Exposure to Chlamydia pneumoniae Infection and Age-Related Macular Degeneration: The Blue Mountains Eye Study

Luba Robman,1 Olaimatu S. Mahdi,2 Jie Jin Wang,1,3 George Burlutsky,4 Paul Mitchell,5 Gerald Byrne,2 Robyn Guymer,1 and Hugh Taylor1

PURPOSE. To assess cross-sectional and longitudinal associations between exposure to Chlamydia pneumoniae infection and age-related macular degeneration (AMD) in the nested case-control sample drawn from the Blue Mountains Eye Study (BMES) cohort.

METHODS. The BMES examined 3654 persons aged 49 to 97 years during 1992 through 1994 (BMES I survey). Survivors from this cohort (n = 2335; 75%) and 1174 persons who moved in this area or reached an eligible age were examined during 1997 through 2000 (BMES II survey, n = 3509). One hundred ninety-seven AMD cases and 453 control subjects matched for age, sex and smoking status, were drawn from the BMES II survey. Photographic macular grading followed the Wisconsin grading system. Plasma samples were analyzed with an enzyme-linked immunosorbent assay to determine antibody titers to the elementary bodies from C. pneumoniae AR39. Associations between seroreactivity to C. pneumoniae and prevalent and incident AMD were assessed by using logistic regression models.

RESULTS. There were 159 early and 38 late AMD cases. Of them, 87 cases of early and 22 of late AMD developed between the baseline and follow-up examinations. After adjustment for age, gender, and smoking, no significant association was evident between C. pneumoniae antibody titer and any prevalent early or late AMD (OR 1.02, 95% CI 0.66–1.56 comparing upper with lower tertile of antibody titer). Findings were similar when early or late AMD was analyzed separately. Analysis confined to incident AMD also showed no significant association with the incidence of either early (OR 0.92, 95% CI 0.52–1.64) or late (OR 1.85, 95% CI 0.57–6.05) AMD. The results did not change after adjustment for family history of AMD and cardiovascular disease.

CONCLUSIONS. In this nested case-control sample of an older Australian population we found no association between C. pneumoniae antibody titers and early AMD. The study has insufficient power to assess an association with late AMD. (Invest Ophthalmol Vis Sci. 2007;48:4007–4011) DOI:10.1167/iovs.06-1434

Increasing evidence supports an association between inflammation and age-related macular degeneration (AMD). Macrophages, lymphocytes, and mast cells have been found in areas of Bruch’s membrane disruptions, atrophic retinal pigment epithelium, and choroidal neovascularization tissue from AMD cases.1 Drusen were shown to contain proteins related to inflammation,2–8 and, in some studies, elevated levels of C-reactive protein have been found to be associated with an increased risk of AMD and its progression.9–12 Recently, genetic variations in complement pathway genes (complement factor H [CFH], and factor B [FB], and C2) were found associated with AMD, providing further evidence to support a role for inflammation in AMD.12–17 Hageman et al.18 proposed that aberrant regulation of the alternative complement pathway, which can be activated by infectious agents, could significantly contribute to AMD pathogenesis. Chlamydia pneumoniae could be one such trigger that activates the alternative complement pathway.

Previous studies conducted to investigate the association between C. pneumoniae infection and AMD,19–20 including AMD progression,21 showed some evidence to support this hypothesis. However, current available evidence on the association between C. pneumoniae infection and AMD has mainly been derived from clinic-based samples.18–20 Also, there was some inconsistency across studies, with a small recent study that did not support this association.22 We sought to study the association between exposure to C. pneumoniae infection and both prevalent and incident AMD in a nested case-control sample of the Blue Mountains Eye Study (BMES) cohort.

