Complement Factor H and the Bilaterality of Age-Related Macular Degeneration

The Blue Mountains Eye Study

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Objective: To determine whether complement factor H (CFH Y402H) genotype influences bilateral involvement of age-related macular degeneration (AMD) lesions.

Methods: The Blue Mountains Eye Study (BMES) followed up 3654 participants 49 years and older (BMES 1, 1992-1994), including 2335 (75.3% of survivors) at the 5-year (BMES 2, 1997-1999) and 1952 (76.5%) at the 10-year (BMES 3, 2002-2004) examinations. Age-related macular degeneration retinal photographic grading used the Wisconsin system. Early and late AMD included prevalent and incident cases from all visits. CFH genotyping used TaqMan assays.

Results: Of 767 AMD cases, 53.3% of early and 53.1% of late AMD cases were bilateral. After adjusting for age and other covariants, the CFH CC (Y402H polymorphism) genotype was associated with an increased likelihood of bilateral compared with unilateral involvement by any soft drusen (odds ratio [OR], 2.5; 95% confidence interval [CI], 1.4-4.5), distinct soft drusen (OR, 2.8; 95% CI, 1.0-8.1), and pigmentary abnormalities (OR, 1.7; 95% CI, 1.0-2.8). We could not establish significant associations between this genotype and the bilaterality of late AMD (OR, 1.8; 95% CI, 0.4-7.7), either geographic atrophy (OR, 0.6; 95% CI, 0.07-4.6) or neovascular AMD (OR, 3.4; 95% CI, 0.3-41.4).

Conclusions: Persons with the CFH CC genotype at any given age have an increased likelihood of bilateral compared with unilateral involvement of some early AMD lesions.


AGE-RELATED MACULAR DEGENERATION (AMD) is the leading cause of blindness in most developed countries.1 Age-related macular degeneration is an irreversible debilitating disease with complex genetic and environmental contributors. Recent research has focused on identifying the genetic predisposition to AMD, and complement factor H (CFH), LOC387715, and HTRA1 are the principal documented gene loci responsible.2-7 A meta-analysis has indicated that CFH CC and CT genotypes are responsible for 58.9% of AMD cases, implying that more than half of AMD cases may be attributable to the CFH Y402H polymorphism.8 Furthermore, this CFH polymorphism has been associated with the exudative and advanced forms of AMD,6 which greatly impair vision. Several published case series have suggested that diseases involving bilateral organs have an underlying genetic predisposition.10,11 Bilateral breast cancer and testicular germ cell tumors are 2 examples. Whether the CFH CC or CT genotypes increase the likelihood or age at onset of bilateral involvement by AMD lesions is unclear, with only limited data from the literature addressing this association. The involvement and age at which this occurs, of late AMD in both eyes rather than only 1 eye, results in legal blindness, leading to substantial disability and an increased burden of care for those affected individuals.12 This question is highly relevant not only to individuals but also to public health and the need for aged care services. We therefore aimed in this report to investigate the contribution of CFH genotypes to the bilaterality of early and late AMD lesions in a population-based
sample of AMD cases from the Blue Mountains Eye Study (BMES).

METHODS

The BMES is a population-based cross-sectional study of common eye diseases and other health outcomes in an urban, elderly Australian population (aged ≥49 years at baseline). The sample is mainly white (98%) and details of the survey method and procedures have been described previously.1,13 The baseline study was conducted during 1992-1994, in which 3654 of 4433 eligible residents (82.4%) identified in a door-to-door census of a defined region, west of Sydney, Australia, participated.

The 5-year follow-up examinations (BMES 2) were conducted during 1997-1999, where 2335 persons (75.3% of survivors) were reexamined, excluding 543 participants who had died since baseline. During 1999-2000, a second door-to-door census was conducted and 1443 newly eligible residents were identified, of whom 1174 (82.4%) participated in the BMES extension study. At 10-year follow-up examinations (BMES 3), conducted during 2002-2004, 1932 persons (76.9% of survivors) were reexamined, after excluding 1103 persons who had died.

All BMES examinations were approved by the Human Research Ethics committees of the Sydney West Area Health Service and the University of Sydney and signed informed consent was obtained from all participants at each examination.

