Genetic Susceptibility, Dietary Antioxidants, and Long-Term Incidence of Age-Related Macular Degeneration in Two Populations

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Objective: To examine effect modification between genetic susceptibility to age-related macular degeneration (AMD) and dietary antioxidant or fish consumption on AMD risk.

Design: Pooled data analysis of population-based cohorts.

Participants: Participants from the Blue Mountains Eye Study (BMES) and Rotterdam Study (RS).

Methods: Dietary intakes of antioxidants (lutein/zeaxanthin [LZ], β-carotene, and vitamin C), long-chain omega-3 polyunsaturated fatty acids, and zinc were estimated from food frequency questionnaires. The AMD genetic risk was classified according to the number of risk alleles of *CFH* (*rs1061170*) or *ARMS2* (*rs10490924*) as low (no or 1 risk allele) or high (≥2 risk alleles). Interactions between dietary intake and genetic risk levels were assessed. Associations between dietary intake and AMD risk were assessed comparing the highest with the 2 lower intake tertiles by genetic risk subgroups using discrete logistic regression, conducted in each study separately and then using pooled data. Participants without AMD lesions at any visit were controls. We adjusted for age and sex in analyses of each cohort sample and for smoking status and study site in pooled-data analyses.

Main Outcome Measures: All 15-year incident late AMD cases were confirmed by chief investigators of the Beaver Dam Eye Study, BMES, and RS. Intergrader reproducibility was assessed in an early AMD subsample, with 86.4% agreement between BMES and RS graders, allowing for a 1-step difference on a 5-step AMD severity scale.

Results: In pooled data analyses, we found significant interaction between AMD genetic risk status and LZ intake ($P = 0.0009$) but nonsignificant interactions between genetic risk status and weekly fish consumption ($P = 0.05$) for risk of any AMD. Among participants with high genetic risk, the highest intake tertile of LZ was associated with a >20% reduced risk of early AMD, and weekly consumption of fish was associated with a 40% reduced risk of late AMD. No similar association was evident among participants with low genetic risk. No interaction was detected between β-carotene or vitamin C and genetic risk status.

Conclusions: Protection against AMD from greater LZ and fish consumption in persons with high genetic risk based on 2 major AMD genes raises the possibility of personalized preventive interventions. *Ophthalmology 2014;121:667-675 © 2014 by the American Academy of Ophthalmology.*

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Genetic predisposition for susceptibility to age-related macular degeneration (AMD) has been confirmed,[1] with estimated heritability ranging from 46% to 71% and environmental exposures explaining some proportion of risk variance.[2]

The Age-Related Eye Disease Study (AREDS), a randomized controlled trial, documented that high-dose zinc and antioxidant vitamin supplementation slowed AMD progression in advanced early AMD cases.[3] Another randomized controlled trial conducted in persons with geographic atrophy demonstrated that a lutein supplement over 12 months improved visual function.[4] Although evidence of the protective association between dietary intake or serum levels of these carotenoids and AMD has been inconsistent, findings from a systematic review and meta-analysis support an association of dietary lutein/zeaxanthin (LZ) intake and reduced risk of late AMD.[5]

The Blue Mountains Eye Study (BMES) and Rotterdam Study (RS) investigators independently documented that high dietary intake of LZ was associated with a reduced

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long-term risk of AMD.[6,7] In addition, in the BMES cohort, weekly consumption of fish was associated with a reduced risk of late AMD only in participants with the *CFH* risk (CC) genotype.[8] In the RS cohort, high dietary intake of antioxidants reduced the risk of early AMD in persons with high genetic risk for AMD.[9] The apparent protective effect observed in persons with genetic susceptibility to AMD[8,9] suggests an effect modification between known AMD genetic variants and dietary long-chain omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) or antioxidants.

Joint contributions and interactions between AMD-related genetic variants and other AMD risk factors (e.g., smoking[10,11] and inflammatory markers[12,13]) have been documented, including an effect modification of dietary docosahexaenoic fatty acids (a component of ω-3 PUFAs) on the risk of geographic atrophy in persons with the risk genotype of the *ARMS2* gene.[14] The interplay between nature and nurture may provide a better understanding of why some, but not all, persons with AMD-related genetic risk variants develop this condition. By using pooled longitudinal data from 2 population-based cohorts, we aimed to assess the consistency of the suggested effect modification between AMD genetic susceptibility and dietary intake of antioxidants or fish in relation to the incidence of early, late, and any (early and late) AMD.

Methods

The BMES and RS are population-based cohort studies with follow-up periods of 15 years. Participants were predominantly white.

Blue Mountains Eye Study

In 1992−1994, 3654 residents (82.4% of those eligible) aged ≥49 years, living in 2 postcode areas west of Sydney, Australia, participated in baseline examinations; 2335, 1952, and 1140 were reexamined after 5 years (1997−1999), 10 years (2002−2004), and 15 years (2007−2010), respectively. There were 2452 baseline participants followed up at least once. Each study visit was approved by the University of Sydney and the Sydney West Area Health Service Human Research Ethics Committees, and written, informed consents were obtained.

