Chlamydia infection status, genotype, and age-related macular degeneration

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Purpose: To evaluate whether Chlamydia (C.) infections are associated with age-related macular degeneration (AMD) and to assess if this association is influenced by the complement factor H (CFH) Y402H or the high temperature requirement A serine peptidase 1 (HTRA1) rs11200638 risk genotypes.

Methods: One hundred ninety-nine AMD patients with early and late forms of the disease and 100 unaffected controls, at least 50 years old were included in the study. Patients in the AMD and control groups were selected based on known CFH Y402H variant genotype status (one third homozygous CC, one third heterozygous CT, and one third wild-type TT). Plasma from all patients and controls was tested for C. pneumoniae, C. trachomatis, and C. psittaci IgG seropositivity using a micro-immunofluorescent assay to establish previous infection status. Assays were conducted blind to risk genotypes and the results analyzed using univariate and multivariate (logistic regression) analysis.

Results: IgG seropositivity to C. pneumoniae was most prevalent (69.2%, n=207), followed by C. trachomatis (7.4%, n=22) and C. psittaci (3.3%, n=10). No association was found between each of the three Chlamydia species IgG seropositivity and AMD status or severity (early/late). There was also no significant association between Chlamydia species IgG seropositivity and AMD status or severity, in patients carrying at least one CFH Y402H risk allele (C) or HTRA1 rs11200638 risk allele (A), with univariate or logistic regression analysis.

Conclusions: Chlamydia infection status does not appear to be associated with AMD status or severity. The presence of CFH Y402H and HTRA1 rs11200638 risk genotypes does not alter this negative association.

Age-related macular degeneration (AMD) is the leading cause of severe visual impairment in developed countries [1, 2], affecting approximately 30–50 million people worldwide (World Health Organization, Visual impairment and blindness). Environmental and genetic factors play a role in AMD pathogenesis [3-8]. However, the exact biochemical and cellular processes involved are not fully known. Several reports have described significant associations between complement genes and susceptibility to AMD. The genes include complement factor H (CFH) [9-12], complement 3 (C3) [13], high temperature requirement A serine peptidase 1 (HTRA1) [14], complement factor B (CFB) [15], and complement factor H-related 1+3 (CFHR1+CFHR3) [16,17]. A previous meta-analysis showed a 35% prevalence of the CFH Y402H (rs1061170, T→C) risk allele (C) in AMD. This increased the risk of AMD significantly (odds ratios of 2.5 and 6.3 for the heterozygous CT and homozygous CC genotypes, respectively) with an estimated population risk of 59% [18].

The association of CFH Y402H with AMD is intriguing as the CFH protein is involved in regulating the alternative complement pathway. By binding to C3b, the CFH protein accelerates the decay of the alternative pathway convertase C3bBb, and acts as a cofactor for complement factor I, another C3b inhibitor [19,20]. Activation of the alternative complement pathway is normally triggered by microbes, including the Chlamydia species [21-23]. This suggests that chronic low-grade Chlamydia infection in the presence of abnormal CFH protein production may lead to enhanced alternative complement pathway activation in the retina, therefore increasing an individual’s risk of developing AMD.

The Chlamydiae include three species that can infect humans: Chlamydia (C.) pneumoniae, C. trachomatis, and C. psittaci. Chlamydiae are obligate intracellular parasites, due to their reliance on host metabolism. They are found in the environment as non-active stable small cells known as elementary bodies (EB). These cells are able to bind to and enter host epithelial cells, forming larger intracellular reticulate bodies (RB). The RB then multiply, deriving energy from host metabolic processes, to form a cytoplasmic inclusion. This inclusion can then release new EBs from the host cell to infect other cells. Typically, Chlamydiae remain in the host on a subclinical level on a prolonged basis [24].
C. pneumoniae causes respiratory tract infections in humans, including pneumonia, bronchitis, pharyngitis, and sinusitis. C. pneumoniae is transmitted airborne, human to human. It is extremely prevalent, with 30%–50% of the population carrying C. pneumoniae antibodies worldwide. Only one species of C. pneumoniae has been described. Chronic infection with C. pneumoniae has been associated with AMD and other degenerative diseases (atherosclerosis [25–29], cardiac valvular stenosis [30], Alzheimer disease [31], and multiple sclerosis [32]). The association between C. pneumoniae and AMD is not fully established in the literature. Various studies, including preclinical and clinical studies, have all shown contradictory results (see the summary in Appendix 1) [33-44]. In addition, the association of C. pneumoniae with CFH polymorphisms in AMD has not been consistently replicated [40,42,45].