At each examination, 30° color stereoscopic retinal photographs of the macula and other retinal fields of both eyes were taken using an FF3 fundus camera (Zeiss, Oberkochen, Germany). Macular photographs were taken of both eyes at baseline and after 5 years in 2277 participants (97.5% of those examined).14 At the 10-year follow-up examination, 1649 of 1952 participants (84.5%) had bilateral retinal photographs taken.15 The retinal photograph grading closely followed the Wisconsin Age-Related Maculopathy Grading System.16 Assessments of intergrader and intragrader reliability demonstrated high consistency.13 In this report, we incorporated all (early or late) AMD cases detected at any of the BMES examinations, including both prevalent and incident cases. After excluding participants without DNA data or with missing retinal photographs, 767 AMD cases were included in this report. Participants without signs of AMD were excluded from this report.

Family history of AMD was recorded using interviewer-administered questionnaires, together with information on smoking status (current, past, never) and other AMD risk factors. Participants were asked to return for fasting blood tests. These included white blood cell (WBC) count and whole blood for DNA extraction.

DEFINITION OF AMD

Late or advanced AMD was defined to include geographic atrophy (GA) or neovascular (NV) AMD, as described in the International AMD Classification.17 When GA and NV AMD were present in the same eye, NV AMD was considered to be the primary diagnosis. All late AMD cases in our study were confirmed by a retinal specialist (P.M.).

Early AMD was defined as the absence of any late macular lesions 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 (hyperpigmentation or hypopigmentation).17

UNILATERAL AND BILATERAL DISEASE

We included the following lesions in assessing the unilateral and bilateral involvement of AMD lesions:

Late AMD lesions:

1. Unilateral disease: any late AMD lesions in one eye and no late AMD in the other eye.

2. Bilateral disease: any late AMD lesions in both eyes.

Late AMD was subclassified as:

   A. GA or NV AMD.
   B. NV AMD.

Cases with NV AMD in one eye and GA in the other were graded as unilateral for both NV AMD and GA, but as bilateral for late AMD.

Early AMD lesions:

1. Unilateral disease: any early AMD lesions present in one eye and no early AMD lesions in the other eye.

2. Bilateral disease: any early AMD lesions present in both eyes.

They were subclassified as:

   A. Early AMD (as per definition),
   B. Any soft drusen, including reticular drusen,
   C. Indistinct soft drusen,
   D. Distinct soft drusen, and
   E. Pigmentary abnormalities (hypopigmentation or hyperpigmentation).

Hyperpigmentation was defined by the presence of discrete areas of retinal depigmentation, without visible choroidal vessels and with adjacent pigment aggregation. Hyperpigmentation was defined by the presence of clumps of dark brown or green pigment, visible beneath the retina.

Any AMD:

1. Unilateral disease: either early or late AMD lesions in one eye and no AMD in the other eye.

2. Bilateral disease: either early or late AMD lesions in both eyes. Cases where there was early AMD in one eye and late AMD in the other eye were not included in the assessment of bilateral early AMD but were categorized as unilateral late AMD and bilateral for any AMD.

CFH GENOTYPING

At the BMES 2 and BMES extension examinations, 3222 participants (88.2%) had DNA collected for CFH genotyping using TaqMan assays. The single-nucleotide polymorphism (SNP) rs1061170 (Y402H) in exon 9 was genotyped using polymerase chain reaction amplification in a volume of 5 μL including 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, California). The overall genotyping error rate was estimated to be less than 1% based on 136 replicates of 3 SNPs, with similar error rates at each SNP. Genotyping completeness ranged from 99.1% to 100%.18 In this report, we examined the risk allele C (histidine variant) and control allele T (tyrosine residue) of the CFH gene and the combined genotypes (TT, CT, and CC) in our sample.