After pharmacologic mydriasis, 30° stereoscopic color transparencies of the macula and optic disc, and nonstereoscopic color transparencies of another 4 subfields were taken using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany) at baseline and 5- and 10-year follow-up visits, and a 40° digital camera (Canon CF-60 DSi with a Canon EoS 1DS Mark II camera body; Canon Inc., Tokyo, Japan) was used at the 15-year follow-up examination.

Rotterdam Study

At baseline (1990−1993), 7983 eligible persons (77.7% participation rate) aged ≥55 years were interviewed and examined. Ophthalmological examinations and retinal photography were performed on 6419 participants. Of these, 4977, 3637, 2674, and 1452 were reexamined at the second (1993−1995), third (1997−1999), fourth (2002−2004), and fifth (2009−2011) visits, respectively. Overall, 3579 participants had genetic and baseline dietary data together with follow-up information, were free of late AMD at baseline, and were included. To correspond with the BMES follow-up visit intervals (every 5 years), participants of the second follow-up visit (1993−1995) were excluded, except for incident late AMD cases that were included as incident AMD cases at the third visit (1997−1999). Each visit was approved by the Erasmus Medical Center Ethics Committee and complied with the Declaration of Helsinki. All participants gave written informed consent before participation.

After pharmacologic mydriasis, 35° stereoscopic color transparencies of the macula (Topcon TRV-50VT fundus camera; Topcon Optical Co., Tokyo, Japan) were taken in each of the first 3 visits, and 35° digital images (Topcon TRC 50EX fundus camera with the Sony DXC-950P digital camera; Topcon Optical Co.) were taken in the fourth and fifth visits.

Age-Related Macular Degeneration Phenotype Definitions and Harmonization

In both studies, retinal photographs of both eyes were initially graded by trained graders of each study[15,16] following the Wisconsin Age-related Maculopathy Grading System. Phenotype harmonization was performed within the Three Continent AMD Consortium.[17] In brief, all late AMD incident cases detected from each study were initially adjudicated and confirmed by the retinal specialists of the corresponding study team and then were confirmed by chief investigators of the BMES, RS, and Beaver Dam Eye Study. A 5-step severity scale (levels 10−50) was developed (Table 1, available at www.aaojournal.org). A subsample of 60 eyes covering various severity levels of early AMD was selected from the Beaver Dam Eye Study and sent to BMES and RS teams to be graded independently. Exact agreement on the 5-step severity scale was 61.0% between BMES and RS graders, noting that a 1-step difference increased agreement to 86.4%.

Assessment of Dietary Intake

In the BMES, a validated[18] 145-item, semiquantitative food frequency questionnaire (FFQ), modified from an early FFQ by Willett et al,[19] was used. The FFQ was completed and returned by 3267 baseline participants (89.4%), of which 2900 (88.8%) were considered usable.[18]

The electronic version of the Australian Tables of Food Composition 1990[20] was used to calculate the intake of most nutrients. The intake of ω-3 PUFA was calculated by adding dietary consumption of eicosapentaenoic (20:5, n-3) and docosahexaenoic (22:6, n-3) fatty acids. Information on fish consumption was obtained from the FFQ, and regular fish consumption was defined as ≥1 serving per week, compared with <1 serving per week.

In the RS, baseline dietary information was collected in 2 stages. First, participants completed a checklist at home. Second, a face-to-face interview was conducted by a trained dietitian at the research center using a 170-item validated semiquantitative FFQ.[21] By using the computerized Dutch Food Composition Table, these dietary data, including intakes of vitamins and zinc, were converted to total energy and nutrient intakes per day.[21] Intake of specific fatty acids was based on a food composition database derived from the TRANSFAIR Study.[22] β-Carotene and LZ were updated using an additional database of the Netherlands Institute of Public Health and Environmental Protection (Vollebregt YCJ, Feskens EJM, personal communication, 1993).[23]

Genotyping

In the BMES, genotyping was performed using the Illumina Human 670-Quadv1 custom genotyping array at the Wellcome Trust Centre for Human Genetics, Sanger Institute, Cambridge, UK, as

Table 3. Baseline Characteristics of the Blue Mountains Eye Study and Rotterdam Study Populations by Incidence of Age-Related Macular Degeneration

