In this study, we examined the relationship between exposure to siblings and 1) the risk of age-related macular degeneration (AMD) and 2) C-reactive protein levels. We retrospectively analyzed pooled cross-sectional data from 2 studies: the Cardiovascular Health and Age-Related Maculopathy Study (2001–2002) and the Age-Related Maculopathy Statin Study (2004–2006). Associations between number of siblings and AMD were assessed by using multinomial logistic regression. Associations between number of siblings and C-reactive protein levels were examined by using a generalized linear model for γ distribution. A higher number of younger siblings was associated with significantly lower odds of early AMD in those with a family history of AMD (odds ratio = 0.2, 95% confidence interval: 0.1, 0.8) (P = 0.022) but was unrelated to AMD for those who had no family history of the disease (odds ratio = 1.0, 95% confidence interval: 0.9, 1.2) (P = 0.874). A higher number of younger siblings correlated with lower C-reactive protein levels (β = −0.19, 95% confidence interval: −0.38, −0.01) (P = 0.036). This supports the theory that immune modulation contributes to AMD pathogenesis and suggests that exposure to younger siblings might be protective when there is a family history of AMD. Age-related macular degeneration; C-reactive protein; inflammation; sibling exposure

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; CRP, C-reactive protein; IQR, interquartile range; OR, odds ratio.
It has been suggested that there is possibly a mal-adaptive parainflammatory response in AMD leading to chronic inflammation and subsequent tissue damage. Other chronic, age-related diseases have also been attributed to deranged parainflammatory responses, including type 2 diabetes (25), cardiovascular disease (26, 27), and Alzheimer’s disease (28, 29). As in AMD, the initial trigger for the chronic inflammatory response in these diseases remains unknown, although infectious agents have been implicated (30, 31).

There has been much interest in the role birth order plays in chronic, acquired diseases, and there is evidence that sibling exposure may play a role. According to the “hygiene hypothesis,” early life infections are important in balancing Th1- and Th2-type immune responses, and allergic disease in particular may be prevented by exposure to childhood illnesses (32). Several studies have examined birth order and the development of different diseases (33–39); young children are a source of common viral antigens and, as such, exposure to them is thought to play an important role in the development of a normal immune system (34, 40).

Given the growing interest in immune regulation in AMD pathogenesis, we hypothesized that exposure to siblings, in particular younger siblings, as a proxy for childhood infections, may be associated with the risk of AMD in later life. We also wanted to explore the relationship between exposure to siblings and CRP levels to look for any supportive evidence that immune/inflammatory modulation had occurred as a result of this exposure. Family history is a surrogate for genetic risk, and given the influence of genetics on AMD, we stratified our results into those with and without a family history to determine whether sibling exposure modified disease severity.

**MATERIALS AND METHODS**

The 2 cohorts for which we had information on siblings as well as other risk factors for AMD were the Cardiovascular Health and Age-Related Maculopathy Study (initial recruitment 1992–1995; follow-up study 2001–2002) and the prospective, randomized controlled trial, Age-Related Macular Degeneration Statin Study (2004–2006). The Cardiovascular Health and Age-Related Maculopathy Study was a case-control study designed to examine the risk factors for prevalent AMD and its progression and has been used to investigate both genetic and environmental influences (21, 41). The Age-Related Macular Degeneration Statin Study included only participants with AMD and was a 3-year randomized, placebo-controlled, double-blind trial designed to determine if 40 mg of simvastatin daily slowed progression of early AMD (42). The Cardiovascular Health and Age-Related Maculopathy Study had well-characterized control participants without any evidence of AMD. Both studies were undertaken in Melbourne, Australia, run by the same group of investigators, and used similar methodology with identical questionnaires, databases, AMD assessment methods, disease definitions, and genotyping. For our study, we used the cross-sectional data on AMD status to examine the possible association between sibling exposure and CRP levels with prevalent AMD. In the Age-Related Macular Degeneration Statin Study, no participant was taking a statin at baseline, but in the Cardiovascular Health and Age-Related Maculopathy Study, the use of statins was permitted. Both studies were approved by the Royal Victorian Eye and Ear Hospital’s Human Research and Ethics Committee and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Methodology for both studies has been published elsewhere (25, 41). In brief, baseline fundus photographs were taken of all participants. Grading of these images was carried out by experienced graders by using OptoMize PRO software (Digital Healthcare, Ltd., Cambridge, United Kingdom). AMD participants either had 1) at least 1 large druse(>125 μm) or extensive intermediate drusen (63–124 μm) with pigment change in both eyes; or 2) late-stage AMD (choroidal neovascularization or geographic atrophy) in 1 eye and any early AMD features in the other eye (drusen >63 μm or pigment change) based on the international classification and grading system (42, 43). Control participants in the Cardiovascular Health and Age-Related Maculopathy Study had no AMD features in either eye (hard drusen <63 μm allowed).

