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Complement factor H gene polymorphisms and Chlamydia pneumoniae infection in age-related macular degeneration

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Abstract

Purpose—To investigate the association of the *complement factor H gene (CFH)* Y402H polymorphism and age-related macular degeneration (AMD) in the Austrian population (Caucasoid descent), and to determine whether there is an association between exposure to *Chlamydia pneumoniae*—responsible for up to 20% of community-acquired pneumoniae—and the AMD-associated *CFH* risk polymorphism.

Methods—Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism analysis in 75 unrelated AMD patients and compared with 75 healthy, age-matched control subjects. *C. pneumoniae* serum IgG was tested by ELISA (R&D) in both groups. The association between the *CFH* Y402H genetic polymorphism and the disease was examined by χ^2-test and logistic regression.

Results—*CFH Y402H genotype* frequencies differed significantly between AMD patients and healthy controls (1277 TT, 22.7%; 1277 TC, 53.3%; and 1277 CC, 22.7% in the AMD group; 1277 TT, 48.0%; 1277 TC, 38.7%; and 1277 CC, 13.3% in the control group) showing a *P*-value <0.005 (OR:2.920/3.811).

No association was found between a positive *C. pneumoniae* titre and AMD ($P = 0.192$), nor was any association found between *C. pneumoniae* and the *CFH* Y402H polymorphism.

Conclusions—Our data confirm that the *CFH* Y402H polymorphism is a risk factor for AMD in the Austrian population with a higher frequency of the Y402 polymorphism in AMD patients. No association between preceding *C. pneumoniae* infection and diagnosed AMD was found.

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Keywords

age-related macular degeneration; complement factor H; polymorphism; *chlamydia pneumoniae*; infection; aetiology

Introduction

Age-related macular degeneration (AMD) is a progressive degenerative disorder of the central macular region of the retina. It is the leading cause of legal blindness in people greater than 55 years at age in developed countries.[1]

The aetiology of AMD is complex. Besides genetic factors, ischaemia, oxidative stress, aging, and inflammation are the proposed aetiologic factors.[2] Candidate gene screening, positional cloning, and full genome-wide scans have defined the association of some gene variants with AMD, major associations including *complement factor H (CFH)*,[3–9] *LOC387715*,[10] and small associations including *ABCA4*,[11] *APOE*,[12] *FBLN5*,[13] *ELOVL4*,[14] and *TLR4*.[15] In contrast, variants in the complement factor B (CFB) gene and in the complement component 2 (C2) gene have been shown to confer a significantly reduced risk of AMD.[16]

The CFH is an inhibitor of the alternative complement activation pathway. The alternative complement pathway is activated by a trigger, which is often of a microbial nature. Different infectious agents are currently implicated in AMD aetiology models.[17,18] One of them is *Chlamydia pneumoniae*, responsible for up to 20% of community-acquired pneumoniae.[19–20] This potential activator has previously been linked to AMD in a few studies, although other studies where unable to identify an association.[21–26]

In this study we determined:

1. The frequency of the *CFH Y402H polymorphism* in patients with AMD in the Austrian population of Caucasian descent; and
2. The association between exposure to *C. pneumoniae* and risk of AMD in patients with the CFH polymorphism.

Materials and methods

Study design: case–control study

A total of 150 patients were recruited from the Department of Ophthalmology at our Rudolf Foundation Hospital (75 unrelated patients with AMD and 75 control subjects (patients assigning for cataract surgery with a good visible fundus) aged 55 years and above). All participants were of Caucasian origin. They lived in the same geographical area of Austria and were seen at the local Department of Ophthalmology. Written informed consent was obtained before enrolment. The study was performed in accordance with the Austrian Gene Technology Act, the tenets of the Declaration of Helsinki, and the guidelines of the local ethics committee.

All AMD patients enrolled in this study were classified according to the Age-Related Eye Disease Study system (drusen, geographic atrophy, predominantly classic choroidal neovascularisation, minimal classic choroidal neovascularisation, occult choroidal neovascularisation, and retinal angiomatous proliferation).[27] AMD was diagnosed by ophthalmoscopic fundus examination, optical coherence tomography, and fluorescein/indocyanine angiography. Excluded from the study were patients with hereditary diseases, polypoidal choroidal vasculopathy, or secondary CNV due to pathologic myopia (>−2 D, spherical equivalent), angioid streaks, inflammatory or infectious chorioretinal disease, trauma, or diabetic retinopathy.

