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Dietary Omega-3 Fatty Acids, Other Fat Intake, Genetic Susceptibility and Progression to Incident Geographic Atrophy

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Abstract

Objective—To investigate associations between dietary omega-3 fatty acids and other fat intake, genes related to age-related macular degeneration (AMD) and progression to geographic atrophy (GA).

Design—Observational analysis of a prospective cohort.

Participants—2531 individuals from the Age-Related Eye Disease Study, among which 525 eyes progressed to GA and 4165 eyes did not.

Methods—Eyes without advanced AMD (GA or neovascular disease) at baseline were evaluated for progression to GA. Behavioral data, including smoking and body mass index measurements were collected at baseline using questionnaires. Dietary data was collected from food frequency questionnaires (FFQ) at baseline. Dietary fats, including omega-3 fatty acids (docosahexaenoic acid or DHA and eicosapentaenoic acid or EPA), omega-6 fatty acids, monounsaturated, saturated, polyunsaturated and total fat were sex and calorie adjusted and divided into quintiles. Eight single nucleotide polymorphisms (SNPs) in 7 genes: *CFH*, *ARMS2/HTRA1*, *CFB*, *C2*, *C3*, *CFI*, *LIPC* were genotyped. Cox proportional hazards models were used to test for associations between incident GA and intake of dietary lipids, and interaction effects between dietary fat intake and genetic variation on risk of GA.

Main Outcome Measures—Associations between dietary fat intake reported from FFQs, genetic variants and incident GA.

Results—Increased intake of DHA was significantly associated with reduced risk of progression to GA in multivariate models with behavioral factors (Model A) and behavioral factors with genetic variants (Model B) (P-trend=0.008 and 0.03, respectively). Total omega-3 long chain polyunsaturated (DHA + EPA) fatty acid intake was significantly associated with reduced risk of progression in Model B variants (P-trend =0.02). Monounsaturated fat was associated with increased risk in Model A (P=0.05).. DHA intake in the 5th quintile was significantly associated

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with reduced risk of incident GA among those with the *ARMS2/HTRA1* homozygous risk genotype (HR = 0.4, P = 0.002, P – interaction between gene and fat intake = 0.05), whereas DHA was not associated with reduced risk of GA among those with the homozygous non-risk genotype (HR = 1.0, P = 0.90).

Conclusions—Increased self-reported dietary intake of omega-3 fatty acids is associated with reduced risk of GA and may modify genetic susceptibility for progression to GA.

Age-related macular degeneration (AMD) is a chronic, progressive disease with two end-stages: neovascular disease (NV) and geographic atrophy (GA), both of which can lead to irreversible blindness.¹ GA and NV are clinically and pathologically different: NV is characterized by angiogenesis which leads to leaking fluid, lipids and blood in the retina, whereas GA is characterized by atrophy of the neurosensory retina and retinal pigment epithelium.^{1,2,3} The presence of drusen, the clinical sign of early and intermediate AMD is associated with increased risk of progression to advanced AMD.^{4,5} AMD has a strong genetic component, and several genes are associated with advanced AMD, including *CFH*, *ARMS2/HTRA1*, *C3*, *C2*, *CFB*, *CFI*, and *LIPC*.^{6–14} Modifiable factors are also associated with slowing progression of early AMD to advanced stage, including antioxidant vitamin supplements, no history of smoking, lower body mass index (BMI), intake of leafy green vegetables which are high in lutein/zeaxanthin, and fish which are high in omega-3 fatty acids.^{1,15–21} Total dietary fat intake, saturated fat, omega-6 fatty acids, monounsaturated fat, and transunsaturated fats have also been shown to be associated with increased risk of advanced AMD.^{17,18,21–23} AMD is associated with inflammatory mechanisms including the complement gene pathway^{6,7,11–13} and systemic inflammatory biomarkers including C-reactive protein.²⁴ Omega-3 long chain polyunsaturated fatty acids have anti-inflammatory and anti-oxidative properties, and increased dietary intake of these nutrients has been shown to slow or reduce development of advanced stages.^{17,21,22,25,26}

The exact mechanisms whereby some individuals never progress beyond the early or intermediate stages, while others go on to develop GA or NV are not completely understood. Pharmacologic treatments exist for the NV form of the disease, but to date there are no such treatments for GA and little is known about risk factors specifically related to this form of advanced AMD.