**Number of infant siblings**

We used 2 measures of sibling exposure in this study: total number of siblings and number of younger siblings. Although we were primarily interested in the relationship between AMD and younger siblings, we used the number of siblings for sensitivity analysis to determine the effect of family size (e.g., due to crowding, low socioeconomic status) on AMD. Birth order and sibling number have been used previously as surrogates for primary and recurrent early life infections (33–35, 37–39). Recalling episodes of childhood infections is notoriously unreliable; therefore, information regarding family size and birth order serves as a useful proxy. In this study, information was collected on participants’ and siblings’ ages (rather than dates of birth) at the time of interview. By using this information, we classified each sibling as “younger” or “not younger” than the participant. Precise calculation of infant-years of exposure was not possible in this study.

**C-reactive protein analysis**

Patients with CRP levels of more than 20.0 mg/L (as measured by a high-sensitivity CRP assay) were excluded because this may represent acute inflammation, and this study was primarily concerned with exploring the relationship between past sibling exposure and chronic inflammation.

**Statistical analysis**

Data were analyzed by using Stata, version 11, software (StataCorp LP, College Station, Texas). Graphs were generated by using SPSS, version 18, software (SPSS, Inc., Chicago, Illinois). A nominal level of statistical significance was set at α = 0.05.
Associations between demographic and clinical characteristics and AMD status were assessed by using Pearson’s χ² statistic for categorical variables (smoking, sex, genotype, family history, and use of antiinflammatory medication) and Kruskal-Wallis tests for continuous predictors (age, total number of siblings, and number of younger siblings). Variables with P values less than 0.20 were then included in a multivariate multinomial logistic regression to assess their relative importance in predicting AMD status. We used a cutoff value of P < 0.20 for inclusion of predictors in the multivariate model because it was expected that variables with P values more than 0.20 would make negligible contributions to the model.

Combined effects of family history and siblings on AMD

The associations between AMD and the total number of siblings and the number of younger siblings were examined by using multinomial logistic regression with age, sex, smoking status, family history of AMD, use of antiinflammatory medication, CRP levels, and use of statins as covariates. Because correlation between the total number of siblings and the number of younger siblings was moderately strong (Spearman’s ρ = 0.6, P < 0.001), separate analyses were conducted for the total number of siblings and the number of younger siblings to avoid problems with multicollinearity. The modifying effects of the total number of siblings and the number of younger siblings on family history of AMD were tested by using a multiplicative interaction test with the product of family history and sibling numbers entered into the model in addition to covariates, family history, and sibling variables. Statistical significance of the interaction was assessed by using a likelihood ratio test with significant interactions followed up with multinomial logistic regression analyses stratified by family history of AMD.

Relationship between number of siblings and C-reactive protein

The relationship between the number of siblings and CRP was assessed by using a generalized linear modeling approach adjusting for age, sex, smoking, use of antiinflammatory medication, and use of statins. Given high positive skewness of CRP levels (skewness = 2.3) and the number of younger siblings (skewness = 2.1), we explored 2 alternative regression models suitable for positively skewed continuous outcomes (linear model with log link and inverse Gaussian model with identity link). The fit of the 2 models was compared by using the ratio of deviance χ² to degrees of freedom with values closer to 1 indicating better fit. For the model that used the number of younger siblings as a predictor of CRP levels, a linear model with log link was rejected because of poor fit with the data (deviance χ²/df = 14.2) with an inverse Gaussian model showing a far better fit (deviance χ²/df = 0.9). Comparable results were obtained when the total number of siblings was used as a predictor. Only the results of the inverse Gaussian regression model are presented.