STATISTICAL ANALYSIS

SAS (version 9.1.3; SAS, Cary, North Carolina) was used for all data analyses, including χ² and logistic regression analyses.19 The additive model was implemented to investigate influences of the CC and CT genotypes on the likelihood of developing bilateral AMD compared with the TT genotype. We
### Table 1. Characteristics of 767 Cases With Early and Late AMD in the Blue Mountains Eye Study Population

<table>
<thead>
<tr>
<th></th>
<th>Early AMD Lesions</th>
<th>Late AMD Lesions</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>686</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>73.9 (8.8)</td>
<td>80.4 (7.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Women, %</td>
<td>56.0</td>
<td>65.4</td>
<td>.10</td>
</tr>
<tr>
<td>CFH genotypes, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>248 (36.2)</td>
<td>14 (17.3)</td>
<td>.002</td>
</tr>
<tr>
<td>CT</td>
<td>314 (45.9)</td>
<td>45 (55.6)</td>
<td>.002</td>
</tr>
<tr>
<td>CC</td>
<td>123 (17.9)</td>
<td>22 (27.2)</td>
<td>.002</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>7.7</td>
<td>12.4</td>
<td>.15</td>
</tr>
<tr>
<td>WBC count, /µL, mean (SD)</td>
<td>6700 (3200)</td>
<td>6700 (1600)</td>
<td>.88</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CFH, complement factor H gene; WBC, white blood cell.

SI conversion factor: To convert WBC count to ×10⁹ per liter, multiply by 0.001.

*a*Early AMD lesions in this Table were defined as any early changes in either eye.

### RESULTS

Of 767 participants with AMD, 686 (89.4%) had early AMD lesions, 81 (10.6%) had late AMD, and 364 (47.5%) had any AMD (early or late AMD). The proportions of bilateral involvement in the early (53.3%) and late (53.1%) AMD subgroups were similar, while 60.4% of 364 cases with any AMD were bilateral. The mean age was significantly older in the late AMD group: 74 years for the early AMD group and 80 years for the late AMD group (P < .001). Women predominated in both the early (56.0%) and late (65.4%) AMD subgroups. Current smoking was more frequent in the late (12.4%) than in the early (7.7%) AMD subgroup. Mean WBC counts were equal and within normal ranges for both early and late AMD cases. The proportion with the *CFH* CC genotype was significantly higher in the late (27.2%) than the early (17.9%) AMD group (P = .002) (Table 1).

**Figure 1** shows the proportions of bilateral early AMD lesions for the 3 *CFH* genotypes. There was a trend of increasing numbers with bilateral involvement as the number of C alleles increased from TT to CT and CC genotypes. A similar trend was also observed for the proportions of late AMD and any AMD cases with bilateral involvement, although not for specific late AMD phenotypes (Figure 2).

After adjusting for age, sex, current smoking, and WBC count, the *CFH* CC genotype was significantly associated with bilateral early AMD lesions, in particular, bilateral any soft drusen (OR, 2.5; 95% CI, 1.4-4.5), distinct soft drusen (OR, 2.8; 95% CI, 1.0-8.1), and pigmentary abnormalities (OR, 1.7; 95% CI, 1.0-2.8) (Table 2). A significant association was found between the *CFH* CC genotype and bilateral involvement by any AMD (OR, 2.4; 95% CI, 1.3-4.5) (Table 3). The CC genotype, however, was not found to be significantly associated with bilateral indistinct soft drusen (OR, 1.5; 95% CI, 0.7-3.1) or bilateral early AMD (OR, 1.8; 95% CI, 0.9-3.5) (Table 2). Figure 3 displays the multivariable-adjusted ORs and CIs for the likelihood of bilateral involvement of early AMD lesions associated with the CC genotype, referenced to the TT genotype.

We were unable to demonstrate significant associations between the *CFH* CC genotype and bilateral late AMD (OR, 1.8; 95% CI, 0.4-7.7), GA (OR, 0.6; 95% CI, 0.07-4.6), or NV AMD (OR, 3.4; 95% CI, 0.3-41.4), after adjusting for age, sex, current smoking, and WBC count (Table 4).