We therefore investigated the impact of specific types of fats on GA controlling for genetic susceptibility, and whether their intake could modify genetic susceptibility to progression to GA. In this study we expand upon previous studies in several ways: investigating progression to GA only, accounting for various rates of progression over time in both eyes, controlling for 8 genetic variants and assessing interactions and effect modification between dietary fats and genetic variants.

Methods

Study Population and Progression Data

Details of the Age-Related Eye Disease Study (AREDS) population are reported elsewhere. Briefly, AREDS included a randomized clinical trial to assess the effect of antioxidant and mineral supplements on risk of AMD and cataract and a longitudinal study of AMD that ended in December, 2005.²⁷ Research adhered to the tenets of the Declaration of Helsinki.

Phenotype data was accessed through the Database of Genotypes and Phenotypes (dbGAP). Data from ocular examinations and fundus photographs were used to define eye phenotypes. Eyes were assigned a grade of no AMD, early, intermediate, or two different forms of advanced or late stage AMD based on the 5 Stage Clinical Age-Related Maculopathy Grading System (CARMS), in order to combine central and non-central GA into one grade

(4), and to separate NV as a separate grade (5), regardless of visual acuity.²⁸ Grades were defined as follows based on fundus and examination data: neovascular disease, or grade 5, if there were any definitive signs of neovascular AMD such as hemorrhagic retinal detachment, hemorrhage under the retina or retinal pigment epithelium, or subretinal fibrosis; geographic atrophy, or grade 4 if there was geographic atrophy either in the center grid or anywhere within the grid and had no record of hemorrhage; large drusen ($\geq 125\mu\text{m}$) were assigned to grade 3; intermediate drusen ($63\text{--}124\mu\text{m}$) were assigned to grade 2, as long as there were no signs of advanced AMD; no drusen or only a few small drusen ($<63\mu\text{m}$) were assigned to grade 1.

Progression was defined as either eye progressing from a grade 1, 2, or 3 to grade 4 (GA), at any point in time. Eyes with the end point (4 or 5) at baseline were excluded from the analysis. Follow-up ended when an eye progressed to GA. Eyes that had no record of GA were censored when they reached grade 5.

Dietary and Behavioral Covariates

Demographic (age and sex), behavioral (BMI, smoking, antioxidant status), and dietary information at baseline was obtained from dbGAP. Antioxidant treatment was defined as “yes” for subjects in the antioxidants alone or the antioxidants plus zinc groups, and “no” for subjects in the placebo or the zinc groups. Antioxidant treatment groups were randomly assigned in the AREDS clinical trial. Diet data were obtained from food frequency questionnaires (FFQs), including measurements of total fat, saturated fat, total polyunsaturated fatty acids, monounsaturated fat, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), combined long chain polyunsaturated fatty acids DHA and EPA, linolenic, and linoleic acid (an omega-6 fatty acid). In our analysis, nutrients were log transformed and adjusted for sex and caloric intake. Individuals with intake < 600 were excluded from the analysis and, men and women with total caloric intake ≥ 4200 or ≥ 3200 , respectively, were excluded from the analyses.