Missing data

Missing data were present for family history of AMD (17.2%), number of younger siblings (12.3%), use of antiinflammatory medication (2.8%), and use of statins (0.2%). In addition, 8.1% of all participants had CRP values below

Table 1. Demographic, Clinical, and Family Characteristics of Study Participants, CHARM Study (2001–2002) and ARMSS (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No.</th>
<th>Median (IQR)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>306</td>
<td>44.9</td>
<td></td>
</tr>
<tr>
<td>Early AMD</td>
<td>310</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>Late AMD</td>
<td>65</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Geographic atrophy</td>
<td>15</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Choroidal neovascularization</td>
<td>50</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>302</td>
<td>44.3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>379</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>343</td>
<td>50.4</td>
<td></td>
</tr>
<tr>
<td>Past or current smoker</td>
<td>338</td>
<td>49.6</td>
<td></td>
</tr>
<tr>
<td>Family history of AMD</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>75.5</td>
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</tr>
<tr>
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<td>50</td>
<td>7.3</td>
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<td>Unknown</td>
<td>117</td>
<td>17.2</td>
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<tr>
<td>Use of antiinflammatory medication</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>529</td>
<td>77.7</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>133</td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>19</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Use of statins</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>533</td>
<td>78.3</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>147</td>
<td>21.6</td>
<td></td>
</tr>
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<td>0.2</td>
<td></td>
</tr>
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<td></td>
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<td>64</td>
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<td></td>
</tr>
<tr>
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<td>617</td>
<td>90.6</td>
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<td>Has younger siblings</td>
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<td></td>
<td></td>
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<tr>
<td>No</td>
<td>223</td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>374</td>
<td>54.9</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
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<td></td>
</tr>
<tr>
<td>Number of younger siblings</td>
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<td>0–2</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>73.45 (68.2–78.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP level, mg/L</td>
<td>1.69 (0.6–3.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CRP, C-reactive protein; IQR, interquartile range.
the limit of detection (<0.05 mg/L). To maximize data available for analysis and minimize potential bias due to missing data, all multivariate analyses were conducted by using a multiple imputations method with estimates averaged across 5 imputed data sets. Univariate associations are based on cases with complete data only.

**RESULTS**

**Participants**

The results of this study are based on data from 681 subjects, including 375 cases with AMD and 306 controls. Among AMD cases, 310 (82.6%) had early AMD and 65 had late AMD (including 50 people with choroidal neovascularization and 15 people with geographic atrophy).

Background characteristics of the study sample are presented in Table 1.

Clinical and demographic characteristics of the groups based on AMD status are summarized in Table 2. The median age was 72.5 (interquartile range (IQR), 68.3–77.8) years for controls, 73.5 (IQR, 67.9–78.1) years for people with early AMD, and 77.5 (IQR, 72.0–81.0) years for people with late AMD. The proportion of women was highest in the early AMD group (59.7%), followed by late AMD group (53.8%) and the control group (52%). Less than half of all controls (46.4%) and early AMD cases (48.7%) were past or current smokers compared with 69.2% in the late AMD group. Only 2.2% of controls had a family history of AMD compared with 15.3% of participants with early AMD and 14.3% of participants with late AMD.