### COMMENT

Bilateral involvement is typical in AMD, increasing in frequency with age.²⁰ In addition, similar to other diseases...
affecting bilateral organs, genetic predisposition may play a role. Published case series and animal experiments have suggested that bilateral involvement of the disease, often the more severe stage of the disease, has genetic predisposition as an underlying mechanism.\textsuperscript{10,11,21-23} Bilateral testicular germ cell tumors,\textsuperscript{11} breast tumors,\textsuperscript{10} vasoproliferative retinal tumors,\textsuperscript{21} acoustic neurofibromatosis,\textsuperscript{22} and Wilms tumors\textsuperscript{23} are some examples. The p53 tumor suppression gene abnormalities were detected in 50.0% of bilateral and only 25.8% of unilateral breast cancer cases.\textsuperscript{10}

It could be expected that a genetic risk factor (and also some environmental risk factors) would exert its influence on age-related diseases like AMD by leading to an earlier age at onset of the signs, an increase in their severity, or a reduced period from first- to second-eye involvement. In other words, risk factors could determine whether unilateral or bilateral involvement is present at any given time.

The CFH gene and its relevant complement cascade are now confirmed to be strongly associated with AMD.\textsuperscript{3,4,24,25} We therefore hypothesized that the CFH Y402H polymorphism would lead to a higher frequency of bilateral involvement by AMD lesions, after accounting for age and other significant risk factors.

Our findings have documented that the homozygous CFH CC genotype is significantly associated with bilateral involvement of early AMD phenotypes (Table 2). Similar findings were also observed for any AMD involvement (Table 3). These results support the hypothesis that genetic risk factors play a role in the development of bilateral AMD.

![Figure 3](https://jamanetwork.com/)

**Figure 3.** Bilateral vs unilateral involvement of early age-related macular degeneration (AMD) phenotypes for the CC genotype, with TT genotype used as the reference genotype, expressed in odds ratios adjusted for age, sex, current smoking, and white blood cell count.
general AMD (any early or late) and bilateral distinct soft drusen. Soft drusen have been shown to signify a high risk of progression to late AMD in the 15-year follow-up data from the Beaver Dam Eye Study in the United States. The Rotterdam Study reinforced these findings of early AMD progression and indicated that as many as 23% of cases with large soft drusen and pigmentary abnormalities progress to a more severe stage of AMD over 2 years. The BMES showed that large drusen and hyperpigmentation are significantly associated with an increased risk of progression to NV or atrophic AMD lesions. Hence, our finding of an association between the \textit{CFH} CC genotype and bilateral early AMD lesions implies inevitably an association of this genotype with bilateral involvement of the more severe stage of AMD down the track, although in our current study sample we could not document the latter association because of small numbers.

Bilateral AMD, especially the advanced stage of AMD, commonly results in legal blindness and, thus, carries greater clinical significance than unilateral disease. Bilateral AMD has been shown to impact greatly a person’s ability to live independently. A multicountry study showed that more than 50% of bilateral AMD cases were dependent on family members or spouses and at least 40% required assistance for daily activities. Patients with bilateral AMD are also at a higher risk of falling than those without bilateral disease, subsequently leading to an increase in health resources use. The health economic burden of bilateral AMD is estimated to be as high as €1300 million (US $1829.25) per annum in some developed countries. Our study results are in keeping with findings from a limited number of previous studies. Tedeschi-Blok and colleagues from the Los Angeles Latino Eye Study showed that the \textit{CFH} Y402H polymorphism was linked to bilaterality of specific early AMD phenotypes. Latino persons with bilateral intermediate to large macular drusen were 1.7 times more likely to carry at least 1 risk allele of the \textit{CFH} gene compared with persons with no AMD. Chen and colleagues showed that \textit{HTRA1} was also responsible for the development of bilateral AMD lesions. Persons with the risk genotype AA at rs11200638 of the \textit{HTRA1} gene were 11.0 times more likely to develop bilateral wet AMD than those with the \textit{HTRA1} GG genotype. Some studies have shown stronger but less consistent associations between AMD and other SNPs that were not examined in our report. We recognize that this is a possible limitation, which in addition to the small sample size, may explain the insignificant association found between bilaterality of late AMD lesions and the CC genotype in our sample.

In conclusion, this study has shown that the \textit{CFH} Y402H homozygous CC genotype is significantly associated with bilateral compared with unilateral presence of any AMD or of soft drusen, independent of age, sex, smoking, and WBC count. These findings suggest a genetic predisposition for an earlier development of bilateral involvement by some early AMD lesions.

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REFERENCES