MATERIALS AND METHODS

The BMES is a population-based study of vision and common eye diseases of nonindustrialized residents, 49 years of age and older, living in two suburban postal code areas, west of Sydney, Australia. A detailed description of the study population and methods has been published.23 In brief, at the baseline BMES examination, 3654 persons aged 49 to 97 years participated from 1992 through 1994 (BMES I survey). Five years later, 2355 (75% of surviving baseline participants), together with 1174 persons who newly moved into the study area or reached the eligible age of the study by that time, participated in the BMES II survey (n = 3509, 1997–2000).24 The BMES study protocols were approved by the University of Sydney Human Research Ethics Committee, and written consent was obtained from all study participants.

Eye examinations during each survey included dilated 30° stereoscopic retinal photographs (model FF3; Carl Zeiss Meditec, GmbH, Oberkochen, Germany), which were graded using the Wisconsin AMD grading system. Early AMD was defined by the appearance of indistinct soft drusen, reticular drusen, or concurrent distinct soft drusen and pigmentary abnormalities in either eye of a person. Late AMD was defined by the appearance of neovascular AMD or geographic atrophy.

From the 1Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia; the 2Department of Molecular Sciences, University of Tennessee Health Science Center, Memphis, Tennessee; and the 3Centre for Vision Research, Westmead Millennium Institute, University of Sydney, Sydney, Australia.

Supported by American Health Assistance Foundation Grant M2005-012; the National Health and Medical Research Council, Australia; and the National Institutes of Health, Bethesda, MD.

Submitted for publication December 3, 2006; revised April 23, 2007; accepted June 12, 2007.

Disclosure: L. Robman, None; O.S. Mahdi, None; J.J. Wang, None; G. Burlutsky, None; P. Mitchell, None; G. Byrne, None; R. Guymer, None; H. Taylor, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Robyn Guymer, Centre for Eye Research Australia, University of Melbourne, 32 Gisborne Street, East Melbourne, 3002, Australia; rbg@unimelb.edu.au.
in either eye. Incident late AMD was defined as occurring in persons who were free of late AMD at the time of the BMES I survey but who had either of the two late lesions at the BMES II survey. Incident early AMD was defined as occurring in persons who were free of early and late AMD at the time of the BMES I survey and were also free of late AMD at the BMES II survey, but who had early AMD at the time of BMES II.

There were 284 cases of early and 65 cases of late AMD identified in the 3509 BMES II survey participants. Of these 349 with AMD, 197 (159 with early and 38 with late AMD) had plasma samples available. The remaining 152 nontested subjects with AMD either did not have blood collected or did not have enough plasma in storage, as some samples had already been used. The 433 age, gender- and smoking-matched control subjects (a window of ±5 years was allowed if there was no exact age-matched control subject) were drawn from the same BMES II survey population. Matching for smoking was based on current smoking status (current smokers versus current nonsmokers). Primarily, the original sample of 210 cases and 420 controls was drawn from the initial AMD grading. At the later stage during adjudication, we excluded 13 cases of previously diagnosed early AMD which were assigned to non-AMD cases. The blood test had been performed; therefore, we adjusted for age, sex, and smoking in the analysis. For analyses of association between seroreactivity to C. pneumoniae and incident AMD, only the participants in the original BMES cohort seen at both the baseline and 5-year examinations were included. Of the 197 prevalent AMD cases, 87 were incident early AMD and 22 were incident late AMD.

The protocol of this research was approved by the Human Research and Ethics Committees of the Royal Victorian Eye and Ear Hospital (Melbourne), the University of Sydney, and the University of Tennessee. At any stage of this research, all procedures adhered to the tenets of the Declaration of Helsinki.

