Original Contribution

Combined Effects of Complement Factor H Genotypes, Fish Consumption, and Inflammatory Markers on Long-Term Risk for Age-related Macular Degeneration in a Cohort

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At baseline in 1992–1994, the authors assessed the combined effects of complement factor H (CFH) genotypes with smoking, fish consumption, and inflammatory markers on the risk of age-related macular degeneration (AMD) in 3,654 persons aged ≥49 years. They reexamined 75% of the survivors after 5 and 10 years, confirming incident AMD by side-by-side photographic grading. Of the 2,452 persons followed in the Blue Mountains Eye Study, 1,881 were genotyped (rs1061170), with CC, CT, and TT identified in 13.6%, 46.7%, and 39.7%, respectively. AMD risk increased with each additional C allele (early AMD: age- and sex-adjusted relative risk (RR) = 1.6, 95% confidence interval (CI): 1.2, 1.9; late AMD: RR = 2.3, 95% CI: 1.5, 3.6). Late AMD risk among current smokers with the CC/CT genotypes (RR = 10.7, 95% CI: 3.4, 33.9) was 5-fold that for genotypically similar nonsmokers (RR = 2.2, 95% CI: 0.9, 5.5) versus current nonsmokers with TT genotypes. Weekly compared with less than weekly consumption of fish was associated with reduced late AMD risk in participants with the CC genotype (RR = 0.15, 95% CI: 0.03, 0.8) but not the CT (RR = 0.7, 95% CI: 0.3, 2.0) or TT (RR = 1.3, 95% CI: 0.2, 7.2) genotypes. This study documents joint contributions from genetic and systemic factors in determining the progression of AMD.

cohort studies; complement factor H; environmental exposure; genetics; macular degeneration; seafood; smoking

Abbreviations: AMD, age-related macular degeneration; BMES, Blue Mountains Eye Study; CFH, complement factor H; CI, confidence interval; RPE, retinal pigment epithelium; RR, relative risk.

Research on age-related macular degeneration (AMD) in recent years has consistently identified certain gene variants and environmental risk or protective factors associated with AMD. Smoking is the major modifiable AMD risk factor consistently found in cross-sectional (1) and longitudinal (2–7) studies. Greater dietary intake of omega-3 polyunsaturated fatty acids was associated with a reduced likelihood of AMD (8). Regular consumption of fish has been associated with both reduced prevalence (9) and incidence (10) of AMD in the Blue Mountains Eye Study (BMES) cohort and other populations (8, 11–13). Although evidence for the involvement of specific inflammatory markers is inconsistent (14–16), there is increasing support for a role of low-grade inflammation in the pathogenesis of AMD. In neovascular AMD mouse models, macrophage accumulation paralleled the extent of choroidal neovascularization (17). Seminal recent findings linking the complement factor H (CFH) Y402H gene variant to AMD (18–22) provide further support for this hypothesis (23). The CFH gene is a key inhibitor of the alternative pathway of complement activation. This, as well as links between other AMD-associated gene variants (BF, C2) and complement pathway-associated genes, points to involvement by local inflammatory processes, stimulated by retinal pigment epithelium (RPE) cell debris and aggregated by activated complement and other inflammatory mediators, in the pathogenesis of AMD (23, 24). It could mimic the processes of chronic inflammation initiated by endothelial injury in the pathogenesis of atherosclerosis (23–25).

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In addition, recent findings indicate that 2 genes (26, 27) or AMD genetic markers together with other modifiable risk factors, such as smoking, greater body mass index, and inflammatory markers, may increase susceptibility to AMD (28–31). The joint effects of smoking combined with other factors contributing to greater AMD risk have emerged (7, 27–34). Carriers of the APOE*E2 allele had a greater risk of neovascular AMD if they smoked, compared with APOE*E4 carriers or APOE*E3 homozygotes who did not smoke (28). For the 2 confirmed AMD-related gene loci (complement factor H (CFH) and LOC387715), smoking appears to exert a much greater effect on AMD risk in persons homozygous for the risk alleles of either of these 2 gene polymorphisms (Y402H in CFH and A69S in LOC387715) than in subjects homozygous for the nonrisk alleles (27, 29–37). Greater effects on the 10-year risk of AMD were observed from combined exposure to smoking plus the lowest quintile level of serum high density lipoprotein cholesterol, the highest total cholesterol:high density lipoprotein cholesterol ratio, or self-reported infrequent consumption of fish (BMES) (7).