Genotype Data

DNA samples were obtained from the AREDS repository, and we genotyped them for 8 single nucleotide polymorphisms (SNPs) in genes demonstrated to be related to AMD: 1) Complement Factor H (*CFH* Y402H (rs1061170) in exon 9 of the *CFH* gene on chromosome 1q32, a change 1277T>C, resulting in a substitution of histidine for tyrosine at codon 402 of the CFH protein, 2) *CFH* rs1410996 an independently associated SNP variant within intron 14 of *CFH*,^{6,7} 3) *ARMS2/HTRA1* rs10490924, a non-synonymous coding SNP variant in exon 1 of LOC387715 on chromosome 10 resulting in a substitution of the amino acid serine for alanine at codon 69,^{9,10} 4) Complement Component 2 or *C2 E318D* (rs9332739), the non-synonymous coding SNP variant in exon 7 of *C2* resulting in a substitution of aspartic acid for glutamic acid at codon 318, 5) Complement Factor B or *CFB R32Q* (rs641153), the non-synonymous coding SNP variant in exon 2 of *CFB* resulting in the substitution of the amino acid glutamine for arginine at codon 32,¹¹ 6) Complement Component 3 or *C3 R102G* (rs2230199), the non-synonymous coding SNP variant in exon 3 of *C3* resulting in the substitution of the amino acid glycine for arginine at codon 102,¹² 7) Complement Factor I or *CFI* (rs10033900), an independently associated SNP located in the linkage peak region of chromosome 4, 2781 base pairs upstream of the 3' untranslated region of *CFI*,¹³ and 8) Hepatic Lipase C or *LIPC* (rs10468017), a promoter variant on chromosome 15q22.¹⁴ (For the genetic variant on chromosome 10, *ARMS2*, it remains a subject of debate whether the gene *HTRA1* adjacent to it may in fact be the AMD susceptibility gene on 10q26; however, the relevant SNPs in these 2 genes have been reported to be nearly perfectly correlated. Thus, while the other SNP is a promising candidate variant, rs10490924 used in this study can be considered a surrogate for the causal

variant which resides in this region.^{8,9,10} For the *C2/CFB* genes, there are two independent associations to the *C2/CFB* locus, but because of linkage disequilibrium we do not know which of the two genes or both are functionally affected).¹¹ Genotyping was performed using primer mass extension and MALDI-TOF MS analysis (MassEXTEND methodology of Sequenom, San Diego, CA) at the Broad Institute Center for Genotyping and Analysis (Cambridge, MA).

Statistical Analysis

The Cox proportional hazards model (PROC PHREG with the covariate aggregate option) was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for progression to geographic atrophy in individual eyes controlling for baseline AMD status, genetic, environmental, demographic, and dietary fat intake. Dietary fat variables were ranked into quintiles by sex. The median value of dietary fats in each quintile was used in multivariate models, and for performing tests to calculate the P-value for trend. P-values ≤ 0.05 were considered statistically significant.

Results

Table 1 (available at <http://aaojournal.org>) shows baseline demographic, behavioral and genetic characteristics among progressors and non-progressors adjusting for age. Among 2128 individuals (4165 eyes), 403 individuals (525 eyes) progressed to GA. The percentage of individuals who progressed to GA over 5 and 10 years was 8.1% and 16.9%, respectively. Individuals with intermediate AMD (grade 3) in the worse eye, or GA in one eye and a non-advanced fellow eye, were at increased risk of progression to GA. Progressors to GA tended to be older, were more likely to have smoked (among those less than 70 years of age), and had higher BMI than non-progressors. Non-progressors (N = 1454, 68%) completed more years of education than progressors (P = 0.002). The risk alleles of *CFH* variants, *ARMS/HTRA1*, *C3* and *CFI* were all significantly associated with increased risk of progression. The protective alleles of *CFB*, *C2*, and *LIPC* were all significantly associated with decreased risk of progression.

In Table 2 (available at <http://aaojournal.org>) we present the baseline distribution of sex and calorie adjusted dietary fat intake among progressors and non-progressors. Controlling for age and initial eye grade, progressors had significantly higher intake of monounsaturated fat (P-trend = 0.02) than non-progressors. Progressors had a lower intake of DHA (P-trend = 0.03). Although not significant, progressors also tended to have a lower intake of EPA and lower combined DHA and EPA intake, higher intake of linoleic acid and total polyunsaturated fatty acids.

Table 3 displays baseline demographic, behavioral and genetic characteristics by sex and calorie adjusted quintile of DHA intake. Individuals with low DHA were more likely to have NV in at least one eye (15%) than those with higher intake (11%). People with low DHA were also more likely to be current smokers (8%) compared to those with higher intake (5%), but this difference was not significant.

Individuals with higher intake of DHA tended to have higher BMI. There were no significant associations between quintile of DHA and genetic factors.