Serologic Tests
Plasma samples were collected during the BMES II survey and stored in plastic cryotubes at −80°C (Forma Scientific Inc., Marietta, OH) at the study center. Plasma samples (0.2 mL) were forwarded (on dry ice) to the Department of Molecular Sciences, University of Tennessee Health Sciences Center (Memphis, TN). Enzyme-linked immunosorbent assay (ELISA) tests were performed with antibody bodies from C. pneumoniae AR39, and titers were expressed as optical density (OD) units. The C. pneumoniae whole organisms (AR39) were grown in HeLa cells, and the infectious stage of the organism known as elementary bodies (EBs) was harvested and stored at −80°C until used. The complete details of the enzyme-linked immunosorbent assay (ELISA) test were previously reported.16,24,25 Briefly, two plates (Immunolon; Dynex Technologies, Chantilly, VA) coated with 0.5 μg antigen in phosphate-buffered saline (PBS) for 48 hours at 4°C were incubated with a 1:250 dilution of patient sera in PBS. The plates were incubated with the alkaline phosphatase-conjugated goat anti-human IgG (Jackson Immunoresearch Laboratories, West Grove, PA) and the substrate, p-nitrophenylphosphate (SigmaFAST tablets; Sigma-Aldrich, St. Louis, MO). Absorbance was read as OD at 405 nm on a plate reader (HTS 7000 Bio Assay Reader; Perkin Elmer, Wellesley, MA). For each serum sample, the OD of a phosphate-buffered saline-coated well that had no antigen (antigen blank) was subtracted from the values for all the test wells for that antigen. Triplicate-blanked test ODs were averaged and reported for each patient. Laboratory personnel were masked to clinical and demographic information on the patients. The partially automated ELISA is a more standardized method than the microimmunofluorescence (MIF) test, and the reading of results is less subjective. It permits obtaining results in the form of a continuous variable from lowest to highest ODs rather than a binary variable based on a certain cutoff point chosen for a test on MIF. The continuous variable on seroreactivity from the ELISA test presents more comprehensive data than the associations in question.

We have previously compared our ELISA method with the MIF gold standard presently used for measuring C. pneumoniae IgG antibody titers in sera from 200 patients with heart disease recruited for the Intermountain Heart Collaborative Study performed in 2002.24 In this study, we compared average ELISA ODs and MIF endpoint titers of IgG antibodies to C. pneumoniae. We found a good (r = 0.59) and significant (P < 0.001) correlation between the MIF and ELISA results, which was in agreement with the published data on the validity of the latter for detection of the exposure to C. pneumoniae infection.24,26–29

Statistical Analysis
Statistical analyses were performed with commercial software (SAS ver. 8.2 for windows; SAS Institute, Cary, NC). Statistical power was estimated with another program (Power and Precision software; Biostatistics, Inc., Morristown, NJ). Power calculations a priori were based on our previous report on association between exposure to C. pneumoniae and AMD progression.31 The proportion of the high/medium (upper and middle tertiles) titers of antibodies to the C. pneumoniae AR39 elementary bodies in the progressive cases of early AMD was 79%, compared to 61% in nonprogressors. To detect a similar 18% difference in proportions with the high/medium (upper and middle) tertiers of the antibodies would require a sample size of 101 subjects per group with 80% power and 132 per group with 90% power (two-tailed α = 0.05). The available numbers of cases with prevalent early AMD from our sample (n = 159) should enable our study to detect a smallest difference of 15% with 80% power, or a difference of 18% with 92% power, between cases and controls. We should be able to detect odds ratios (ORs) of 1.7 at a significance level of 0.05 for any (early or late) AMD, comparing the upper to the lower tertiles of the antibody titer. In regard to late AMD, the available number of cases with prevalent or incident late AMD in this study were too small to be able to detect such a small difference, with a power of only 55% for prevalent and a power of 27% for incident late AMD. Logistic regression analyses of associations between the C. pneumoniae antibody level and AMD were adjusted for age and smoking status, the two major risk factors known to be independently associated with AMD. Since family history of AMD may reflect heritability of the disease, whereas history of cardiovascular events may be associated with both the outcome and covariates of interest, additional adjustment for AMD family history and histories of heart attack or stroke was performed to minimize potential confounding effects. We used analysis of covariance (PROC GLM, SAS) to compare the means of the log-transformed antibody titers adjusted for age, gender, and current smoking. Tests with P < 0.05 were considered significant.