However, although 1 study has documented a statistically significant interaction between the LOC387715 gene variant and smoking on AMD risk (29), most studies investigating the interplay of gene and environmental factors have not found significant statistical interaction at a multiplicative scale by use of logistic regression (27, 28, 30–39). Nevertheless, consistent observations from multiple studies showing that combined exposure to 2 factors is associated with substantially greater late AMD risk (27, 28, 30–39) suggest that joint effects of multiple, independent risk factors are likely frequently involved in AMD pathogenesis. A small number of effects, however, could have contributed, and therefore replicating these findings is essential.

In this study, we aimed to confirm the joint effects of smoking with CFH (Y402H) on the long-term risk of AMD in the BMES cohort and also to explore the joint effects and potential effect modification (statistical interaction) of CFH gene variants with inflammatory markers or dietary fish consumption on the long-term risk of AMD. According to Greenland et al., “…statistical interaction-effect measure modification should not be confused with biologic interaction” (40, p. 82). Effect modification refers to a situation when the effect of X on Y depends on the presence or absence of a third factor. Biologic interaction covers a wide range of possible interaction response types of 2 exposures on the disease process, provided each factor has biologically plausible mechanism(s) for its potential causal role to the disease of interest (40).

MATERIALS AND METHODS

Study population

The BMES is a population-based cohort study of eye diseases and other health outcomes in an urban population aged 49 years or older. In 1992–1994, 3,654 residents (82.4% of those eligible) aged 49 or more years, living in 2 postcode areas near Sydney, Australia, participated; 2,335 (75.1% of survivors) were reexamined after 5 years in 1997–1999, and 1,952 (76% of survivors) were reexamined after 10 years in 2002–2004. There were 2,452 participants who were followed at least once since the baseline examinations. The study was approved by the University of Sydney and the Sydney West Area Health Service human research ethics committees, and written, informed consent was obtained (41).

At each visit, 30° stereoscopic color retinal photographs of the macula and other retinal fields of both eyes were taken, as described (42), by using a Zeiss FF3 Fundus Camera (Carl Zeiss, Oberkochen, Germany). Photographs were obtained for both eyes in 97%–98% (3,543/3,654, 2,289/2,335), or of at least 1 eye in 98%–99% (3,583/3,654, 2,307/2,335), of the baseline and 5-year examination participants (43), and of both eyes in 84.5% (1,649/1,952), or of at least 1 eye in 86.5% (1,689/1,952), of 10-year examination participants. Masked photographic grading for AMD lesions was performed (42), closely following the Wisconsin Grading System (44). After initial grading, a side-by-side grading, comparing the baseline and 5-year photographs (43) and then the baseline and 10-year photographs, was performed on participants with lesions identified at either follow-up examination. The assessment of inter- and intragrader reliability showed good agreement for AMD grading (42).

Late AMD was defined as the presence of neovascular or atrophic AMD. Neovascular AMD was defined as serous or hemorrhagic detachment of the sensory retina or RPE, presence of subretinal or sub-RPE hemorrhage, or subretinal fibrous scarring. Geographic atrophy was defined as a discrete area, at least 175 μm in diameter, characterized by a sharp border and presence of visible choroidal vessels (42), using the definitions of the International ARM Classification (45). If both geographic atrophy and neovascular AMD were present in the same eye, neovascular AMD was considered the diagnosis for that eye. All late AMD cases detected from each examination were adjudicated and confirmed by a retinal specialist (P. M.). Incident late AMD was defined by the appearance at either follow-up examination of neovascular AMD or geographic atrophy in either eye of persons in whom no late AMD lesions were present at baseline.

Early AMD was defined as the absence of late AMD and the presence of either 1) large (>125-μm diameter), indistinct, soft or reticular drusen or 2) both large, distinct, soft drusen and retinal pigmentary abnormalities (hyper- or hypopigmentation) (42, 45) within the macular area. This definition followed the definition used in the Beaver Dam Eye Study from Wisconsin (46). Incident early AMD was defined by the appearance at follow-up examinations of either indistinct soft or reticular drusen or the co-presence of distinct soft drusen and retinal pigmentary abnormalities, in either eye of persons in whom no late or early AMD was present in either eye at baseline and no late AMD at follow-up examinations.