Table 4 displays multivariate associations between dietary fat intake and progression to GA in two models: Model A controlling for baseline AMD grade, sex, age, AREDS treatment, education, smoking, BMI, and caloric intake and Model B with all the covariates in Model A in addition to the genetic variants. In Model A there was a significant trend for reduction in risk of progression to GA with increasing intake of DHA (P-trend = 0.03). This trend

remained significant (P-trend = 0.008, HR, 95% CI (Quintile (Q) 5 vs Q1) = 0.68 (0.48 – 0.94)) after adjustment for genetic variants (Model B). In Model B the trend between a combination of DHA + EPA intake and reduced risk of progression was significant (P=0.02). There was a trend for increased risk of progression with increasing intake of monounsaturated fat in Model A (P-trend= 0.05). In Model B, Q2, Q4, and Q5 of monounsaturated fat were significantly associated with increased risk of progression compared to Q1, but the overall trend was not significant. Total fat and saturated fat were not significantly associated with risk of progression to GA.

In Table 5 we display the effect of DHA intake on progression to GA according to genotype, controlling for baseline AMD grade, demographic, environmental factors, DHA and a single gene (Model A), and all covariates in Model A and all 8 genetic variants in Model B. There was a significant protective effect of DHA among people with the *ARMS2/HTRA1* homozygous risk genotype (Model B: HR = 0.4, P = 0.002) while no association was seen among individuals with the homozygous non-risk genotype (Model B: HR = 1.0, P = 0.9, P – interaction = 0.05). In contrast, there was a significant protective effect of DHA among individuals with the *CFH:Y402H* homozygous non-risk genotype (Model B: HR = 0.5, P = 0.02), but no significant effect of DHA among those with the *CFH:Y402H* homozygous risk genotype. Although there was a suggestion that the effect of DHA was stronger for the TT *CFH:Y402H* genotype (non-risk) than the CC genotype (risk), the test for interaction for this gene was not statistically significant (P= 0.16). Figure 1 displays the hazard ratios for DHA intake (Q5 vs Q1) according to *CFH* and *ARMS2/HTRA1* homozygous risk and non-risk genotypes from Table 5, Model B.

Table 6 (available at <http://aaojournal.org>) displays the number of progressors and non-progressors, and proportion which progress to GA according to genotype, by intake of DHA. Among those with the *ARMS2/HTRA1* risk genotype, the proportion of individuals who progress to GA is higher for DHA intake in the lowest quintile compared to those with intake in the highest quintile (30% vs 20%), whereas there was little difference for individuals with the homozygous non-risk genotype (11% vs 9%).

Discussion

This study presents new findings regarding dietary intake of DHA, reported from FFQs, and incident GA using expanded methods: multivariate Cox proportional hazards models including all non-advanced eyes at baseline, behavioral risk factors, 8 genetic variants in 7 genes, and effect modification and interactions between genes and DHA. Increased DHA intake was associated with reduced risk of progression to GA controlling for behavioral risk factors and genetic variants. EPA and DHA significantly reduced risk of progression to GA in multivariate models which controlled for both genetic and behavioral risk factors. Increased DHA intake also significantly reduced risk of progression among individuals with the *ARMS2/HTRA1* homozygous risk genotype, but not the non-risk *ARMS2* genotype, with a suggestive interaction between DHA intake and *ARMS2/HTRA1*.

Since the first report of an inverse association between dietary intake of omega-3 fatty acids and AMD in a case control study in 1994 (Seddon J, Ajani U, Sperduto R et al. Dietary fat intake and age-related macular degeneration [abstract]. Invest Ophthalmol Vis Sci 1994; 35:2003), the association between DHA, omega-3 fatty acids and progression to advanced AMD has been explored in several studies. DHA and omega-3 fatty acids have a protective effect on progression to advanced AMD in both case-control and prospective study designs with an estimated reduction in risk of 30% to 50%.^{17,21–23,25,26,29} Previous studies using the AREDs cohort classified progression as overall progression within a person, regardless of whether 1 or 2 eyes advanced, and used logistic regression for statistical analysis.^{25,26} One

investigated progression among those with mild to moderate risk of progression at baseline,²⁵ and the other among those with moderate to high risk of progression at baseline.²⁶ In these studies DHA alone was not significantly associated with reduced risk of progression to GA, but did trend in that direction, but EPA and EPA and DHA combined were significantly associated with decreased risk of progression to GA in these studies.^{25,26} Models in both studies did not control for genetic variants, and did not include all non-advanced eyes at baseline. Chiu et al. found a protective effect for DHA and progression to GA, but the trend was not significant.²⁹ Our study of progression differs from these studies by including up to 12 years of follow-up, adjusting for several genetic variants, testing for gene-nutrient interactions, and including all non-advanced eyes at baseline in the analyses.