C. trachomatis can cause a range of diseases in humans, including trachoma, inclusion conjunctivitis, non-gonococcal urethritis, salpingitis, cervicitis, and lymphogranuloma venereum. C. trachomatis is transmitted person to person, including by sexual contact and from mother to baby during delivery. At least 15 antigen-specific species (“serovars”) of C. trachomatis have been described, including B, Ba, C-K, and L1-L3 [24]. The prevalence of C. trachomatis in a general European population aged 15–40 is around 3% [46], but can be up to 17% in young women [47]. C. trachomatis is endemic in poorer countries, where it is a leading cause of blindness through trachoma. Only one study has investigated the association between C. trachomatis and AMD but found no association [33]. No study has examined the association with the CFH genotype and C. trachomatis in AMD.

The natural hosts for C. psittaci are birds, especially parrots and parakeets. C. psittaci can be transmitted via bird excretions to humans, causing a disease known as psittacosis, which primarily causes atypical pneumonia. At least four serovars of C. psittaci have been described [24]. C. psittaci prevalence in the general population is the least common of the Chlamydia species, but is typically more common in bird handlers [48]. No studies have as yet investigated the association between C. psittaci and AMD.

In this study, we investigated the association of all three Chlamydia species (C. pneumoniae, C. trachomatis, and C. psittaci) with the CFH Y402H AMD risk variant in AMD. We also investigated the association of the Chlamydia species with another genetic variant strongly associated with AMD, the HTRA1 gene (rs11200638, G→A) [49]. The function of this gene is not yet known, and has not been previously associated with Chlamydia.

METHODS

Patients were selected from a preexisting database of AMD and control patients. Selection was restricted to those of Caucasian origin, at least 50 years old, and with confirmed genotyping [50]. Recorded height and weight were used to calculate body mass index (BMI). All patients were recruited under UK National Research Ethics Service approval, and had previously given full informed consent.

AMD status was determined according to the Age-Related Eye Disease Study (AREDS) grading system using clinical examination, stereoscopic fundus photographs, and fluorescein angiography (Topcon TRC50IX) [51]. AREDS grades 1–4 corresponded to AMD. AREDS grades 1–3 corresponded to early AMD, and AREDS 4 to late/advanced AMD.

Patients were selected based on AMD and CFH Y402H genotype status. In the AMD and control groups, one third of patients carried the homozygous (CC) risk CFH Y402H genotype, one third the heterozygous (CT) genotype, and the remaining one third the wild-type (TT) genotype. This was done to facilitate comparisons between genotypes. DNA was previously extracted using the salting-out method [52], and stored at −20 °C. CFH Y402H [50] and HTRA1 rs11200638 genotype status were determined using TaqMan allelic discrimination assay probe kits on an Applied Biosystems 3730 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA).

Determining plasma Chlamydia serology: Stored plasma samples were used for Chlamydia serology testing. These samples were previously obtained by collecting 10 ml of peripheral blood in lithium heparin tubes. These were then centrifuged within a few hours at 1,300 xg for 10 min, and the supernatant stored at −80 °C until analysis. Defrosted plasma samples were tested for the presence of Chlamydia IgG antibodies toward C. pneumoniae, C. trachomatis, and C. psittaci using a micro-immunofluorescent (MIF) assay kit (Focus Diagnostics, Cypress, CA). MIF is considered the laboratory gold standard for serological testing [53]. The IgG assay was selected as it indicates any previous Chlamydia infection and although titers do decrease after infection, the rate of decline is minimal.