Characteristics	Incident Age-related Macular Degeneration							
	Blue Mountains Eye Study				*Rotterdam Study*			
	Controls (Level 10)	*Early (Levels 20–40)*	*Late (Level 50)*	*Any (Levels 20–50)*	*Controls (Level 10)*	*Early (Levels 20–40)*	*Late (Level 50)*	*Any (Levels 20–50)*
N	723	467	88	555	2006	657	115	772
Mean age, years (SD)	61.3 (7.6)	64.9 (7.7)	70.6 (7.1)	65.8 (7.9)	65.01 (6.41)	65.57 (6.46)	69.67 (6.55)	66.18 (6.63)
Men, %	45.1	39.4	31.8	38.2	42.1	41.3	38.3	40.8
Smoking status, %								
Never	47.9	55.1	51.1	54.5	33.0	33.2	29.8	32.7
Past	36.7	34.4	29.1	33.6	45.4	46.2	43.9	45.8
Current	15.4	10.5	19.8	11.9	21.6	20.6	26.3	21.4
At least weekly fish consumption, %	64.1	60.4	44.3	57.8	39.9	40.9	37.4	40.4
Genotype, %								
CFH (rs1061170)								
TT	42.3	34.9	21.8	32.8	46.4	33.8	22.6	32.1
CT	46.2	46.9	50.6	47.5	43.9	49.7	44.4	48.9
CC	11.5	18.2	27.6	19.7	9.8	16.5	33.0	19.0
ARMS2 (rs10490924)								
GG	64.9	57.7	49.4	56.3	65.2	56.7	40.0	54.2
TG	30.8	37.2	42.5	38.1	31.3	38.4	52.2	40.5
TT	4.3	5.1	8.1	5.6	3.5	4.9	7.8	5.3
Genetic risk level (% with 0, 1, or 2 risk alleles of *CFH and ARMS2*)								
0	27.5	18.9	9.3	17.3	29.2	20.8	5.2	18.5
1	41.5	42.9	36.1	41.7	45.1	38.0	37.4	37.9
2	31.0	38.2	54.7	40.9	25.7	41.2	57.4	43.6
AMD phenotype, %*								
Early AMD	–	–	61.4	–			62.6	
Large soft drusen	–	–	46.3	–			47.0	
Retinal pigmentary abnormality	–	–	47.6	–			31.3	

AMD = age-related macular degeneration; *CFH* = complement factor H; *ARMS2* = age-related maculopathy susceptibility 2; SD = standard deviation.
*Among participants who developed late AMD over the follow-up period, proportions with early AMD or early AMD lesions at study baseline.

part of the Wellcome Trust Case Control Consortium 2. After quality checking,[24] genotypes of 2534 participants (544,802 single nucleotide polymorphisms [SNPs]) were used for imputation. Genotypes were imputed from the 1000 Genomes (version 1) reference using IMPUTE software (available at: https://mathgen.stats.ox.ac.uk/impute/impute.html, accessed June 4, 2013).

In addition, genotype data were obtained previously for *rs1061170* in *CFH*, and *rs10490924* in *ARMS2* for participants who attended the 5-year follow-up visit.[8] We therefore used these 2 genotyped SNPs whenever available. The concordance rate between typed and imputed genotypes was 99.61% for *rs1061170* and 99.26% for *rs10490924* based on participants who had both typed and imputed data of these 2 SNPs.

In the RS, genotyping was performed using TaqMan assays (Applied Biosystems, Foster City, CA). The 2 SNPs (*rs1061170* in *CFH* and *rs10490924* in *ARMS2*) were successfully genotyped in 6345 and 6411 participants, respectively, and 6260 participants had both SNPs typed.[9] In addition, for participants without genotype data, imputed data of these 2 SNPs were obtained from a genome-wide association scan dataset, genotyped using the Illumina Infinium II HumanHap5. Imputation was performed using Markov Chain Haplotyping software version 1.0.15 (available at: http://www.sph.umich.edu/csg/abecasis/MACH/, accessed June 4, 2013) and HapMap CEU data (NCBI build 36, release 22, The International HapMap Project). There were 6478 participants with both SNPs typed or imputed.

Statistical Analysis

Of the 2534 BMES participants with dietary and genotype data available, 680 who had not been followed participated in the BMES Extension Study (1999–2000), leaving 1854 included in this report. Of the 3579 RS baseline participants with follow-up information and dietary and genotype data available, 2778 were followed at the third, fourth, or fifth visits and thus included. Characteristics between participants who were included and excluded were compared by each study (Table 2, available at www.aaojournal.org). Distributions of baseline AMD risk factors by incident AMD categories are shown in Table 3.

We compared dietary intakes between Australian and Netherlandish people using national survey data of the 2 countries (Table 4, available at www.aaojournal.org). The most recent published food survey data available are from the 1995 Australian National Survey[25,26] and the Dutch National Food Consumption Survey 2007–2010.[27] Main macronutrient intakes were also compared between BMES and RS baseline populations (Table 5, available at www.aaojournal.org).