Smoking history was obtained from an interviewer-administered questionnaire. Current smokers were compared with those who had either never smoked or had
smoked in the past (current nonsmokers). Eight-hour fasting blood specimens were drawn from 3,222 (88%) baseline participants for hematology and clinical biochemistry assessments. Plasma fibrinogen was determined by using the prothrombin time-derived technique on an ACL 300R coagulometer (ACL, Inc., Elk Grove Village, Illinois). The white cell count was determined by using Coulter Counter (Beckman Coulter, Inc., Fullerton, California) methods; reliability coefficients based on blind replicate control data ranged from 0.96 to 1.00.

A 145-item, semiquantitative food frequency questionnaire was modified from an early food frequency questionnaire by Willett et al. (47), taking into account variations of the Australian food supply from that in the United States. It has been validated against nutrient estimates from 4-day weighted food records collected 3 times over the period of 1 year in a random subsample (n = 79) (48, 49), and energy-adjusted Spearman’s correlations were above 0.5 for most nutrients (48). The food frequency questionnaire was attempted and returned by 3,267 participants (89.4%) at baseline, with 2,900 usable (88.8% of those attempted), after excluding those considered unreliable (i.e., if >12 food frequency questionnaire questions or an entire page remained blank or daily energy intakes were <2,500 kJ or >18,000 kJ). Characteristics of the food frequency questionnaire respondents and exclusion criteria were reported previously (48, 50).

Analyses for the frequency of fish consumption, regardless of portion size, were based on a smaller sample (n = 1,520), after excluding subjects with missing data on this dietary variable (n = 271, 15.1%). Comparing subjects with and without data on fish consumption, we excluded those who were older (mean age, 66.0 years vs. 63.5 years; P < 0.0001), but there were no differences in the proportions of men (40.2% vs. 43.2%) and current smokers (12.1% vs. 14.3%) or in mean white cell count (6.32 × 10^9/L vs. 6.38 × 10^9/L). Regular consumption of fish was defined as 1 or more servings per week.

Genotyping

Of the 2,452 participants followed at least once, 1,881 had DNA available and were genotyped for CFH by using TaqMan assays (Applied Biosystems, Foster City, California). There were no differences in mean age (64.2 years vs. 64.3 years), mean white cell count (6.37 × 10^9/L vs. 6.44 × 10^9/L), or in proportions of male gender (42.7% vs. 41.1%), current smokers (12.3% vs. 15.2%), and persons who consumed fish weekly (59.5% vs. 59.8%) between those with and without DNA available. Among participants for whom first-degree family members were also study participants, only the youngest member of each family was included (90 subjects were excluded). We genotyped the CFH single-nucleotide polymorphism rs1061170 (Y402H) in exon 9 using polymerase chain reaction amplification in a volume of 5 μL including 1× TaqMan Universal PCR Master Mix (Applied Biosystems). The overall genotyping error rate was estimated at less than 1% based on 136 replicates (51). Genotyping completeness was 97.4% for this single-nucleotide polymorphism.

Statistical methods

Of the 1,881 participants with genotype data, 1,791 had complete data on other selected study factors and no late AMD at baseline, and thus they were at risk of late AMD. After exclusion of 47 participants with incident late AMD at follow-up visits and 39 with early AMD at baseline, 1,705 were at risk of early AMD.

SAS, version 9, software (SAS Institute, Inc., Cary, North Carolina) was used for all analyses. Discrete logistic models (52) were used to assess genetic and environmental factors associated with 10-year incident late or early AMD. Hardy-Weinberg equilibrium was tested by using chi-square. We examined additive, dominant, and recessive models for gene variant effects on AMD risk and dominant models for combined effects from CFH Y402H and other environmental/systemic risk factors, as well as interaction terms. We tested for statistical interaction by adding a product term of the gene variant with 1 of the chosen systemic/environmental factors in multivariable-adjusted discrete logistic regression models. Stratified analysis by genotype was indicated by significant product terms (statistical interaction). We also assessed joint effects from combined exposure of the gene variant with 1 of the chosen systemic/environmental factors, regardless of whether statistical interaction was significant. We previously reported that the baseline white cell count was associated with long-term incidence of AMD, and we detected a significant effect on the highest compared with the lower 2 tertiles (53). We have therefore used the same cutpoint for the inflammatory markers in this report. All selected systemic/environmental factors have been hypothesized to have biologically plausible mechanism(s) for their roles in the development of AMD. All statistical models were constructed by adjusting for age (continuous variable) and sex for the findings presented in Tables 1 and 2 and by additionally adjusting for current smoking, white cell count, and the frequency of fish consumption (all categorical variables) for the findings presented in Tables 3 and 4.