The MIF assay was a two-stage “sandwich” procedure, using slides enclosed with the kit containing pretreated wells. A single well contained four individual spots. Three of these spots contained EBs of each Chlamydia species (each incorporating one strain of C. pneumoniae, two strains of C. psittaci, and eight serotypes (D–K) of C. trachomatis), diluted in 3% yolk sac (to provide background contrast). The fourth spot was composed of yolk sac alone and acted as a control. Each plasma sample was diluted 1:16 with phosphate buffered saline (PBS 0.01 M, containing sodium chloride 137 mM, phosphate buffer 10 mM, potassium chloride 2.7 mM), and 25 µl added to each well. The slide was covered and incubated at room temperature for 60 min. The slide was then washed with PBS for 10 min to remove unbound plasma antibodies, dipped in distilled water, and then air-dried. Twenty-five µl of fluorescein-labeled anti-IgG antibody was then added to
each well. The slide was covered and re-incubated at room temperature for 30 min. The slide was then washed twice (as before). “Mounting medium” (supplied with the kit) was then added to the slide, and wells viewed using a fluorescence microscope at 400× magnification. Positive reactions appeared green, indicating fluorescent EBs on a background of yolk sac (Figure 1). A positive result was confirmed when the observed fluorescence level in any of the EB-containing spots was greater than that seen in the corresponding control yolk sac, no matter how minimal this difference was. A negative result was confirmed when there was no fluorescence, or the fluorescence equaled that observed in the corresponding yolk sac control spot or in the negative control well. Any samples with positive tests for all three Chlamydia species were retested for verification.

The sensitivity and specificity of this test for detecting C. pneumoniae IgG seropositivity were specified as 62.5% and 99.8%, respectively; the corresponding values for C. trachomatis and C. psittaci were not available (Focus Diagnostics, Chlamydia MIF IgG assay instructions). All assays were performed blind to patient AMD CFH/HTRA1 genotype status.

Statistics: One hundred and ninety-nine patients with early and late AMD, and 100 controls without AMD were selected. Univariate analysis of differences between the AMD and control groups were performed using the \( \chi^2 \) test (and Fisher Exact test when appropriate) for categorical data. Continuous data was analyzed using the Student \( t \) test. Multivariate analysis was performed using binary logistic regression to assess whether covariates (including CFH Y402H/HTRA1 genotype status, Chlamydia species serology status, age, sex, and BMI) could predict AMD status or severity. The level of statistical significance in the study was defined as \( p < 0.05 \). When Chlamydia species were compared, a \( p \) value of <0.0167 was used to determine statistical significance, taking into account the Bonferroni correction for multiple testing. All statistical analyses were performed using SPSS (version 18.0, IBM, New York, NY).

RESULTS

Baseline demographics and prevalence of Chlamydia infection: Baseline demographics are described in Table 1. Compared to controls, patients with AMD were older (78.3 versus 75.6 years, \( p=0.009 \)), with a higher proportion of women (72.9% versus 56%, \( p=0.003 \)). Overall, C. pneumoniae infection was common (69.2%), whereas C. trachomatis and C. psittaci were less prevalent (7.4% and 3.3%, respectively). There was no difference in age between seropositive and seronegative patients, for C. pneumoniae (78.1±8.1 versus 78.6±8.6, \( p=0.688 \)) and C. trachomatis (80.1±6.3 versus 78.1±8.4, \( p=0.344 \)), although patients seropositive for C. psittaci were older (84.0±8.0 versus 78.0±8.2, \( p=0.044 \)). There was also no difference in age between patients with early versus late AMD (Table 2).

Chlamydia and AMD: Univariate analysis demonstrated no statistical association between IgG seropositive status for all three Chlamydia species and AMD status. A subgroup analysis of the AMD group also demonstrated no association between Chlamydia species seropositivity and AMD severity (early or late; Table 2).

Chlamydia and CFH genotype: No association was found between Chlamydia species seropositivity and CFH Y402H polymorphism, either when comparing genotypes (TT versus CT versus CC) or the presence or absence of the risk allele (C; Table 3). There was also no association between Chlamydia species seropositivity and AMD severity in those carrying the CFH Y402H risk allele (C; Table 4).

Chlamydia and HTRA1: We also looked at patients carrying at least one HTRA1 rs11200638 risk allele (A) to explore any association with Chlamydia seropositivity status and AMD. There was no association with Chlamydia species seropositivity and AMD status or severity in this group (Table 5).

Chlamydia, CFH genotype, and AMD: Multivariate logistic regression analysis was then performed to identify covariates that could predict AMD status or severity. C. trachomatis and C. psittaci status was not included as covariates since IgG+ status was of low prevalence overall. When covariates were
restricted to \textit{C. pneumoniae} IgG status (positive versus negative) and \textit{CFH} Y402H genotype (CC versus CT versus TT), \textit{C. pneumoniae} IgG status was not predictive of AMD status (p=0.842) or AMD severity (p=0.078). The outcome was similar when the presence of the \textit{CFH} Y402H risk allele (C: present versus absent) was used as a covariate instead of the \textit{CFH} genotype.