We used energy-adjusted dietary intakes of antioxidants (LZ, β-carotene, vitamin C), ω-3 PUFA, zinc, and fish consumption as dietary exposures, and incident early, late, or any (early and late combined) AMD as outcome variables. Controls were participants who had no early or late AMD lesions at all visits (level 10 on the severity scale; Table 1, available at www.aaojournal.org). Given the substantial differences in

Table 6. Incident Age-Related Macular Degeneration Cases and Controls by Tertiles of Baseline Nutrient Intake in the Blue Mountains Eye Study and Rotterdam Study Cohorts

Dietary Antioxidant Intake/Day	Blue Mountains Eye Study				Rotterdam Study			
	Population, Mean (SD)	*First Tertile*	*Second Tertile*	*Third Tertile*	*Population, Mean (SD)*	*First Tertile*	*Second Tertile*	*Third Tertile*
LZ, mean μg (range)	912 (490)	442 (0–642)	810 (642–1005)	1425 (1005–4870)	2365 (1070)	1478 (101–1918)	2252 (1919–2610)	3362 (2610–32,645)
Persons by tertile, %								
Controls		30.7	32.0	37.3		31.2	34.4	34.4
Incident early AMD		28.9	36.8	34.3		30.9	33.5	35.6
Incident late AMD		31.8	39.8	28.4		33.9	38.3	27.8
Incident any AMD		29.4	37.3	33.3		31.3	34.2	34.5
β-carotene, mean μg (range)	7311 (4156)	3290 (0–5159)	6703 (5159–8299)	11512 (8299–43,699)	4009 (2227)	2400 (200–3193)	3754 (3193–4363)	5871 (4364–55,376)
Persons by tertile, %								
Controls		33.3	34.0	32.6		32.3	33.3	34.4
Incident early AMD		29.1	34.9	36.0		32.9	32.7	34.4
Incident late AMD		25.0	34.1	40.9		32.2	38.3	29.6
Incident any AMD		28.5	34.8	36.8		32.8	33.5	33.7
Vitamin C, mean mg (range)	182 (88)	98 (0–136)	167 (136–201)	272 (201–999)	120 (53)	70 (0–95)	113 (95–133)	177 (133–659)
Persons by tertile, %								
Controls		30.6	32.9	36.5		33.2	32.9	33.9
Incident early AMD		31.7	34.7	33.6		29.2	36.4	34.4
Incident late AMD		31.8	34.1	34.1		30.4	38.3	31.3
Incident any AMD		31.7	34.6	33.7		29.4	36.7	33.9
ω-3 PUFAs, mean g (range)	0.22 (0.25)	0.05 (0–0.11)	0.16 (0.11–0.22)	0.45 (0.22–3.30)	0.15 (0.21)	0.024 (0.0–0.05)	0.10 (0.05–0.15)	0.32 (0.15–6.34)
Persons by tertile, %								
Controls		31.5	32.9	35.6		32.3	32.7	35.0
Incident early AMD		33.0	34.5	32.5		33.6	33.0	33.3
Incident late AMD		29.6	37.5	32.9		32.2	36.5	31.3
Incident any AMD		32.4	35.0	32.6		33.4	33.6	33.0
Zinc, mean mg (range)	12 (2.3)	9.6 (4.4–10.8)	11.6 (10.8–12.5)	14.2 (12.5–31.4)	11 (2.0)	8.5 (3.7–9.7)	10.6 (9.7–11.4)	12.8 (11.4–24.9)
Persons by tertile, %								
Controls		33.3	33.2	33.5		32.9	32.6	34.6
Incident early AMD		28.9	34.3	36.8		28.9	35.8	35.3
Incident late AMD		21.6	34.1	44.3		41.7	29.6	28.7
Incident any AMD		27.8	34.2	38.0		30.8	34.8	34.3

AMD = age-related macular degeneration; LZ = lutein/zeaxanthin; ω-3 PUFA = long-chain omega-3 polyunsaturated fatty acid; SD = standard deviation.

estimates for population means of dietary antioxidant intake between the 2 studies, we used population-specific tertiles in analyses and examined tertile distributions of dietary antioxidant intake by incident early, late, and any AMD (Table 6). We initially examined the associations of these dietary intakes with risk of AMD across 3 tertiles and detected a threshold between the highest and the 2 lower intake tertiles. We therefore decided to compare the highest with the 2 lower tertiles in all models and reference them to controls.

To assess whether there were effect modifications between the selected dietary intakes and the genetic susceptibility to AMD, using the 2 variants of major AMD-related genes (*CFH, rs1061170* and *ARMS 2, rs10490924*) we grouped participants' genetic risk of AMD into 3 levels: (1) having no risk alleles of *CFH* (*rs1061170*) or *ARMS2* (*rs10490924*); (2) having 1 risk allele from either of the 2 genes; and (3) having ≥2 risk alleles from either or both of these genes. We tested for statistical interactions between genetic risk status according to the grouping and the dietary exposures by adding product terms of the gene risk levels with 1 dietary exposure at a time, together with the genetic risk status and the dietary exposure in each of the discrete logistic regression models. We adjusted for age, sex, and smoking in analyses performed within each study and for study site in analyses of pooled data of the 2 studies (Table 7, available at www.aaojournal.org).