RESULTS
Characteristics of the 630-subject sample selected for this analysis, such as the gender distribution and proportion of smokers, were similar to those for remaining BMES II participants, apart from age. The mean age of this sample was 8 years older (73 vs. 65 years of age) than that of the rest of the cohort, which would be expected because of the older age of AMD-affected individuals and age-matched control subjects. The AMD carriers tested in the course of this study were on average 2.5 years younger than those whose blood was not tested, and there was no statistically significant difference in gender and smoking status between these two groups (Table 1). Measurements of antibody titer ranged from 0.04 to 2.13 OD units, with a mean of 0.42 ± 0.29 OD units; measurements became normally distributed after logarithm transformation.

Seroreactivity to C. pneumoniae and Prevalent AMD
The mean age of the patients in the 197 prevalent AMD cases (74.8 years) was not significantly different from the mean age of the 433 control subjects (72.8 years; Table 2). Both cases and controls were predominantly women (59% and 58%, re-
spectively). There was no significant difference in the proportions of smokers between cases and controls. The mean age of 38 patients with late AMD was 78.1 years and 16% of them were current smokers.

The mean level of antibodies to C. pneumoniae was 0.42 OD units for the 197 patients with any (early and late) AMD and 433 control subjects. After adjustment for age, gender, and smoking status, no significant association was evident between C. pneumoniae antibody titers and any AMD (OR 1.02; 95% CI 0.66–1.69), when comparing the upper to the lower tertile of the antibody titers. The results were similar, with no association detected, in the separate analyses for prevalent early or late AMD (Table 3).

Additional adjustment for family history of AMD and history of cardiovascular disease did not alter the results (data not shown).

Seroreactivity to C. pneumoniae and Incident Early AMD

The mean age of the 87 patients with incident early AMD (74.7 years old) was similar to the mean age of the control subjects (76.7 years old; Table 2), with about two thirds of the samples being women. The proportions of people who ever smoked were 52% of cases and 45% of controls, whereas only 13% of cases and 9% of controls were current smokers. The mean age of the 22 patients with incident late AMD was 76.3 years; 55% were women and 14% were current smokers.

The mean level of antibodies to C. pneumoniae was 0.43 and 0.35 OD among incident cases of early and late AMD, respectively, compared with 0.42 OD units in the controls.

After adjusting for age, gender, and current smoking, no significant association was evident between C. pneumoniae antibody titer and incident early AMD (OR 1.08, 95% CI 0.68–1.99, comparing the upper to the lower tertile of antibody titer; Table 3). There was a modest increased risk of incident late AMD for the highest tertile of antibody titer (OR 1.85, 95% CI 0.57–6.05), but this was not statistically significant.

Additional adjustment for family history of AMD and history of cardiovascular disease did not alter the results (data not shown).

### DISCUSSION

In this older Australian case–control sample drawn from the population-based BMES, we were unable to find an association between seroreactivity to C. pneumoniae and either the prevalence or incidence of early AMD. Our study sample should have been sufficient to detect associations with OR of 1.7 at a significance level of 0.05 for any (early or late) AMD comparing the upper to the lower tertiles of the antibody titer. These negative findings do not exclude the possibility that an association smaller than 1.7-fold exists between C. pneumoniae antibody and AMD. Our study did not have sufficient power to assess this association for prevalent or incident late AMD cases.