### Table 2. Associations Between AMD Status and Clinical and Demographic Characteristics, CHARM Study (2001–2002) and ARMSS (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Median (IQR)</th>
<th>%</th>
<th>No.</th>
<th>Sex</th>
<th>Median (IQR)</th>
<th>%</th>
<th>No.</th>
<th>Late AMD (Geographic Atrophy or CNV)</th>
<th>Median (IQR)</th>
<th>%</th>
<th>P Value*</th>
</tr>
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<tbody>
<tr>
<td>268</td>
<td>Male</td>
<td>147</td>
<td>48.0</td>
<td>125</td>
<td>Male</td>
<td>30</td>
<td>40.3</td>
<td>30</td>
<td>Male</td>
<td>46.2</td>
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<td>0.149</td>
</tr>
<tr>
<td>159</td>
<td>Female</td>
<td>159</td>
<td>52.0</td>
<td>185</td>
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<td>35</td>
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<td>35</td>
<td>Female</td>
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<td></td>
<td></td>
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<tr>
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<td>Smoking status</td>
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<td>53.6</td>
<td>159</td>
<td>Smoking status</td>
<td>20</td>
<td>51.3</td>
<td>20</td>
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<tr>
<td>142</td>
<td>Current or past smoker</td>
<td>142</td>
<td>46.4</td>
<td>151</td>
<td>Current or past smoker</td>
<td>45</td>
<td>48.7</td>
<td>45</td>
<td>Current or past smoker</td>
<td>69.2</td>
<td></td>
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<td>268</td>
<td>Family history of AMD</td>
<td>268</td>
<td>97.8</td>
<td>210</td>
<td>Family history of AMD</td>
<td>36</td>
<td>84.7</td>
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<td>6</td>
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<tr>
<td>52</td>
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<td>65</td>
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<td>16</td>
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<td>26.2</td>
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<tr>
<td>231</td>
<td>Use of satins</td>
<td>231</td>
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<td>247</td>
<td>Use of satins</td>
<td>55</td>
<td>79.9</td>
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<td>Yes</td>
<td>95.4</td>
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<td>61.1</td>
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<td>Yes</td>
<td>75.9</td>
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<td>Number of younger siblings</td>
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<td>Number of younger siblings</td>
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</tr>
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<td>72.5 (68.3–77.8)</td>
<td>73.5 (67.9–78.1)</td>
<td>77.5 (72.0–81.0)</td>
<td>Age, years</td>
<td>0.001</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1.4</td>
<td>CRP level, mg/L</td>
<td>1.4 (0.5–3.2)</td>
<td>1.8 (0.7–4.0)</td>
<td>2.1 (1.2–3.8)</td>
<td>CRP level, mg/L</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CNV, choroidal neovascularization; CRP, C-reactive protein; IQR, interquartile range.

a From χ² or Kruskal-Wallis test, as appropriate.
Although the majority of participants in all study groups had siblings, the proportion of people with any siblings was highest for the late AMD group (95.4%), followed by the early AMD group (91.6%) and the controls (88.6%), although this was not statistically different among the groups ($P=0.106$). The median number of siblings was 2 (IQR, 1–4) for controls and the early AMD group and 3 (IQR, 2–4) for the late AMD group. Among those for whom information on the number of younger siblings was available, the proportion who had younger siblings was 61.5% ($n = 168$) in the control group, 61.1% ($n = 165$) in the early AMD group, and 75.9% ($n = 41$) in the late AMD group. The median number of younger siblings was 1 (IQR, 0–2) for the controls and the early AMD group and 1 (IQR, 1–2) for the late AMD group.

### Siblings and family history in AMD

After adjusting for age, sex, smoking, CRP level, use of antiinflammatory medication, and use of statins, neither the total number of siblings nor the number of younger siblings was associated with AMD (see Tables 3 and 4). The multiplicative interaction between the total number of siblings and family history of AMD was also not significant (likelihood ratio $\chi^2 = 1.9$, df = 2) ($P = 0.386$) (see Table 3). However, adding the multiplicative interaction between family history of AMD and the number of younger siblings into the model that already contained age, sex, smoking, CRP level, use of antiinflammatory medication, use of statins, CRP levels, and total number of siblings as predictors, the multiplicative interaction remained small, it appeared as though for each additional younger sibling on early AMD in patients with a family history indicated that a higher number of younger siblings had a significantly lower odds of early AMD (odds ratio (OR) = 0.3, 95% confidence interval (CI): 0.1, 0.8) ($P = 0.022$) but was unrelated to early AMD for those with no family history of AMD (OR = 0.2, 95% CI: 0.1, 0.8) ($P = 0.008$). The interaction was significant for those with early AMD (odds ratio (OR) = 0.3, 95% confidence interval (CI): 0.1, 0.8) ($P = 0.015$) but not for those with late AMD (OR = 0.8, 95% CI: 0.3, 2.4) ($P = 0.749$) (see Table 4).