We further compared the highest intake tertile with the 2 lower tertiles in subgroups stratified by the 3 AMD genetic risk levels, with 15-year incident early, late, or any AMD as the dependent variable and time to event as the discrete variable in logistic regression models. We adjusted for age and sex in all models (1 model for each dietary exposure) in analyses of data from each study separately (Table 8) and adjusted for age, sex, smoking status, and study indicator in pooled data analyses (Table 9).

Risk estimations are presented as odds ratios and 95% confidence intervals. SAS (version 9.2; SAS Inc., Cary, NC) was used for all analyses. Odds ratios >1 or <1 indicated an increase (or decrease) in risk associated with the highest intake tertile relative to the risk associated with the lower intake tertiles.

Results

Comparisons of characteristics between the 1854 included and the 1800 excluded BMES participants, and between the 2778 included and the 3641 excluded RS participants, showed that those excluded were older and more likely to have a history of diabetes or cardiovascular conditions and early AMD. After adjusting for age, the significant differences in early AMD and AMD lesion distributions between the 2 groups disappeared (Table 2, available at www.aaojournal.org). In the BMES, excluded participants had

Table 8. Associations between Dietary Antioxidants and Incidence of Early and Late Stage Age-Related Macular Degeneration by Subgroups of the Population with None, One Only, or Two or More Risk Alleles of the *CFH* and *A9RMS2* Genes Combined

Risk Factor	Blue Mountains Eye Study			Rotterdam Study		
	Early AMD	*Late AMD*	*Any AMD*	*Early AMD*	*Late AMD*	*Any AMD*
Genetic Risk Group = 0 Risk Alleles from *CFH* or *ARMS2*						
	n=80	*n=8*	*n=88*	*n=136*	*n=6*	*n=142*
Age, per 10 yrs	2.26 (1.61–3.17)	11.55 (2.86–46.72)	2.36 (1.71–3.27)	1.67 (1.23–2.26)	9.24 (2.40–35.65)	1.80 (1.34–2.41)
Female sex	1.41 (0.86–2.32)	—	1.53 (0.94–2.48)	0.98 (0.68–1.41)	0.39 (0.07–2.25)	0.94 (0.66–1.35)
Current smoker	1.54 (0.67–3.57)	—	1.53 (0.66–3.54)	1.05 (0.66–1.65)	1.41 (0.15–13.66)	1.07 (0.68–1.67)
Fish, ≥1 serving/wk†	1.12 (0.68–1.85)	1.79 (0.36–8.80)	1.10 (0.68–1.78)	1.20 (0.84–1.72)	0.35 (0.04–3.10)	1.16 (0.81–1.65)
LZ*	0.99 (0.60–1.65)	0.30 (0.03–2.64)	0.93 (0.57–1.53)	1.74 (1.21–2.50)	1.18 (0.20–6.82)	1.69 (1.18–2.41)
Vitamin C*	0.96 (0.57–1.62)	0.31 (0.04–2.76)	0.91 (0.55–1.50)	0.88 (0.60–1.31)	1.30 (0.21–7.88)	0.90 (0.61–1.32)
Genetic Risk Group = 1 Risk Allele from *CFH* or *ARMS2*						
	n=181	*n=31*	*n=212*	*n=248*	*n=43*	*n=291*
Age, per 10 yrs	2.46 (1.95–3.10)	12.39 (6.13–25.01)	2.59 (2.10–3.20)	1.83 (1.48–2.27)	5.61 (3.39–9.29)	2.08 (1.70–2.54)
Female sex	1.21 (0.85–1.71)	2.74 (1.12–6.69)	1.24 (0.89–1.72)	1.26 (0.96–1.67)	1.31 (0.68–2.52)	1.25 (0.97–1.63)
Current smoker	1.41 (0.84–2.37)	2.28 (0.67–7.78)	1.38 (0.85–2.23)	1.19 (0.85–1.66)	2.14 (1.02–4.50)	1.26 (0.93–1.72)
Fish, ≥1 serving/wk†	0.94 (0.66–1.34)	0.90 (0.39–2.06)	0.96 (0.68–1.34)	0.84 (0.63–1.11)	1.14 (0.60–2.16)	0.87 (0.67–1.13)
LZ*	0.85 (0.60–1.21)	1.34 (0.55–3.23)	0.90 (0.64–1.27)	0.94 (0.71–1.24)	0.90 (0.47–1.73)	0.94 (0.72–1.22)
Vitamin C*	0.91 (0.64–1.31)	1.82 (0.78–4.24)	0.98 (0.70–1.38)	0.90 (0.67–1.20)	0.87 (0.44–1.72)	0.89 (0.68–1.17)
Genetic Risk Group ≥2 Risk Alleles from *CFH* or *ARMS2*						
	n=161	*n=47*	*n=208*	*n=269*	*n=66*	*n=335*
Age, per 10 yrs	2.15 (1.69–2.72)	5.94 (3.59–9.83)	2.45 (1.96–3.06)	2.00 (1.60–2.51)	6.37 (4.07–9.97)	2.48 (2.01–3.06)
Female sex	0.99 (0.69–1.42)	2.22 (1.09–4.52)	1.06 (0.76–1.48)	0.87 (0.66–1.14)	1.18 (0.68–2.05)	0.89 (0.69–1.15)
Current smoker	1.02 (0.57–1.83)	3.43 (2.50–7.84)	1.20 (0.74–1.94)	1.03 (0.74–1.44)	2.03 (1.11–3.74)	1.16 (0.86–1.57)
Fish, ≥1 serving/wk†	0.71 (0.49–1.03)	0.18 (0.08–0.38)	0.62 (0.44–0.87)	0.99 (0.76–1.30)	1.05 (0.61–1.81)	1.00 (0.78–1.29)
LZ*	0.76 (0.51–1.13)	0.58 (0.28–1.20)	0.72 (0.50–1.04)	0.78 (0.59–1.05)	0.70 (0.38–1.29)	0.77 (0.59–1.01)
Vitamin C*	0.66 (0.44–0.98)	0.42 (0.20–0.87)	0.67 (0.47–0.96)	1.02 (0.77–1.35)	0.73 (0.41–1.29)	0.97 (0.75–1.25)