We also conducted analyses on age-stratified subgroups to determine whether the interplay of genes and other factors exerted a stronger effect on AMD in younger than in older groups, given our previous finding that late AMD in current smokers developed an average of 5–10 years earlier than in cases in nonsmokers (6, 7). We speculated that these risk factors may play a more important role in AMD cases having an earlier than a later age at onset. Relative risks with 95% confidence intervals are presented.

To better reflect the magnitude of joint effects, data are presented in 3 groups, by using the format recommended by Botto and Khoury (54), 2 with either the genetic or other factor assessed alone and 1 with both factors compared with the group with neither factor. We also evaluated the Rothman synergy index (55) to determine whether the joint effects from CFH genotypes plus another factor exceeded the sum of effects from each factor alone:

\[
S_{ab} = (R_{ab} - 1)/\left( (R_{a} + R_{b}) - 2 \right).
\]

R_{ab} is the relative risk of combined exposures, while R_{a} and R_{b} are relative risks for exposure to CFH genotypes...
and another factor, respectively. The synergy index represents the ratio of increased risk due to combined exposures to the sum of increased risks due to each exposure alone.

RESULTS

The mean baseline age of the 1,791 participants was 63.9 years, 42.8% were men, and 12.5% were current smokers at baseline. Late AMD developed in 47 of 1,791 persons (2.6%), and early AMD developed in 185 of 1,705 persons (10.9%) over the 10-year period. The CFH CC genotype was found in 13.6% (n = 244), the CT genotype in 46.7% (n = 836), and the TT genotype in 39.7% (n = 711). Table 1 shows that persons with either incident early or late AMD were older, more likely to have the CC genotype, and less likely to have the TT genotype. The CC genotype was found in 12.6% of subjects who did not have incident early or late AMD. The proportion of persons who consumed fish at least weekly was significantly lower among the 47 persons who developed incident late AMD (45%) than among the 1,520 persons who did not develop either early or late AMD (61%) during the follow-up period of our study (Table 1).

After adjustment for age and sex, each additional C allele was associated with a 60% greater risk for early AMD (relative risk [RR] = 1.6, 95% confidence interval [CI]: 1.2, 1.9) and more than double the risk for late AMD (RR = 2.3, 95% CI: 1.5, 3.6) (Table 2). Although point estimates for these relative risks show a dose gradient pattern, the confidence intervals were wide and overlapping, likely because of the small numbers.

Table 3 displays the combined exposures of CFH risk genotypes with the chosen study factors on the risk of late and early AMD. After adjustment for age, sex, white cell count, and regular fish consumption, there was no significant interaction (P = 0.96), but a joint effect of CFH risk genotypes with current smoking on the risk of late AMD was observed. Among noncurrent smokers, the relative risk was 2.2 (95% CI: 0.9, 5.5) for the CC/CT genotypes (for the CT genotype: RR = 1.7, 95% CI: 0.6, 4.6; for the CC genotype: RR = 4.2, 95% CI: 1.4, 12.6); among current smokers, the corresponding relative risk was 10.7 (95% CI: 3.4, 33.9) for the CC/CT genotypes (Table 3) (for the CT genotype: RR = 10.9, 95% CI: 3.1, 38.1; for the CC genotype: RR = 9.7, 95% CI: 1.7, 54.8). The synergy index for late AMD between CFH genotypes and smoking was not obtainable as no participants in the smoking-alone group developed late AMD. A joint effect of CFH risk genotypes and current smoking on the risk of early AMD was not apparent (Table 3).

Similar joint effects of CFH Y402H with white cell count or plasma fibrinogen on incident late AMD were suggested (Table 3). The synergy indices for the risk of late AMD were 1.10 for combined exposure to both high white cell count and CFH risk genotypes and 1.48 for combined exposure to both high fibrinogen and CFH risk genotypes. The joint effects of this gene variant with both inflammatory markers appeared weaker for early than for late AMD (Table 3).

