement activation,[49,67] which can be counteracted by zinc, as mentioned earlier. Not all nutrients interacting with *CFH* showed a significant interaction with *LOC388715,* and the reduced statistical power due to a lower frequency of the risk allele may account for this. It is also biologically plausible, since β-carotene and lutein/zeaxanthin do not accumulate preferentially in mitochondria,[68] while zinc and EPA/DHA do.[69,70]

In conclusion, our study shows that high dietary intake of antioxidants, zinc, and ω-3 fatty acids may reduce the risk of early AMD among those at high genetic risk. What diet is recommended? Fortified cereals, meats, dairy products, nuts, and seeds are a good source of zinc; dark-green leafy vegetables such as spinach and kale and orange vegetables including carrots and pumpkin are rich in β-carotene and lutein/zeaxanthin; and oily fish such as herring, salmon, sardines, trout, and tuna provide EPA/DHA. These nutrients are all recommended in the Food Guide Pyramid and should be part of a regular diet for older adults. Given that there are no other interventions that are readily available, or offer prevention at such low cost, our findings stress the importance of sufficient intake of these nutrients in young susceptible individuals to postpone or prevent the devastating effects of AMD.

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