AMD = age-related macular degeneration; *ARMS2* = age-related maculopathy susceptibility 2; *CFH* = complement factor H; LZ = lutein/zeaxanthin.
Data are shown as age- and sex-adjusted odds ratios (95% confidence intervals).
*Population-specific tertiles with the highest versus other 2 (middle and lowest) tertiles, after adjusting for energy intake.
†Compared with persons with fish consumption <1 serving/wk.

lower mean intake levels of dietary antioxidants and ω-3 PUFA compared with those who were included. In the RS, excluded and included participants had similar mean intake levels of dietary oxidants and ω-3 PUFA (Table 2, available at www.aaojournal.org). Of the 1800 BMES excluded participants, 32% died before the 5-year follow-up examination. Of the 3641 RS excluded participants, 22% died before the 6-year follow-up examination.

Of the 1854 BMES participants, 723 had no AMD at all during visits, 467 had incident early AMD, and 88 had incident late AMD. Of the 2778 RS participants, the corresponding numbers were 2006 without AMD, 657 with incident early AMD, and 115 with incident late AMD.

As expected, the mean ages of the incident AMD groups were higher compared with controls. In addition, crude data comparisons confirmed that in both the incident early and late AMD groups, the proportions of participants homozygous for the risk genotypes of *CFH* and *ARMS2* were higher compared with controls, as were the proportions of current smokers in the late AMD groups (Table 3).

Comparison of Australian[25,26] and Dutch[27] national food consumption survey data (Table 4, available at www.aaojournal.org) showed that Australian men and women aged 45 to 64 years consumed more fruits, seafood, and meat/poultry products than Dutch men and women aged 51 to 69 years. Although vegetable consumption levels were similar between Australian and Dutch men and women of similar age groups, Australians consumed fewer leafy vegetables than the Dutch, and Australian men consumed more carrots/root vegetables than Dutch men. Consumption levels of energy and other dietary items/food groups, macronutrients, and micronutrients were similar, except for iron intake, which was substantially lower among Dutch men and women. We could not find data on LZ consumption levels from the national survey reports.[25–27]

Energy and main macronutrient intake levels were similar between the BMES and RS populations (Table 5, available at www.aaojournal.org). Although the mean intakes of ω-3 PUFA and zinc were similar between the 2 populations, the mean intakes of LZ (higher in the RS) and β-carotene (higher in the BMES) differed substantially (Table 6). A consistent pattern was evident in both study samples that relatively lower proportions of participants in the incident late AMD group were in the highest intake tertile of LZ and ω-3 PUFA (Table 6).