There was a significant interaction between the CFH risk allele and fish consumption on late AMD risk (P = 0.03). After adjustment for age, sex, smoking, and white cell count, the risk of developing late AMD doubled among subjects with either CT or CC genotypes who consumed less than 1 serving of fish per week (RR = 3.6, 95% CI: 1.2, 11.2), compared with genotypically similar subjects who

<table>
<thead>
<tr>
<th>Model for Genotype</th>
<th>Early AMD</th>
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<th>Late AMD</th>
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<tr>
<td></td>
<td>No. at Risk</td>
<td>No. of Cases</td>
<td>Relative Risk, Age and Sex Adjusted</td>
<td>95% Confidence Interval</td>
<td>No. at Risk</td>
<td>No. of Cases</td>
<td>Relative Risk, Age and Sex Adjusted</td>
<td>95% Confidence Interval</td>
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<tr>
<td>TT/CT/CC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,705</td>
<td>185</td>
<td>1.55</td>
<td>1.24, 1.94</td>
<td>1,791</td>
<td>47</td>
<td>2.33</td>
<td>1.50, 3.62</td>
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<tr>
<td>CT/TT</td>
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<td>149</td>
<td>1.0</td>
<td>1.52, 3.77</td>
<td>1,547</td>
<td>34</td>
<td>1.0</td>
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<tr>
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<td>36</td>
<td>2.40</td>
<td>1.52, 3.77</td>
<td>244</td>
<td>13</td>
<td>2.75</td>
<td>1.40, 5.39</td>
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<tr>
<td>TT</td>
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<td>53</td>
<td>1.0</td>
<td>1.26, 2.45</td>
<td>711</td>
<td>7</td>
<td>1.0</td>
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<tr>
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<td>132</td>
<td>1.76</td>
<td>1.26, 2.45</td>
<td>1,080</td>
<td>40</td>
<td>3.49</td>
<td>1.54, 7.88</td>
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</tbody>
</table>

Abbreviation: AMD, age-related macular degeneration.
<sup>a</sup> The referent group is subjects with the TT genotype.

### Table 3. Combined Effect of Current Smoking, Elevated White Cell Count, Elevated Plasma Fibrinogen, or Infrequent Fish Consumption at Baseline (1992–1994) and the Complement Factor H Gene Variant (Y402H) on the 10-Year Incidence of Age-related Macular Degeneration in the Blue Mountains Eye Study Population<sup>a</sup>

| AMD Type (no. of no exposure, no. of exposure to nongenetic factors) | Genotype | No Exposure to Nongenetic Factors | | | Exposure to Nongenetic Factors | | |
|---------------------------------------------------------------|----------|----------------|-----------------|----------------|----------------|-----------------|-----------|----------------|
|                                                              |          | Relative Risk, Age and Sex Adjusted | 95% Confidence Interval | | Relative Risk, Age and Sex Adjusted | 95% Confidence Interval |
| Nonsmoker<sup>b</sup>                                        |          |                               |            | | Smoker<sup>b</sup> |                               |            |
| Early AMD (n = 1,450, n = 202)                               | TT       | 1.0                          | 1.29       | 0.37, 4.49 |
| Late AMD (n = 1,518, n = 216)                                | CT/CC    | 1.66                         | 2.04       | 0.99, 4.21 |
| WCC < 6.8 × 10<sup>9</sup>/L<sup>d</sup>                     |          | 2.18                         | 10.67      | 3.36, 33.91 |
| Early AMD (n = 1,060, n = 546)                               | TT       | 1.0                          | 1.57       | 0.83, 2.96 |
| Late AMD (n = 1,108, n = 577)                                | CT/CC    | 1.74                         | 2.50       | 1.45, 4.31 |
| Fibrinogen < 4.3 g/L<sup>d</sup>                              |          | 4.23                         | 6.92       | 1.52, 31.49 |
| Early AMD (n = 1,077, n = 520)                               | TT       | 1.0                          | 0.54       | 0.27, 1.11 |
| Late AMD (n = 1,124, n = 550)                                | CT/CC    | 1.20                         | 1.72       | 1.05, 2.82 |
| Fish Consumption ≥ 1 Serving per Week<sup>e</sup>            |          | 2.68                         | 4.11       | 1.13, 14.89 |
| Early AMD (n = 873, n = 577)                                 | TT       | 1.0                          | 1.21       | 0.64, 2.29 |
| Late AMD (n = 908, n = 612)                                  | CT/CC    | 1.73                         | 3.62       | 1.17, 11.20 |