Controlling for multiple variables (\textit{C. pneumoniae} IgG status, age, gender, BMI, \textit{CFH} Y402H genotype, \textit{HTRA1} rs11200638 genotype), logistic regression analysis indicated predictors for AMD status included increased BMI (p=0.015, OR 1.08, 95% CI: 1.01–1.14), increased age (p=0.015, OR 1.04, 95% CI: 1.01–1.08), female gender (p=0.011, OR 2.04, 95% CI: 1.18–3.51), and \textit{HTRA1} rs11200638 homozygous...
Again, these same covariates (BMI, C. pneumoniae seropositivity and AMD status or severity, either independently or when taking into account CFH risk genotypes, in addition to other variables (age, gender, BMI). We then performed logistic regression to predict whether these same covariates (C. pneumoniae IgG status, age, gender, BMI, CFH Y402H genotype, HTRA1 rs11200638 genotype) were predictors of AMD severity. The only positive association in this model was CFH Y402H heterozygous (CT) status, associated with late AMD (p=0.006, OR 3.43, 95% CI 1.42–8.27).

Table 4. Chlamydia species seropositivity status and AMD status/severity in CFH Y402H C allele carriers

| Chlamydia IgG +ve status | Control (n=67) | AMD (n=133) | p value (control versus AMD) | Early AMD (n=30) | Late AMD (n=103) | p value (early versus late AMD) |
|--------------------------|---------------|-------------|-----------------------------|---------------|----------------|-------------------------------|
| C. pneumoniae +ve (%, n) | 70.1% (47)    | 72.2% (96)  | 0.724                       | 60.0% (18)    | 75.7% (78)    | 0.091                         |
| C. trachomatis +ve (%, n) | 6.0% (4)      | 7.5% (10)   | 0.686*                     | 6.7% (2)      | 7.8% (8)      | 1.000*                        |
| C. psittaci +ve (%, n)   | 1.5% (1)      | 5.3% (7)    | 0.272*                     | 13.3% (4)     | 2.9% (3)      | 0.045*                        |

AMD=Age-related macular degeneration, CFH=Complement Factor H gene, C.=Chlamydia NB: \( \chi^2 \) test performed for all comparisons, except for those indicated by * (Fisher test used). P values for Chlamydia species seropositivity associations are uncorrected.

Table 5. Chlamydia species seropositivity status and AMD status/severity in HTRA1 rs11200638 A allele carriers

| Chlamydia IgG +ve status | Control (n=43) | AMD (n=122) | p value (control versus AMD) | Early AMD (n=31) | Late AMD (n=91) | p value (early versus late AMD) |
|--------------------------|---------------|-------------|-----------------------------|---------------|----------------|-------------------------------|
| C. pneumoniae +ve (%, n) | 72.1% (31)    | 69.7% (85)  | 0.765                       | 67.8% (21)    | 70.3% (64)    | 0.787                         |
| C. trachomatis +ve (%, n) | 7.0% (3 )    | 11.5% (14 ) | 0.563*                     | 12.9% (4)     | 11.0% (10)    | 0.751*                        |
| C. psittaci +ve (%, n)   | 0% (0)        | 3.3% (4)    | 0.574*                     | 9.7% (3)      | 1.1% (1)      | 0.050*                        |

C. pneumoniae IgG+ve status (p=0.768) was not a predictor of AMD status.

DISCUSSION

In this study, there was no association with Chlamydia species seropositivity and AMD status or severity, either independently or when taking into account CFH and HTRA1 risk genotypes, in addition to other variables (age, gender, BMI).