Significant interaction between AMD genetic risk status and LZ intake with respect to risk of early or any AMD was evident in the RS but not the BMES. In pooled data analyses of the 2 cohorts, we found a significant interaction between AMD genetic risk status and LZ intake with respect to risk of early AMD ($P = 0.002$) or any AMD ($P = 0.0009$) and a marginally nonsignificant interaction

Table 9. Associations between Dietary Antioxidants and Incidence of Early and Late Age-related Macular Degeneration by Subgroups of Population with None, One, or Two or More Risk Alleles of the *CFH* and *ARMS2* Genes (Pooled Data of the Blue Mountains Eye Study and Rotterdam Study, Adjusting for Age, Sex, Smoking, Energy Intake, and Study Site Indicator)

Dietary Factor	Early AMD	Late AMD	Any AMD
Genetic Risk Group = 0 Risk Alleles from CFH *or* ARMS2			
	n=215	*n=14*	*n=229*
Fish, ≥1 serving/wk†	1.17 (0.87–1.57)	0.91 (0.29–2.84)	1.14 (0.85–1.51)
LZ*	1.47 (1.09–1.97)	0.65 (0.17–2.43)	1.40 (1.05–1.87)
Vitamin C*	0.91 (0.67–1.24)	0.78 (0.20–2.95)	0.91 (0.67–1.23)
Genetic Risk Group = 1 Risk Allele from *CFH* or *ARMS2*			
	n=429	*n=74*	*n=503*
Fish, ≥1 serving/wk†	0.87 (0.70–1.08)	0.98 (0.59–1.63)	0.90 (0.73–1.10)
LZ*	0.91 (0.73–1.13)	1.06 (0.63–1.79)	0.92 (0.75–1.13)
Vitamin C*	0.94 (0.75–1.17)	1.33 (0.79–2.24)	0.96 (0.78–1.19)
Genetic Risk Group = 2+ Risk Alleles from *CFH* or *ARMS2*			
	n=430	*n=112*	*n=542*
Fish, ≥1 serving/wk†	0.89 (0.71–1.10)	0.54 (0.35–0.85)	0.84 (0.69–1.03)
LZ*	0.78 (0.62–0.99)	0.64 (0.40–1.03)	0.75 (0.60–0.93)
Vitamin C*	0.87 (0.70–1.10)	0.67 (0.43–1.06)	0.86 (0.70–1.06)

AMD = age-related macular degeneration; *ARMS2* = age-related maculopathy susceptibility 2; *CFH* = complement factor H; LZ = lutein/zeaxanthin.
Data are shown as odds ratios (95% confidence intervals).
*Population-specific tertiles with the highest versus the other 2 (middle and lowest) tertiles, adjusted for age, sex, smoking, energy intake, and study site.
†Compared with persons with fish consumption <1 serving/wk.

between AMD genetic risk status and weekly consumption of fish ($P = 0.05$) (Table 7, available at www.aaojournal.org). These interaction P values are smaller than the corresponding significance levels required after Bonferroni correction for 3 interaction tests for the 3 dietary factors ($P < 0.05/3 = 0.017$). There was no significant interaction found between vitamin C, β-carotene, or zinc intake and AMD genetic risk status in relation to the risk of AMD.

We next stratified the study samples according to their genetic risk levels and investigated the associations between baseline weekly consumption of fish, dietary intakes of LZ or vitamin C, and incidence of early, late, or any AMD in each study (Table 8) and then in pooled data of the 2 studies (Table 9). In the subgroup with ≥2 risk alleles of *CFH* or *ARMS2*, weekly fish consumption and the highest tertile intake of vitamin C were associated with reduced risk of any AMD in the BMES but not the RS (Table 8). There was a marginally nonsignificant association between the highest tertile intake of LZ and the reduced risk of any AMD in both studies, where the association magnitude was 28% and 23% risk reduction in the BMES and RS, respectively (Table 8).

In a pooled data analysis of the 2 cohorts, among participants with ≥2 risk alleles of the *CFH* or *ARMS2*, a significant association between weekly consumption of fish and a 46% reduction in late AMD risk was evident. Likewise, significant associations were evident between the highest tertile intake of LZ and 22% to 25% risk reduction in early and any AMD. The highest tertile intakes of LZ and vitamin C were nonsignificantly associated with an approximately 35% risk reduction in late AMD (Table 9). In the other 2 subgroups with no or 1 risk allele, no similar associations were evident (Tables 8 and 9).

Discussion

By using data from 2 population-based cohorts, we showed consistent evidence that participants with ≥2 risk alleles of either or both the *CFH-rs1061170* or *ARMS2-rs10490924* had a significantly reduced risk of early or any AMD if they frequently consumed food items rich in LZ.

Findings from the BMES and RS individually were less consistent for the effect modification of weekly consumption of fish or high dietary vitamin C intake with AMD genetic risk level on AMD risk (Table 8, bottom). By pooling data from the 2 cohorts and incorporating 3 follow-up visits with similar time intervals, we demonstrated a significant association of regular fish consumption with a 46% reduction in late AMD risk and a marginally nonsignificant association between high intake of vitamin C and reduced risk of late or any AMD among participants with high genetic risk of AMD (Table 9, bottom). However, these 2 effect modifications seem to be driven by findings from the BMES. In contrast, the association between the highest tertile of LZ intake and the reduced risk of early or any AMD in those with high genetic risk was driven by the findings from the RS, but the direction of the protective association was relatively consistent across the 2 cohorts, with risk estimates approximately 0.7 to 0.8, although not reaching statistical significance (Table 8, bottom). This effect modification became significant when data were pooled, with a 22% risk reduction in early AMD in participants with high genetic risk (Table 9, bottom).