Subsequent multinomial regression analyses stratified by family history indicated that a higher number of younger siblings was associated with significantly lower odds of early AMD in those who had a family history of AMD (OR = 0.2, 95% CI: 0.1, 0.8) ($P = 0.022$) but was unrelated to early AMD for those who had no family history (OR = 1.0, 95% CI: 0.9, 1.2) ($P = 0.874$) (see Table 5; results for late AMD are presented for completeness). The protective effect of younger siblings on early AMD in patients with a family history of AMD seemed to increase with increasing numbers of siblings. Although numbers in some subgroups were small, it appeared as though for each additional younger sibling that a patient with a family history had, the odds of having early AMD decreased 5-fold.
To further explore the interaction between family history of AMD and having younger siblings, we conducted additional logistic regression analyses stratified by younger siblings status (yes/no; see Table 6). The results indicated a substantial decrease in the odds of developing early AMD in those who had younger siblings. For those who had no younger siblings, there was a 30-fold increase in the odds of early AMD in the presence of family history (OR = 30.1, 95% CI: 3.7, 247.1) (P = 0.002) compared with those with no family history of the disease. For those who had younger siblings, family history of AMD was associated with only a 4-fold increase in the odds of developing early AMD (OR = 3.9, 95% CI: 1.3, 11.4) (P = 0.015) compared with those without a family history of AMD. For those who did have younger siblings, there were 5 controls and 17 cases of early AMD with family history of AMD.

A smaller but similar direction of risk effect was observed for late AMD. For those with a family history of the disease, the odds for developing late AMD in patients who had younger siblings (OR = 7.3, 95% CI: 1.7, 31.6) (P = 0.007) was less than for those who did not have younger siblings (OR = 9.5, 95% CI: 0.4, 214.6) (P = 0.156). However, the overall number of cases of late AMD was small, and there was only 1 case and 1 control in the subgroup with no family history of AMD and no younger siblings.

### Relationship between CRP levels and siblings

Levels of CRP (skewness = 2.3) and the number of younger siblings (skewness = 2.1) were strongly positively skewed. A scatterplot of CRP levels and the number of younger siblings indicated a general trend toward lower levels of CRP for those who had younger siblings. The relationship also appeared to have a nonlinear component, with a decrease in CRP levels becoming more rapid with a higher number of younger siblings (see Figure 1). A similar shape but weaker relationship was also observed between CRP levels and the total number of siblings (not shown).

### Table 4. Results of Multinomial Logistic Regression Analyses Examining Association Between the Number of Younger Siblings and AMD, CHARM Study (2001–2002) and ARMSS (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th></th>
<th>Early AMD (n = 310)*</th>
<th>Late AMD (Geographic Atrophy or CNV) (n = 65)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1b</td>
<td>Model 2c</td>
</tr>
<tr>
<td><strong>OR 95% CI P Value</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age^d</td>
<td>1.02 0.99, 1.04 0.175</td>
<td>1.02 0.99, 1.05 0.159</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.30 0.87, 1.95 0.206</td>
<td>1.33 0.88, 2.01 0.169</td>
</tr>
<tr>
<td>Current or past smoker</td>
<td>1.10 0.74, 1.65 0.627</td>
<td>1.13 0.75, 1.69 0.567</td>
</tr>
<tr>
<td>Family history of AMD</td>
<td>6.95 2.80, 17.27 0.000</td>
<td>29.96 5.38, 166.93 0.000</td>
</tr>
<tr>
<td>Use of antiinflammatory medication</td>
<td>0.95 0.57, 1.59 0.851</td>
<td>1.00 0.60, 1.68 0.993</td>
</tr>
<tr>
<td>Use of statins</td>
<td>1.05 0.67, 1.64 0.844</td>
<td>1.07 0.68, 1.68 0.766</td>
</tr>
<tr>
<td>CRP level</td>
<td>1.02 0.97, 1.08 0.402</td>
<td>1.02 0.96, 1.08 0.594</td>
</tr>
<tr>
<td>Number of younger siblings^e</td>
<td>0.97 0.83, 1.12 0.649</td>
<td>1.01 0.87, 1.18 0.889</td>
</tr>
<tr>
<td>Number of younger siblings x family history of AMD</td>
<td>0.26 0.09, 0.77 0.015</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CI, confidence interval; CNV, choroidal neovascularization; CRP, C-reactive protein; OR, odds ratio.

* Reference group: controls (n = 306).

b Model 1 included age, sex, smoking status, family history of AMD, use of anti-inflammatory medication, use of statins, CRP levels, and number of younger siblings as predictors.

c Model 2 included multiplicative interaction between the number of younger siblings and family history of AMD, in addition to the other predictors included in Model 1.

d Age represents 10-year groupings (<50 years; 50–59 years; 60–69 years; 70–79 years; ≥80 years).

e Number of siblings (including half-siblings) that are younger than the respondent; twins were not counted as younger siblings.
The use of statins, number of siblings, and interactions between number of siblings and family history of AMD (Tables 3 and 4). However, the association between CRP levels and late AMD was significant only in the adjusted models.