A few studies have explored dietary intake and gene interactions and progression to advanced AMD in other populations using different methods.^{30,31} The Blue Mountain Eye Disease population-based study investigated diet and the *CFH* genotype, and found that weekly consumption of fish reduced risk for progression to late AMD among those who had the homozygous risk genotype for *CFH*, however there were only 47 late AMD cases for both GA and NV combined and they did not find any associations with early AMD.³¹ The Rotterdam study assessed *CFH* and *ARMS2/HTRA1* interactions with zinc and omega-3 fatty acids and incident early AMD.³⁰ Although the Rotterdam study did not report results for DHA separately, they did find that those with the highest combined EPA and DHA intake had a reduced risk of progression to early AMD if they had the *ARMS2/HTRA1* homozygous risk genotype.³⁰

Fish contain omega-3 fatty acids and leafy green vegetables are rich in lutein/zeaxanthin and both have anti-inflammatory and anti-oxidative properties. These foods and nutrients are associated with reduced risk of developing advanced AMD as described above and higher intakes are also associated with reduced serum levels of CRP.³² It is plausible that these anti-inflammatory nutrients favorably impact AMD by modulating the immune and inflammatory responses.^{33,34}

Interestingly, in our study, increased intake of DHA had a significant protective effect on GA progression among those with the *ARMS2/HTRA1* homozygous risk genotype. Our previous reports show that variants in this gene, although significantly related to both advanced forms, are actually more strongly related to NV than GA.^{2,3} Variants associated with *ARMS2/HTRA1* are not part of the complement pathway⁸⁻¹⁰ but a marker of systemic inflammation, CRP, was found to be elevated in individuals in a Japanese population with *ARMS2/HTRA1* risk alleles.³⁵

Several groups have examined the potential function of the *ARMS2/HTRA1* gene, although the exact mechanism is still not established.^{10,36-41} The ARMS2 protein has been found in the outer membrane of the mitochondria in rods and cones.^{10,36} Mitochondrial dysfunction can cause generation of reactive oxygen species, activation of the apoptotic pathway in addition to other regulatory problems.^{10,34} Mutations in some mitochondrial proteins are associated with optic neurodegenerative disorders, and altered function of the LOC387715/ARMS2 protein could enhance aging-associated degeneration of photoreceptors.¹⁰

In animal models, DHA has been shown to prolong survival of photoreceptors and also has a protective effect on signs of apoptosis such as fragmented photoreceptor nuclei and mitochondrial dysfunction.³⁴ Omega-3 fatty acids, especially DHA, are found in brain and retina tissues, with a high concentration in the photoreceptor outer segments.^{34, 42,43,44} One could speculate that DHA intake could reduce the presence of reactive oxygen species and/or apoptosis, possibly caused by dysregulation of the ARMS2 protein.

Strengths of this study include a well-defined cohort of individuals and a large number of individuals who progressed to geographic atrophy, a long follow-up time, analyses accounting for varying times of progression and for different types of progression in each eye, and inclusion of genotypes for 8 genetic variants associated with advanced AMD. Data collected from FFQs may result in reporting bias, over or underestimating the calculation of DHA which is consumed, although these questionnaires have been used in ranking levels of intake in many large studies.⁴⁵ Based on results of these analyses eating 60 mg/day may reduce risk of progression to GA. This amount corresponds to approximately 2 oz of fish per week, which provide an average consumption of 63 mg/day. Fish with high omega-3 fatty acid content are salmon, mackerel, sardines and herring.⁴⁶

In summary, results indicate that higher intake of DHA reported from FFQs is associated with reduced risk of progression to GA, controlling for known genetic variants associated with AMD. Increased intake of DHA may also reduce genetic susceptibility for developing incident GA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Effect of Docosahexaenoic Acid (DHA) on Progression to Geographic Atrophy According to Genotype. Hazard ratios and P values from Table 5, Model B.