These findings are contrary to those in several previous reports, which demonstrated an association between C. pneumoniae infection and AMD18–20 or AMD progression.21

Although it is possible that findings in these studies represented merely C. pneumoniae colonization of the already damaged macular tissues or even chance findings, it is also possible that the different findings were due to the difference in sample selection across studies. Samples in these previous studies, including the case–control serologic studies on late AMD,18,19

### Table 1. Characteristics of the Tested and Nontreated AMD Cases from the BMES Cross-section II Survey Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Not Tested (n = 152)</th>
<th>Tested (n = 197)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Mean (STD)</td>
<td>77.2 (8.72)</td>
<td>74.8 (7.90)</td>
<td>0.01</td>
</tr>
<tr>
<td>Gender</td>
<td>Male, n (%)</td>
<td>103 (67.8)</td>
<td>117 (59.4)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>49 (32.2)</td>
<td>80 (40.6)</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Nonsmokers, n (%)</td>
<td>141 (92.8)</td>
<td>177 (89.9)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>11 (7.2)</td>
<td>20 (10.1)</td>
<td></td>
</tr>
<tr>
<td>Never smoked, n</td>
<td>82 (53.6)</td>
<td>96 (48.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Ever smoked, n</td>
<td>70 (46.4)</td>
<td>101 (51.3)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Characteristics of the Study Sample by AMD Status and by Comparison with the BMES Cross-section II Survey Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BMES II Subjects Not Included in Analysis</th>
<th>Prevalent AMD</th>
<th>Incident AMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. pneumoniae antibody titer, mean (CI)²</td>
<td>NA</td>
<td>0.43 (0.41–0.46)</td>
<td>0.41 (0.38–0.44)</td>
</tr>
<tr>
<td>Age mean, SD, y</td>
<td>65.2 (9.12)</td>
<td>72.8 (8.10)</td>
<td>74.8 (7.90)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>1629 (56.6)</td>
<td>252 (58.2)</td>
<td>177 (59.4)</td>
</tr>
<tr>
<td>Ever smoked, n (%)</td>
<td>1460 (51.2)</td>
<td>217 (50.1)</td>
<td>101 (51.3)</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>286 (10.0)</td>
<td>48 (11.1)</td>
<td>20 (10.1)</td>
</tr>
</tbody>
</table>

² Means and confidence intervals (CI) of the antibody titers to CPhAR39 expressed in optical density units, adjusted for age, gender, and smoking.
as well as the histologic study that identified this organism in the neovascular membrane from the AMD-affected eyes, were all recruited from clinic-based settings, where patients would more likely be at an active stage of the disease. Our study sample was drawn from a generally healthy older population. Our AMD cases included a considerable number of early AMD cases that may not progress to late AMD and also a certain number of prevalent late AMD cases that had reached the end stage a long time before the study started.

Strengths of the present study include that both cases and controls were drawn from the same population-based sample, detailed photographic grading for AMD with all cases confirmed by a retinal specialist (PM), and the same ELISA testing methodology was used by the same researchers in the laboratory that reported on a positive link between exposure to C. pneumoniae infection in both prevalent AMD and AMD progression.

This study focused mainly on the association between exposure to C. pneumoniae infection and prevalence or incidence of early AMD, which would help to assess whether this organism plays a primary causal role in AMD pathogenesis. However, the incident late AMD cases in this study are of greater interest, since the cases had progressed to late-stage AMD relatively recently. Regrettably, the size of this group provided insufficient power for study.

Also, we did not collect blood samples to measure seroreactivity to C. pneumoniae infection before the onset of the disease. However, seroreactivity to this organism is known to increase rapidly during childhood, with a very slow increase later in life. Given that the BMES participants were aged 49 years or older, when seroreactivity to this organism is relatively stable, we believe that this limitation would not have a major effect on our findings.

The recent finding that the complement pathway genes have a strong association with AMD prevalence strengthens the notion that inflammation plays an important role in the pathogenesis of AMD. The alternative complement pathway is activated mainly by microbial infection, and C. pneumoniae could be a trigger to activate complement activities. Although previous findings supported this hypothesis, our study does not provide support for the involvement of C. pneumoniae in the pathogenesis of early AMD, and although it may play a role in the progression from early to late AMD, we had insufficient study power to test this hypothesis. C. pneumoniae may simply be a commensal organism that resides in damaged tissues without playing a significant pathologic role. Further work is needed to resolve this question.

### References