Results of generalized linear modeling showed that after adjusting for age, sex, smoking, and use of statins and anti-inflamatory medication, the total number of siblings was not associated with CRP levels ($\beta = -0.01$, 95% CI: $-0.09$, $-0.01$).

### Table 5. Results of Multinomial Logistic Regression Analyses Examining Association Between the Number of Younger Siblings and AMD, Stratified by Family History, CHARM Study (2001–2002) and ARMSS (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th></th>
<th>No Family History of AMD (n = 514)</th>
<th>Family History of AMD (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early AMD (n = 210)$^a$</td>
<td>Late AMD (Geographic Atrophy or CNV) (n = 36)$^a$</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age$^c$</td>
<td>1.02</td>
<td>0.99, 1.05</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.39</td>
<td>0.92, 2.11</td>
</tr>
<tr>
<td>Current or past smoker</td>
<td>1.14</td>
<td>0.75, 1.72</td>
</tr>
<tr>
<td>Use of antiinflammatory medication</td>
<td>0.97</td>
<td>0.56, 1.65</td>
</tr>
<tr>
<td>Use of statins</td>
<td>0.97</td>
<td>0.56, 1.65</td>
</tr>
<tr>
<td>CRP level</td>
<td>1.01</td>
<td>0.87, 1.18</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CI, confidence interval; CNV, choroidal neovascular membrane; CRP, C-reactive protein; OR, odds ratio.

$^a$ Reference group: controls (n = 268).

$^b$ Reference group: controls (n = 6).

$^c$ Age represents 10-year groupings (<50 years; 50–59 years; 60–69 years; 70–79 years; ≥80 years).

$^d$ Number of siblings (including half-siblings) that are younger than the respondent; twins were not counted as younger siblings.

### Table 6. Results of Multinomial Logistic Regression Analyses Examining Association Between Family History and AMD, Stratified by Having Younger Siblings, CHARM Study (2001–2002) and ARMSS (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th></th>
<th>No Younger Siblings (n = 223)</th>
<th>Have Younger Siblings (n = 374)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early AMD (n = 105)$^a$</td>
<td>Late AMD (Geographic Atrophy or CNV) (n = 13)$^a$</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age$^c$</td>
<td>1.03</td>
<td>0.99, 1.08</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.53</td>
<td>0.78, 2.98</td>
</tr>
<tr>
<td>Current or past smoker</td>
<td>0.97</td>
<td>0.51, 1.88</td>
</tr>
<tr>
<td>Family history of AMD</td>
<td>30.07</td>
<td>3.66, 247.13</td>
</tr>
<tr>
<td>Use of antiinflammatory medication</td>
<td>1.47</td>
<td>0.65, 3.36</td>
</tr>
<tr>
<td>Use of statins</td>
<td>1.55</td>
<td>0.75, 3.19</td>
</tr>
<tr>
<td>CRP level</td>
<td>0.97</td>
<td>0.87, 1.08</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CI, confidence interval; CNV, choroidal neovascular membrane; CRP, C-reactive protein; OR, odds ratio.

$^a$ Reference group: controls (n = 105).

$^b$ Reference group: controls (n = 168).

$^c$ Age represents 10-year groupings (<50 years; 50–59 years; 60–69 years; 70–79 years; ≥80 years).

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C-reactive protein.

The formation of the number of younger siblings into the model required an inverse transformation in the inverse relationship between the number of siblings and the level of CRP by adding an inverse transformation ($\beta = -0.19$, 95% CI: $-0.38$, $-0.01$) ($P = 0.036$) (see Table 7).

We tested for the presence of a potentially nonlinear component in the inverse relationship between the number of siblings and the level of CRP by adding an inverse transformation of the number of younger siblings into the model that already had the number of younger siblings (untransformed) and covariates so that we could examine the incremental contribution of the nonlinear term to the regression model. The inverse transformation of the number of younger siblings made a significant contribution to the model ($\beta = -1.6$, 95% CI: $-2.7$, $-0.6$) ($P = 0.003$) over and above age, sex, smoking, use of antiinflammatory medication, use of statins, and the number of younger siblings, supporting the existence of an inverse nonlinear component in the relationship between the number of siblings and the level of CRP. At the same time, the number of younger siblings remained a significant predictor in this extended model ($\beta = -0.4$, 95% CI: $-0.5$, $-0.2$) ($P < 0.001$).