Only one previous study has reported a similar retrospective case-control study to ours, examining the association of C. pneumoniae status with CFH genotype on AMD status. Shen et al. looked at 148 AMD patients and 162 controls, and found an association between C. pneumoniae and AMD (OR 2.17, p=0.017), but not when a CFH risk genetic variant was taken into account. However, important differences exist between this study and ours. AMD patients were included if they had late AMD only (geographic atrophy/CNV). C. pneumoniae status was determined with PCR to identify C. pneumonia DNA in peripheral blood cells, and the rs380390 CFH single nucleotide polymorphism was selected as the risk CFH genotype. Furthermore, there was no comparison between AMD subtypes based on severity [42]. Haas et al. also performed a case-control study with 75 patients with AMD (any stage) and 75 controls, using enzyme-linked immunosorbent assay (ELISA) to determine C. pneumoniae seropositivity status. They found no association between C. pneumoniae seropositivity and AMD, or between C. pneumoniae and the CFH Y402H genotype. This study did not perform further analysis of C. pneumoniae together with CFH genotype status in AMD due to insufficient numbers [41]. Baird et al. performed a prospective study following up 233 patients over a mean of seven years. The authors found patients were more likely to progress if serum C. pneumoniae IgG levels, as measured with ELISA, were at the upper tertile (42.5%) compared to the lower tertile (20.8%). They also found an additive risk of AMD progression in patients with the CFH Y402H risk allele (C) and the upper tertile of C. pneumoniae titers (OR 11.8, p=0.005) compared to those with T allele and lowest C. pneumoniae titer [40].

Far less has been reported on the association of the other two Chlamydia species and AMD, which may be a reflection of the lower prevalence in the developed world. Kayalagu et al. performed a small case-control study looking at serum levels of C. trachomatis heat shock protein in 25 patients with AMD and 13 controls, and found no association [33]. Our study reached the same conclusions with a comparatively larger sample size, and additionally found no association with...
AMD risk genotype. No studies have investigated the association of C. psittaci and AMD. Although our study found no association between C. psittaci seropositivity and AMD, the low prevalence highlights the difficulty of studying this microorganism.

The prevalence of previous Chlamydia infection in this study group was higher for all three species compared to published data. This finding may reflect false positives from the MIF assay, or may indicate Chlamydia species prevalence rates are population-specific. PCR does have a higher specificity and sensitivity as a test for Chlamydia species, but was not chosen due to its reliance on the presence of Chlamydia DNA in the peripheral blood. Serological tests enable any previous infection to be detected, which was our aim. Serological techniques include complement fixation, indirect immunofluorescent assays, and ELISAs. The MIF test was chosen since it is considered the “gold standard” of Chlamydia serology testing [54]. Drawbacks of this method include the dependence on the binding antigen used, as well as variability in the experience and subjectivity of the person reading and interpreting the results [55]. The use of purified EBs to detect species and serovar-specific Chlamydia antibodies means cross-reactivity does not happen often with MIF but can occur especially between C. psittaci and C. pneumonia due to certain antigenic similarities [24,56].

In summary, Chlamydia infection status did not appear to be associated with AMD status or with severity in this study. The presence of CFH Y402H or HTRA1 risk genotypes did not alter this negative association. The negative association reported here is consistent with some previous reports but ideally should be replicated in a larger AMD cohort.

ACKNOWLEDGMENTS

This work was funded by the American Health Assistance Foundation (AHAF), T.F.C. Frost Charitable Trust and the Gift of Sight Appeal.