A recent report from the AREDS2 documented a protective effect of LZ supplement use over 5 years compared with no LZ supplement use on AMD progression in persons in the lowest quintile of dietary LZ intake.[27] The relatively short follow-up duration and lack of stratified analyses conducted in genetic risk subgroups may explain the nonsignificant findings in primary analyses.[28]

A beneficial effect of ω-3 PUFA and fish consumption on AMD[29–31] has been reported, where the anti-inflammatory property of ω-3 PUFA[32] is considered to be one of the underlying mechanisms. In addition, there is increasing recognition that a lipid metabolism pathway may be a key element in the course of AMD development.[33] The outer segments of photoreceptors, subjected to high photo-oxidative stress, have high concentrations of PUFAs and high oxygen tension, and PUFAs are susceptible to oxidation in the presence of oxygen or oxygen-derived radical species.[34] It is possible that lipid oxidation/peroxidation products activate or amplify local inflammatory processes via the complement system[33,35,36] and that ω-3 PUFAs and antioxidants may counteract

these processes. Evidence for possible mechanisms is emerging.[37]

Lutein and zeaxanthin are components of macular xanthophylls and dihydroxy-carotenoids. The light-filtering capability is a passive antioxidant function of LZ and thus potentially prevents blue light from generating reactive oxygen species.[38] Lutein/zeaxanthin also may have an anti-inflammatory property.[39] Our findings are in keeping with these known functions of ω-3 PUFAs and LZ. The effect modification of LZ on participants with high AMD genetic risk suggests the possibility that susceptibility to activation and amplification of the complement pathways can be compensated for by these antioxidants. An analogous observation is the significant association between blue light exposure and neovascular AMD in persons with low levels of plasma LZ and vitamins C and E.[40]

The reliability of dietary data collected in nutritional epidemiology studies may be a concern. We compared dietary consumption survey data between 2 countries[25–27] (Table 4, available at www.aaojournal.org) and between 2 study samples (Table 5, available at www.aaojournal.org) that showed similar intake levels of energy, most main food groups, and macronutrients between the 2 countries and the 2 study populations. The concordance of most dietary intakes between the 2 populations suggests that the FFQs used and the resulting dietary data are likely to be robust, regardless of which specific FFQ was used. Nevertheless, we noticed differences in the intake levels of some food items between the 2 countries. The relatively high intake level of leafy vegetables by the Dutch and the relatively high intake level of carrots/root vegetables by Australian men, together with higher proportions of Australians eating these vegetables, may partly explain the differences in population mean intake levels of LZ and β-carotene (Table 4, available at www.aaojournal.org). Different LZ intake estimation methods used by the 2 studies also may contribute to the difference in mean intake estimates of this nutrient.

Study Strengths and Limitations

Strengths of this study include long-term follow-up of population-based samples, photographic documentation of AMD status with incident late AMD cases cross-validated, and reasonable intergrader reproducibility on early AMD detection between study graders. A major limitation of this study is a degree of heterogeneity in dietary intake patterns and consumption levels of some micronutrients between the 2 populations. We have used relative measures for dietary intakes and adjusted for different study sites in the statistical models. Findings for relative measures are directly applicable to populations of specific geographic locations regardless of absolute intake levels. Other limitations include survival bias to which our cohorts are subject, nonavailability of serum or plasma nutrient levels, and lack of specific data for oily fish consumption. Misclassification or reduced power from these limitations will tend to bias the associations toward the null. There was no evidence supporting associations between the mortality and the 2 genotypes or the dietary antioxidants under investigation, so survival bias should have only minimal effect on the associations. Even with pooled data from 2 cohorts, we had limited numbers of late AMD cases and therefore insufficient power to detect a significant association between LZ intake and late AMD incidence in the high genetic risk subgroup (Table 9).

Caution is needed in interpreting these findings. Nutrients do not work alone but interact with genes and the internal environment of the host, which may be influenced by many factors, such as lifestyle, intestinal microorganisms, and the uptake ability of the host, all of which may lead to differences in bioavailability of specific nutrients on disease pathways.

In conclusion, we showed that dietary intake of LZ is associated with an approximate 20% reduction in risk of developing early AMD among persons with a high genetic risk of AMD. The relatively consistent pattern of the effect modifications between LZ intake and AMD-related genetic risk levels in our 2 cohorts may have clinical implications in the management of patients with AMD. Future studies are warranted to confirm this effect modification of major AMD-related genes and dietary intake of antioxidants on the risk of AMD. Our findings also highlight the importance of incorporating information from both genetic and environmental exposures to capture the complexity of disease pathways and pathogeneses of conditions such as AMD.

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