**DISCUSSION**

In this study, we examined the relationship between number of siblings and the risk of development of AMD later in life as well as the relationship between sibling number and CRP level. For patients with a family history of AMD, having younger siblings reduced the risk of development of early AMD by a factor of 25 ($P = 0.013$). This relationship was not present for participants without a family history of AMD. A smaller but similar direction of risk effect was observed in patients who developed late AMD, but this was only a small cohort, and conclusions may be difficult to draw. Regardless of family history status, there was no relationship between total number of siblings and AMD development. These findings suggest that it is birth order rather than total family size that is associated with reduced risk of early AMD in patients with a family history of the disease. Similarly, it was also the greater number of younger siblings and not the total number of siblings that was associated with a lower level of CRP for all study participants. Therefore, our results suggest that the number of

![Figure 1. Relationship between CRP levels and the number of younger siblings, CHARM Study (2001–2002) and ARMS Study (2004–2006), Melbourne, Australia. ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CRP, C-reactive protein.](https://academic.oup.com/aje/article-abstract/177/9/933/144888)

### Table 7. Results of Generalized Linear Regression Analysis Examining Association Between CRP Level and Number of Younger Siblings, CHARM Study (2001–2002) and ARMS Study (2004–2006), Melbourne, Australia

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$ Coefficient</td>
<td>95% CI</td>
<td>$z$ Score</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>Age $^c$</td>
<td>0.01</td>
<td>$-0.03$, 0.04</td>
<td>0.42</td>
<td>0.673</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.49</td>
<td>0.79, 2.19</td>
<td>4.16</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Current or past smoker</td>
<td>1.16</td>
<td>0.53, 1.79</td>
<td>3.62</td>
<td>0.000</td>
</tr>
<tr>
<td>Use of antiinflammatory medication</td>
<td>0.47</td>
<td>$-0.47$, 1.41</td>
<td>0.97</td>
<td>0.331</td>
</tr>
<tr>
<td>Use of statins</td>
<td>$-0.63$</td>
<td>$-1.12$, $-0.13$</td>
<td>$-2.49$</td>
<td>0.013</td>
</tr>
<tr>
<td>Number of younger siblings $^d$</td>
<td>$-0.19$</td>
<td>$-0.38$, $-0.01$</td>
<td>$-2.10$</td>
<td>0.036</td>
</tr>
<tr>
<td>Inverse transformation of the number of younger siblings</td>
<td>$-1.60$</td>
<td>$-2.65$, $-0.55$</td>
<td>$-2.99$</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: ARMSS, Age-Related Maculopathy Statin Study; CHARM, Cardiovascular Health and Age-Related Maculopathy Study; CRP, C-reactive protein.

$^a$ Model 1 included age, sex, smoking status, family history of AMD, use of anti-inflammatory medication, use of statins, and number of younger siblings as predictors.

$^b$ Model 2 included inverse transformation of the number of younger siblings, in addition to the other predictors included in Model 1.

$^c$ Age represents 10-year groupings ($<50$ years; 50–59 years; 60–69 years; 70–79 years; $\geq 80$ years).

$^d$ Number of siblings (including half-siblings) that are younger than the respondent; twins were not counted as younger siblings.

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youngster siblings may influence the development of AMD in 2 ways. First, in those with a family history of AMD, increasing numbers of younger siblings may modify the genetic risk and reduce the development of early AMD, perhaps through repeated exposure to early infectious and inflammatory stimuli. Second, by lowering CRP levels, greater numbers of younger siblings might indirectly affect the risk of progression to late AMD (given that higher levels of CRP are associated with late AMD).

One limitation of the study was that it centered on secondary analysis of data that were not collected to address the aims of this study specifically. Therefore, the sample size in each group was relatively small. This was important given that the relationship between sibling exposure and late AMD was not significant, possibly because of small numbers. Another consideration was that no information was gathered regarding potential exposure to other children, such as in the setting of child care. This information is not routinely gathered in AMD questionnaires, and we know of no other study with this information available. In general, patients who currently have AMD are of a generation in which child care outside the family home was not routine; therefore, this is not likely to be a significant confounder. Other studies that used data sets with patients in this age group have also acknowledged external child care to be rare (37).