Abbreviation: AMD, age-related macular degeneration; WCC, white cell count.
<sup>a</sup> The referent group is subjects exposed to neither genetic nor nongenetic factors.
<sup>b</sup> Relative risks were adjusted for age, sex, white cell count, and fish consumption.
<sup>c</sup> There were no incident cases of late AMD among smokers with the TT genotype.
<sup>d</sup> In the highest versus 2 lower tertiles, relative risks were adjusted for age, sex, smoking, and fish consumption.
<sup>e</sup> Relative risks were adjusted for age, sex, smoking, and white cell count.

consumed at least 1 serving of fish per week (RR = 1.7, 95% CI: 0.5, 5.5) (Table 3). This joint effect on the incidence of AMD persisted after further adjustment for vitamin C intake and higher occupational prestige (late AMD: RR = 3.4, 95% CI: 1.1, 10.7; early AMD: RR = 1.9, 95% CI: 1.1, 3.2). The synergy index for the risk of late AMD was 6.4, and for early AMD it was 0.95, from combined exposure to infrequent fish consumption and CFH genotypes.

In subgroup analysis stratified into the ages of less than 70 years and 70 years or more, the joint effects on late AMD from infrequent fish consumption and CFH CC/CT genotypes were similar for the 2 age subgroups (RR = 4.0, 95% CI: 0.8, 19.5 and RR = 3.4, 95% CI: 0.7, 17.0, respectively). However, the joint effect on early AMD appeared to be stronger in the older (RR = 3.8, 95% CI: 1.4, 9.8) than in the younger (RR = 1.3, 95% CI: 0.7, 2.6) age group.

In subgroup analysis stratified by CFH genotype, at least weekly fish consumption was associated with a reduced risk of late AMD in persons with only the CC genotype (RR = 0.15, 95% CI: 0.03, 0.8) but not the CT genotype (RR = 0.74, 95% CI: 0.3, 2.0) or the TT genotype (RR = 1.3, 95% CI: 0.2, 7.1) (Table 4). After further adjustment for vitamin C intake and higher occupational prestige, the protective association from weekly consumption of fish among persons with the CC genotype persisted (RR = 0.15, 95% CI: 0.03, 0.9). No similar effect was evident for early AMD (Table 4).

We also repeated analyses to include only the oldest family member of participants for whom first-degree family members were also study participants, and we found no difference from that when the youngest family member was included.

**DISCUSSION**

In this population-based, older, Australian cohort, we documented a dose-dependent risk pattern for the association of CFH C alleles with the incidence of both early and late AMD. Additionally, we found possible joint effects on the long-term risk of AMD, particularly late AMD, from combined exposure to both CFH Y402H gene variants and environmental/systemic factors with known links to AMD. Not all participants with the CFH CC/CT genotypes developed AMD over the 10-year period. However, participants with these risk genotypes who currently smoked or who consumed fish infrequently at the baseline examination had an approximate doubling of the risk of late AMD, compared with those who had the genotypes but did not have such exposures. Compared with subjects who neither had the CFH CC/CT genotype nor were current smokers, the long-term late AMD risk was 10-fold higher among subjects who currently smoked and also had the risk genotypes. Because of the small number of incident late AMD cases in this population-based sample, most documented risk estimates had wide and overlapping confidence intervals. Replication of these findings is therefore needed in substantially larger studies or, alternatively, by pooling data from multiple studies.

Cigarette smoking is considered an important trigger or promoter in the development of both early and late AMD (56–58). Emerging evidence indicates that the combined effects from smoking together with other AMD risk factors likely contribute to the high risk of late AMD (7, 27–34). Our findings from this prospective, population-based sample provide additional evidence supporting the hypothesis of a causal role for cigarette smoking in promoting late AMD risk among genetically susceptible subjects. Although no statistically significant interaction at a multiplicative scale was detected in our study and in most previous studies (27, 28, 30–39) and 95% confidence intervals for the long-term risk of incident late AMD overlapped, a biologic joint effect of the 2 factors on an additive scale (7, 27, 28, 30–39) and 95% confidence intervals for the long-term risk of incident late AMD is possible (40). The strong relation of smoking to AMD progression in persons genetically at risk also suggests that smoking cessation might reduce the public health burden of AMD in persons genetically susceptible to AMD.