Table 3

Baseline Ocular, Demographic, Environmental and Genetic Characteristics by Quintile of Docosahexaenoic Acid (DHA) Intake

	Quintile of DHA			P value*
	1 N= 496 N(%)	3 N=498 N (%)	5 N= 497 N (%)	
Median of quintile (g/day)	0.016	0.043	0.096	
Baseline Grade in Each Eye [‡]				
1,1/1,2/2,2	234(47)	241 (48)	248 (50)	
1,3/2,3/3,3	176(35)	187 (38)	181 (36)	
1,4/2,4/3,4	10 (2)	8 (2)	11 (2)	
1,5/2,5/3,5	76 (15)	62 (12)	57 (11)	0.27
Baseline Age				
< 70	265(53)	306 (61)	288 (58)	
70	231(47)	192 (39)	209 (42)	0.24
Gender				
Females	275 (55)	276 (55)	275 (55)	
Males	221 (45)	222 (45)	222 (45)	0.99
Education				
High School	200(40)	167 (34)	130 (26)	
> High School	296(60)	331 (66)	367 (74)	<0.0001
Smoking				
Never	233(47)	233 (47)	219 (44)	
Past	221(45)	224 (49)	252 (51)	
Current	42 (8)	21 (4)	26 (5)	0.90
Body Mass Index				
<25	162(33)	168 (34)	151 (30)	
25 – 29.9	217(44)	211 (42)	201 (41)	
30 – 34.9	89 (18)	84 (17)	98 (20)	
35+	28 (6)	35 (7)	46 (9)	0.05
Age-Related Eye Disease Study Treatment				
No Antioxidants	244(49)	234 (47)	269 (54)	
Antioxidants	252(51)	264 (53)	228 (46)	0.23
CFH : rs1061170				
T T	143 (29)	135 (27)	141 (28)	
C T	230 (46)	237 (48)	234 (47)	
C C	123 (25)	126 (25)	122 (25)	0.19
CFH :rs1410996				
T T	63 (13)	53 (11)	63 (13)	
C T	186 (38)	204 (41)	210 (42)	
C C	247 (50)	241 (48)	224 (45)	0.92
ARMS2/HTRA1 :rs10490924				

	Quintile of DHA			
	1 N= 496 N(%)	3 N=498 N (%)	5 N= 497 N (%)	P value*
G G	259(52)	266 (53)	248 (50)	0.99
G T	176 (35)	189 (38)	195 (39)	
T T	61 (12)	43 (9)	54 (11)	
C2 : rs9332739				
G G	457(92)	470 (94)	459 (92)	0.54
C C & C G	39 (8)	28 (6)	38 (8)	
CFB :rs641153				
C C	419 (84)	439 (88)	428 (86)	0.42
C T & T T	77 (16)	59 (12)	69 (14)	
C3 :rs2230199				
C C	289 (58)	284 (57)	293 (59)	0.92
C G	179(36)	184 (37)	176 (35)	
G G	28 (6)	30 (6)	28 (6)	
CFI: rs1003390				
C C	121 (24)	134 (27)	127 (26)	0.13
C T	225 (45)	239 (48)	252 (51)	
T T	150 (30)	125(25)	118 (24)	
LIPC :rs10468017				
C C	253 (51)	250 (50)	266 (54)	0.73
C T	211 (43)	207 (42)	188 (38)	
TT	32 (6)	41 (8)	43 (9)	

* P-values calculated using Mantel - Haenszel chi square.

Quintiles of DHA adjusted for sex and calories.

[†]Represents the Clinical Age-Related Maculopathy Staging System (CARMS) grade in each eye:

1,1 (no age-related macular degeneration (AMD), no AMD)/1,2 (no AMD, early AMD)/2,2 (early AMD, early AMD)

1,3 (no AMD, intermediate AMD)/2,3 (early AMD, intermediate AMD)/3,3 (intermediate AMD, intermediate AMD)

1,4 (no AMD, geographic atrophy)/2,4 (early AMD, geographic atrophy)/3,4 (intermediate AMD, geographic atrophy)

1,5 (no AMD, neovascular disease)/2,5 (early AMD, neovascular disease)/3,5 (intermediate AMD, neovascular disease)

Table 4

Multivariate Associations Between Dietary Fats and Progression to Geographic Atrophy