To date, no other study has used younger sibling number as a proxy for childhood infection exposure and the risk of AMD. In addition, there are no papers describing the relationship between CRP levels and exposure to younger siblings. On the other hand, many studies have indicated that there are relationships among chronic inflammation, infection, and age-related illnesses. The role of inflammation in atherosclerosis, cardiovascular disease (44), and diabetes (45) has been well described, as has the possible association of these diseases, as well as AMD, with infectious agents such as Chlamydia pneumonia (46). Chronic inflammation may act as a promoter of AMD initiation and disease progression via the innate immune system through complement activation (47, 48), microglial activation (49), and stimulation of choroidal macrophages. The adaptive immune response may also be involved, with retinal autoantibodies having been detected in some AMD retinas (24). Recently, interest has also focused on the role of parainflammation in chronic illness. This is the host’s normal response to noxious stimuli. If such stimuli persist, a maladaptive response may occur and overt inflammation may ensue, resulting in widespread tissue destruction (23, 24). It is now recognized that unregulated parainflammatory responses play an important role in acquired ocular pathology such as AMD, glaucoma, and diabetic retinopathy (24).

Birth order and disease prevalence have been extensively studied with different conclusions drawn depending on the specific disease in question. Greater numbers of both older (39, 50) and younger (36, 37) siblings have been shown to influence certain diseases. Most studies implicate a role for early childhood infections regardless of the direction of the relationship. Siblings provide a source of common childhood viral infections, and reexposure to such infections has been shown to result in increasing immunoglobulin G levels in seropositive patients (51). Reexposure has also been shown to have effects on B-cell line maturation (52) and T-cell phenotype (53). There is a suggestion that reduced childhood infections result in a delayed transition from the infantile Th2 helper phenotype to Th1 immunity (40). However, experimental evidence supporting this reduction in immune suppression resulting in increasing prevalence of diseases such as atopy remains elusive.

Several studies show that higher birth order (i.e., greater number of older siblings) is associated with a reduced risk of atopic disease (39, 54). On the other hand, there has been shown to be a reduced risk of multiple sclerosis with a lower birth order (i.e., greater number of younger siblings) (37, 38). This is thought to be caused by a protective effect of viral infections, particularly Epstein-Barr virus, which has been correlated with a risk of multiple sclerosis (55). A recent paper investigating infant exposure and multiple sclerosis showed a strong inverse, dose-dependent relationship between infant exposure and multiple sclerosis risk (37). Patients with multiple sclerosis also had very high Epstein-Barr virus immunoglobulin G titers. In addition, the researchers found that this protective effect of younger sibling exposure was lost if there were more than 6 years between the index child and the next younger sibling, hypothesizing that cross-infection within households may be less important once children start school.

Early life infections and exposure to siblings have also been studied for their roles in other diseases such as type 1 diabetes (36) and childhood cancers (33, 50). Some studies have shown that lower birth order is associated with an increased risk of childhood tumors (50). Additionally, a recent meta-analysis of 31 observational studies investigating childhood onset type 1 diabetes showed a reduced risk with higher birth order (36).

It is of interest that, in our study, we found a protective effect of younger siblings only in participants with a family history of AMD. As variants in complement-related genes are, to date, the most commonly associated with AMD, this suggests a role for early priming of the immune system in those with a genetic predisposition to variations in the complement pathway. Those without a family history of AMD—and hence those who are less likely to have a complement-related genetic defect—may not be affected by this sibling exposure. The fact that we found, in the same study, that participants with greater numbers of younger siblings had lower CRP levels further supports the theory that early life immune modulation may result in less late-onset, chronic inflammatory disease.

In conclusion, our study indicated that there is a relationship between younger sibling exposure and AMD development in those with a family history of the disease. We also found lower CRP levels in participants who had greater numbers of younger siblings. Increasing numbers of younger siblings may result in more frequent early childhood infections, which in turn could result in immune modulation. Our results provide some evidence to support a hypothesis that exposure to younger siblings, and thus exposure to childhood infections, may modulate the risk of developing AMD in later life in those who are genetically susceptible. These findings are novel in their associations with AMD but are consistent with findings in other chronic diseases and further implicate immune modulation in the pathogenesis of AMD.
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Inflammation and Siblings: Establishing Risk of AMD