REFERENCES

1. Congdon N, O’Colmain B, Klaver CC, Klein R, Munoz B, Friedman DS, Kempen J, Taylor HR, Mitchell P. Causes and prevalence of visual impairment among adults in the United States. Arch Ophthalmol 2004; 122:477-85. [PMID: 15078664]
2. Foran S, Wang JJ, Mitchell P. Causes of incident visual impairment: the Blue Mountains Eye Study. Arch Ophthalmol 2002; 120:613-9. [PMID: 12003611]
3. Vingerling JR, Dielemans I, Hofman A, Grobbee DE, Hjmering M, Kramer CF, de Jong PT. The prevalence of age-related maculopathy in the Rotterdam Study. Ophthalmology 1995; 102:205-10. [PMID: 7862408] [see comment]
4. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. Ophthalmology 1995; 102:1450-60. [PMID: 9097791] [Review 26 refs]
5. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy. The Beaver Dam Eye Study. Ophthalmology 1992; 99:933-43. [PMID: 1630784]
6. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. Ophthalmology 2007; 114:253-62. [PMID: 17270675]
7. Klein R, Peto T, Bird A, Vannewkirk MR. The epidemiology of age-related macular degeneration. Am J Ophthalmol 2004; 137:486-95. [PMID: 15013873]
8. Klein R, Knudtson MD, Cruickshanks KJ, Klein BE. Further observations on the association between smoking and the long-term incidence and progression of age-related macular degeneration: the Beaver Dam Eye Study. Arch Ophthalmol 2008; 126:115-21. [PMID: 18195228]
9. Klein RJ, Zeiss C, Chew HY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science 2005; 308:385-9. [PMID: 15761122]
10. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noursredine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. Science 2005; 308:419-21. [PMID: 15761120]
11. Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farris LA. Complement factor H polymorphism and age-related macular degeneration. Science 2005; 308:421-4. [PMID: 15761121]
12. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005; 102:7227-32. [PMID: 15870199]
13. Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, Clayton DG, Hayward C, Morgan J, Wright AF, Armbrucht AM, Dhillon B, Deary IJ, Redmond E, Bird AC, Moore AT. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med 2007; 357:553-61. [PMID: 17634448]
14. Yang Z, Camp NJ, Sun EY, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howe K, Zhang K. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Nat Engl J Med 2007; 357:553-61. [PMID: 17634448]
15. Moore AT. Complement C3 variant and the risk of age-related macular degeneration. Science 2005; 308:421-4. [PMID: 15761120]
16. Hageman GS, Hancox LS, Taiber AJ, Gehrs KM, Anderson DH, Johnson LV, Radeke MJ, Kavanagh D, Richards A, Atkinson J, Meri S, Bergeron J, Zernant J, Merriam J, Gold
B, Allikmets R, Dean M. Extended haplotypes in the complement factor H (CFH) and CFH-related (CFHR) family of genes protect against age-related macular degeneration: characterization, ethnic distribution and evolutionary implications. Ann Med 2006; 38:592-604. [PMID: 17438673]

Hughes AE, Orr N, Esfandiary H, Diaz-Torres M, Goodship T, Chakravartthy U. A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. Nat Genet 2006; 38:1173-7. [PMID: 16998489]

Thakkinstian A, Han P, McEvoy M, Smith W, Hoh J, Magnusson K, Zhang K, Atit J. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. Hum Mol Genet 2006; 15:2784-90. [PMID: 16905558]

Ault BH. Factor H and the pathogenesis of renal diseases. Pediatr Nephrol 2000; 14:1045-53. [PMID: 10975323]

Müller-Eberhard HJ, Schreiber RD. Molecular biology and chemistry of the alternative pathway of complement. Adv Immunol 1980; 29:1-53. [PMID: 6158260]

Cortes C, Ferreira VP, Pangburn MK. Native properdin binds to Chlamydia pneumoniae and promotes complement activation. Infect Immun 2011; 79:724-31. [PMID: 21134964]

Hall RT, Strugnell T, Wu X, Devine DV, Stiver HG. Characterization of kinetics and target proteins for binding of human complement component C3 to the surface-exposed outer membrane of Chlamydia trachomatis serovar L2. Infect Immun 1993; 61:1829-34. [PMID: 8478073]

Lin JS, Yan LL, Ho Y, Rice PA. Early complement components enhance neutralization of Chlamydia trachomatis infectivity by human sera. Infect Immun 1992; 60:2547-50. [PMID: 1587622]

Brooks GF, Jawetz, Melnick, and Adelberg-Æs Medical Microbiology (24th Edition). Blacklick, OH: McGraw-Hill Publishing Division; 2007.

Saikku P, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Makela PH, Huttunen JK, Valtonen V. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. Lancet 1988; 2:983-6. [PMID: 2902492]

Thom DH, Grayston JT, Siscovick DS, Wang SP, Weiss NS, Daling JR. Association of prior infection with Chlamydia pneumoniae and angiographically demonstrated coronary artery disease. JAMA 1992; 268:68-72. [PMID: 1608116]

Linnamäki E, Leinonen M, Mattila K, Nieminen MS, Valtonen V, Saikku P. Chlamydia pneumoniae-specific circulating immune complexes in patients with chronic coronary heart disease. Circulation 1993; 87:1130-4. [PMID: 8484830]

Shor A, Kuo CC, Patton DL. Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. South African Medical Journal Suid-Afrikaanse Tydskrif Vir Geneeskunde 1992; 82:158-61. [PMID: 1519134]

Kuo CC, Gown AM, Benditt EP, Grayston JT. Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. Arterioscler Thromb 1993; 13:1501-4. [PMID: 7691166]
age-related macular degeneration. Eye (Lond) 2009; 23:2228-32. [PMID: 19169230]

42. Shen D, Tuo J, Patel M, Herzlich AA, Ding X, Chew EY, Chan CC. Chlamydia pneumoniae infection, complement factor H variants and age-related macular degeneration. Br J Ophthalmol 2009; 93:405-8. [PMID: 18996904]

43. Enzmann V, Hess R, Jordi F, Wolf S, Wolf-Schnurrbusch UEK. Detection of Chlamydia Pneumoniae and Factors of the Complement System in Neovascular Membranes of Patients With Age-Related Macular Degeneration. ARVO Annual Meeting; 2010 May 2–6; Fort Lauderdale (FL).