	Model A*		Model B†	
	HR (95 % CI)	† P Value(Trend)	HR (95 % CI)	† P Value (Trend)
Total Fat (g)				
Quintile 1	1.0	0.16	1.0	0.28
Quintile 2	1.14 (0.82 – 1.59)		1.20 (0.86 – 1.68)	
Quintile 3	0.99 (0.70 – 1.39)		1.02 (0.73 – 1.44)	
Quintile 4	1.54 (1.13 – 2.11)		1.58 (1.16 – 2.15)	
Quintile 5	1.18 (0.85 – 1.64)		1.15 (0.83 – 1.61)	
Saturated Fat (g)				
Quintile 1	1.0	0.38	1.0	0.47
Quintile 2	1.09(0.78 – 1.51)		1.05 (0.76 – 1.46)	
Quintile 3	1.42 (1.03 – 1.95)		1.45 (1.05 – 2.00)	
Quintile 4	1.18 (0.85 – 1.64)		1.15 (0.83 – 1.59)	
Quintile 5	1.19 (0.87 – 1.64)		1.17 (0.85 – 1.61)	
Monounsaturated Fat (g)				
Quintile 1	1.0	0.05	1.0	0.10
Quintile 2	1.37 (0.98 – 1.91)		1.44 (1.03 – 2.02)	
Quintile 3	1.22 (0.86 – 1.71)		1.24 (0.87 – 1.74)	
Quintile 4	1.38 (0.99 – 1.94)		1.40 (1.00 –1.96)	
Quintile 5	1.47 (1.05 – 2.05)		1.43 (1.01 – 2.01)	
Total Polyunsaturated Fatty Acids (g)				
Quintile 1	1.0	0.17	1.0	0.32
Quintile 2	0.95 (0.68 – 1.33)		0.92 (0.66 – 1.29)	
Quintile 3	1.10 (0.80 – 1.52)		1.04 (0.76 – 1.43)	
Quintile 4	1.34 (0.97 –1.85)		1.24 (0.90 – 1.72)	
Quintile 5	1.13 (0.82 – 1.55)		1.07 (0.78 – 1.47)	
Omega-3 Fatty Acids				
Eicosapentaenoic Acid (EPA)(g)				
Quintile 1	1	0.32	1	0.26
Quintile 2	0.92 (0.65 – 1.30)		0.88 (0.62 – 1.25)	
Quintile 3	1.16 (0.86 – 1.58)		1.09 (0.80 – 1.48)	
Quintile 4	1.00 (0.71 – 1.39)		0.95 (0.68 – 1.33)	
Quintile 5	0.84 (0.59 – 1.18)		0.81 (0.58 – 1.15)	
Docosahexaenoic Acid (DHA)(g)				
Quintile 1	1	0.03	1	0.008
Quintile 2	0.99 (0.73 – 1.36)		0.95 (0.69 – 1.30)	
Quintile 3	1.14 (0.84 – 1.53)		1.09(0.81 – 1.46)	
Quintile 4	0.93 (0.68 – 1.27)		0.88 (0.64 – 1.20)	
Quintile 5	0.72 (0.52 – 1.01)		0.68 (0.48 – 0.94)	
DHA + EPA (g)				

	Model A [*]		Model B [‡]	
	HR (95 % CI)	[†] P Value(Trend)	HR (95 % CI)	[†] P Value (Trend)
Quintile 1	1	0.06	1	0.02
Quintile 2	0.98 (0.70 – 1.38)		0.96 (0.68 – 1.34)	
Quintile 3	1.20 (0.88 – 1.64)		1.13 (0.83 – 1.54)	
Quintile 4	0.91 (0.64 – 1.29)		0.87 (0.61 – 1.23)	
Quintile 5	0.79 (0.55 – 1.12)		0.76 (0.53 – 1.08)	
Linolenic Acid (g)				
Quintile 1	1	0.44	1	0.71
Quintile 2	0.90 (0.64 – 1.23)		0.86 (0.62 – 1.21)	
Quintile 3	1.02 (0.74 – 1.42)		1.02 (0.74 – 1.41)	
Quintile 4	1.06 (0.77 – 1.47)		0.94 (0.69 – 1.29)	
Quintile 5	1.08(0.80 – 1.46)		1.01(0.75 – 1.51)	
Omega-6 Fatty Acids				
Linoleic Acid (g)				
Quintile 1	1	0.20	1	0.36
Quintile 2	0.98 (0.70 – 1.37)		0.95 (0.68 – 1.33)	
Quintile 3	1.04 (0.75 – 1.44)		1.02 (0.74 – 1.40)	
Quintile 4	1.36 (0.99 – 1.87)		1.30 (0.95 – 1.78)	
Quintile 5	1.11 (0.81 – 1.53)		1.05 (0.76 – 1.45)	
ArachidonicAcid (g)				
Quintile 1	1	0.32	1	0.21
Quintile 2	0.92 (0.67 – 1.26)		0.90 (0.66 – 1.23)	
Quintile 3	0.85 (0.62 – 1.17)		0.82 (0.60 – 1.12)	
Quintile 4	0.91 (0.66 – 1.25)		0.88 (0.64 – 1.21)	
Quintile 5	0.84 (0.62 – 1.14)		0.81 (0.60 – 1.09)	