An elevated white cell count was associated with long-term AMD incidence in both the Beaver Dam Eye Study (14) and our cohort (53). Evidence has been inconsistent on whether an association exists between C-reactive protein and incident AMD (16, 59–62). A joint effect of the CFH CC genotype with elevated C-reactive protein or white cell count on the progression of AMD was suggested by Rotterdam Study data (30). We found that the combined effect of inflammatory markers with the CFH gene variant was weak and that no synergism was evident.
Another consistently documented protective factor associated with a reduced risk of late AMD is the regular consumption of fish (9–13) or of dietary omega-3 fatty acids (8), although no similar associations were reported for early AMD or for the primary prevention of AMD (63). Although there are no clinical trial data to demonstrate that regular consumption of fish or a healthy diet will contribute to a reduced long-term risk of late AMD, the existing epidemiologic data suggest potential benefits from consuming fish. We previously reported that joint exposure to current smoking and a diet low in fish was associated with a greater long-term risk of late AMD than that from either exposure alone (7). In this current report, we could demonstrate only a protective effect on the development of late AMD from at least weekly fish consumption among subjects with genetic susceptibility to AMD due to the Y402H variant, an effect modification of this gene variant on the protective influence of fish consumption on late AMD. We did not, however, observe a similar protective effect on the development of early AMD (Table 3). This finding is in keeping with a recent report by Chong et al. (63) in a meta-analysis. We speculate that mechanisms for the development of early AMD (primary prevention) may not be the same as those involved in the progression from early to late AMD (secondary prevention).

We should, however, be cautious when interpreting this finding, as such “healthy” dietary behavior could be a surrogate marker of an overall healthier diet and lifestyle, which together could contribute to the overall health status of older persons, including healthier eyes. Although data from our study and those of others show promising relations of diet with AMD (7, 8, 12, 13, 64–67), it is unknown whether multiple interventions, including “healthy” diet changes (frequent fish consumption, foods with higher levels of antioxidants, lower saturated and unsaturated fats, and a lower glycemic index of foods consumed), would result in a reduced risk of early and/or late AMD.

All of the exposures assessed in this study showed a stronger combined effect with CFH Y402H on the development of late than early AMD. Apart from the small number effect, it could be possible that these combined exposures exert a more prominent role in promoting AMD progression from its early to late stages. Gene variants and the exposures related to the onset of early AMD are less clear and may involve different pathogenetic factors (68, 69).

The strengths of our study include its population-based cohort and careful documentation of AMD incidence by use of photographic grading, the current “gold standard.” An important limitation of our study, however, is the relatively small number with late AMD incidence, resulting in only 25% power to detect a significant statistical interaction with an odds ratio of 1.5 for combined exposures to smoking and CFH CC genotypes. Similarly, the study power to detect a significant statistical interaction with an odds ratio of 1.5 for combined exposures to infrequent fish consumption and CFH CC genotypes was 36%. We were also not able to separately examine the 2 late AMD subtypes (neovascular AMD and geographic atrophy). Uncontrolled confounding could have influenced our findings, and selective survival could have biased the associations in either direction.

In conclusion, joint contributions from multiple risk factors, including gene variants, environmental triggers, and systemic conditions, may explain a high proportion of late AMD cases. The exact mechanisms by which genes and environmental factors interact to promote the development and progression of late AMD are still not well understood. Our study may provide new insights into possible pathogenetic mechanisms for late AMD, among the diverse biologic processes likely involved in its etiology (70, 71). Because of the small number of incident late AMD cases, these findings are suitable only to generate hypotheses. Further studies with larger sample sizes (or pooled cohort study data) will be needed to replicate our findings, together with experimental studies to test the hypotheses. If confirmed, such knowledge could be used to develop effective preventive strategies and treatments that target genetically at-risk individuals, by aiming to prevent the development of late AMD or to assist in delaying its onset (71).

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