44. Fujimoto T, Sonoda KH, Hijioka K, Sato K, Takeda A, Hasegawa E, Sato K, Takeda A, Hasegawa E, Oshima Y, Ishibashi T. Choroidal neovascularization enhanced by Chlamydia pneumoniae via Toll-like receptor 2 in the retinal pigment epithelium. Invest Ophthalmol Vis Sci 2010; 51:4694-702. [PMID: 20393111]

45. Haas P. Chlamydia Pneumoniae Infection and Complement Factor H Gene Polymorphism in Age-Related Macular Degeneration - Is There an Association? ARVO Annual Meeting; 2008 April 27-May 1; Fort Lauderdale (FL).

46. Wilson JS, Honey E, Templeton A, Paavonen J, Mårdh PA, Stray-Pedersen B. EU Biomed Concerted Action Group. A systematic review of the prevalence of Chlamydia trachomatis among European women. Hum Reprod Update 2002; 8:385-94. [PMID: 12206472]

47. Harkinezhad T, Vernimmen K, De BM, Rietzschel E, Bekae S, Vanrompay D. Prevalence of Chlamydia psittaci infections in a human population in contact with domestic and companion birds. J Med Microbiol 2009; 58:1207-12. [PMID: 19528151]

49. Tong Y, Liao J, Zhang Y, Zhou J, Zhang H, Mao M. LOC387715/HTRA1 gene polymorphisms and susceptibility to age-related macular degeneration: A HuGE review and meta-analysis. Mol Vis 2010; 16:1958-81. [PMID: 21031019]

50. Goverdhan SV, Hannon S, Newsom RB, Luff AJ, Griffiths H, Lotery AJ. An analysis of the CFH Y402H genotype in AMD patients and controls from the UK, and response to PDT treatment. Eye (Lond) 2008; 22:849-54. [PMID: 17464302]

51. Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report Number 6. Am J Ophthalmol 2001; 132:668-81. [PMID: 11704028]

52. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215. [PMID: 3344216]

53. Dowell SF, Peeling RW, Boman J, Carlone GM, Fields BS, Guarnier J, Hammerschlag MR, Jackson LA, Kuo CC, Maass M, Messmer TO, Talkington DF, Tondella ML, Zaki SR. Workshop Participants Cp. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). Clin Infect Dis 2001; 33:492-503. [PMID: 11462186]

54. Dowell SF, Peeling RW, Boman J, Carlone GM, Fields BS, Guarnier J, Hammerschlag MR, Jackson LA, Kuo CC, Maass M, Messmer TO, Talkington DF, Tondella ML, Zaki SR. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). Clin Infect Dis 2001; 33:492-503. [PMID: 11462186]

55. Persson K, Boman J. Comparison of five serologic tests for diagnosis of acute infections by Chlamydia pneumoniae. Clin Diagn Lab Immunol 2000; 7:739-44. [PMID: 10973447]

56. Villegas E, Sorlozano A, Gutierrez J. Serological diagnosis of Chlamydia pneumoniae infection: limitations and perspectives. J Med Microbiol 2010; 59:1267-74. [PMID: 20724512]
Appendix 1. Association of *C. pneumoniae* with AMD - summary of results from previously published studies.

To access the data, click or select the words “Appendix 1.” This will initiate the download of a compressed (pdf) archive that contains the file. Abbreviations: Early AMD: AMD excluding geographic atrophy (GA)/choroidal neovascularisation (CNV), Advanced/late AMD: Presence of GA/CNV, Wet AMD: Presence/past history of macular CNV, Dry AMD: Presence of macular drusen/pigmentary change/atrophy, OR: Odds Ratio, ELISA: Enzyme-Linked Immunosorbent Assay, IgG: Immunoglobulin G, IHC: Immunochemistry, N/A: Not Applicable, PCR: Polymerase Chain Reaction.