HR = Hazard Ratio; CI = Confidence Interval.

* Model A = adjusted for baseline grade, demographic and environmental characteristics: age, gender, education, smoking, antioxidants and body mass index.

[‡]Model B= adjusted for covariates in Model A + all genes shown in table 3.

[†] P-trend calculated using median values with in each quintile.

Table 5
Effect of Docosahexaenoic Acid (DHA) Intake on Progression to Geographic Atrophy According to Risk/Non-Risk Genotypes

Gene (homozygous risk/homozygous non-risk)	5th Quintile DHA vs 1st Quintile DH A					
	Model A *			Model B †		
	HR (95% CI)	P Value	P Value (Interaction)	HR (95% CI)	P Value	P Value (Interaction)
CFH : rs1061170 (Y402H)						
T T	0.5 (0.3 – 0.8)	0.01		0.5 (0.3 – 0.9)	0.02	
C C	0.9 (0.6 – 1.3)	0.52	0.10	0.8 (0.6 – 1.2)	0.37	0.16
CFH : rs1410996						
T T	0.4 (0.2 – 1.0)	0.05		0.4 (0.2 – 1.0)	0.05	
C C	0.8 (0.6 – 1.1)	0.22	0.18	0.8 (0.6 – 1.1)	0.12	0.20
ARMS2/HTRA1 : rs10490924 (A69S)						
G G	1.0 (0.6 – 1.6)	0.99		1.0 (0.6 – 1.6)	0.90	
T T	0.5 (0.3 – 0.8)	0.004	0.06	0.4 (0.3 – 0.7)	0.002	0.05
C2 : rs9337239 (E318D) †						
GG	0.7 (0.6 – 1.0)	0.04		0.7 (0.5 – 0.9)	0.02	
CG/CC	0.05(0.0 – 15.6)	0.30	0.36	0.03 (0.0 – 10.8)	0.25	0.30
CFB : rs641153 (R32Q) †						
C C	0.7 (0.5 – 0.9)	0.02		0.7 (0.5 – 0.9)	0.01	
C T/T T	1.6 (0.2 – 11.1)	0.64	0.42	1.9 (0.3 – 12.3)	0.52	0.30
C3 : rs2230199 (R102G)						
CC	0.7 (0.5 – 1.1)	0.15		0.7 (0.5 – 1.1)	0.14	
GG	0.7 (0.3 – 1.3)	0.26	0.84	0.6 (0.3 – 1.2)	0.17	0.16
CFI : rs10033900						
C C	0.6 (0.3 – 1.0)	0.07		0.6 (0.3 – 1.0)	0.06	
T T	0.9 (0.5 – 1.3)	0.49	0.40	0.8 (0.5 – 1.2)	0.29	0.49
LIPC : rs10468017						
C C	0.7 (0.5 – 1.0)	0.05		0.7 (0.5 – 1.0)	0.03	
T T	0.8 (0.4 – 1.8)	0.64	0.72	0.7 (0.3 – 1.6)	0.40	0.91

HR = Hazard Ratio; CI = Confidence Interval

* Model A = adjusted for single gene, DHA, baseline grade, demographic and environmental characteristics: age, gender, education, smoking, antioxidants, and body mass index.

[‡] Model B= adjusted for covariates in Model A + all genes shown in Table 3.

[‡] Heterozygous genotype combined with homozygous risk due to small numbers.