The age- and sex-matched control group had a thorough eye examination with a detailed fundus examination. Exclusion criteria for controls were evidence of any stage of age-related maculopathy, macular haemorrhages of any cause, or media opacities resulting in impaired visualisation of the macula—cataract grades 3 and 4 according to the Lens Opacities Classification System III (LOCS III).[28]

Genotyping

Genotyping was carried out in a random order by an experienced technician who was masked to the disease status of the samples. An aliquot of 5 ml venous blood from each subject was withdrawn and collected in an EDTA-containing tube. Genomic DNA was isolated from whole blood using a commercial kit (QIA-AMP DNA blood mini kit, Qiagen, Vienna, Austria). The single-nucleotide polymorphism (Y402H; rs 1061170) located in exon 9 of CFH was genotyped by PCR-directed sequencing using the following primer sequences: 5′-CTT TAG TTC GTC TTC AGT TAT AC-3′ (forward) and 5′-GTC ATC TAT GTT ACT TAG AAA GT-3′ (reverse). PCR products were then purified and sequenced by Single-Read Sequencing reaction (VBC-Biotech, Vienna/Austria).

C. pneumoniae

Peripheral venous blood samples were collected according to the standard hospital procedure. Red-topped tubes were centrifuged at 3000 r/min for 10 min at 4°C, and then the serum was separated and stored at − 70°C.

Plasma samples were analysed with an enzyme-linked immunosorbent assay to determine IgG antibody titres to the elementary bodies from *C. pneumoniae* using a quantitative sandwich enzyme immunoassay technique according to the manufacturer's guidelines (R&D Systems Inc., 614 McKinley Place, Minneapolis, MN 55413, USA).

Statistics

The statistical analysis was performed using R 2.4.0 and SAS 9.1. All *P*-values <0.008333 were considered statistically significant. The critical boundary of 0.008333 results from the correction for multiplicity according to Bonferroni assessment due to the number of tests (six tests were performed, 0.05/6 = 0.008333).

Continuous variables were analysed by *t*-test and presented as mean±SD. Categorial variables were presented as percentages and were compared by χ^2-test. A stepwise binary

logistic regression analysis was performed to assess the influence of *C. pneumoniae* and CFH genotypes on AMD risk. Odds ratios (ORs) estimated from logistic regression were reported with corresponding 95% confidence intervals (95% CI). Where there was no statistical significant effect, a *t*-test was performed.

Results

A total of 150 patients were included in this study (75 unrelated AMD patients compared with 75 healthy controls). Age and gender were similar in both groups. The AMD group consisted of 41 (54.6%) female and 34 (45.3%) male patients, and the mean age was 77.0±7.3 years (range: 56–92). The control group consisted of 37 (49.3%) female and 38 (50.6%) male subjects, and the mean age was 76.5±6.5 years (range: 62–94).

Genotypes were successfully determined in all participants. The prevalence of the *CFH polymorphism* was significantly higher in patients with AMD than in the control group ($P<0.05/P = 0.0054$).

Table 1 shows the genotype distribution in both groups (AMD and healthy controls).

An OR of 2.92 (95% CI: 1.381–6.177) for AMD patients was found among carriers of the CFH 402YH genotype, whereas homozygosity for the CFH polymorphism showed an OR of 3.811 (95% CI: 1.453–9.997).

Neither age ($P = 0.9140$) nor sex ($P = 0.1724$) showed any statistically significant difference on the polymorphism. Men had a higher tendency of having a risk polymorphism than women.

The genotype distribution according to the form of AMD (dry/wet) showed no statistically significant difference ($P = 0.5075$; Table 2).

Genotype distribution according to the angiographic type of CNV is shown in Table 3.

No statistically significant difference ($P = 0.3781$) between the individual groups of AMD could be shown. In eyes with occult CNV, homozygosity and heterozygosity were found to be nearly equally. Homozygosity was more prevalent in eyes with occult CNV compared to those with RAP and predominantly classic and minimal classic CNVs.

C. pneumoniae serology

Serological data were available from all 75 AMD patients and 75 healthy controls. Regarding positive *C. pneumoniae*-specific IgG, there was no statistical significance between the two groups ($P = 0.192$), Figure 1. The control group even showed slightly higher incidence of *C. pneumoniae* IgG-positive samples.

Distribution of *C. pneumoniae*-specific IgG

Out of 69 patients and controls with heterozygosity 53 were positive and 16 were negative. Out of 28 patients and controls with homozygosity 22 were positive for *C. pneumoniae*-

specific IgG and 6 negative. Out of 53 patients and controls with normal genotype 43 were positive for *C. pneumoniae*-specific IgG and 10 were negative.

Looking only on patients and controls with a *C. pneumoniae*-specific IgG, following distribution was registered: in AMD patients, 32 were heterozygote, 13 were homozygote, and 14 had a normal genotype, whereas in the healthy control group, 21 were heterozygote, 9 were homozygote, and 29 had a normal genotype.

The study population was too small to make serious conclusions, and therefore, further statistical analysis for the CFH polymorphism and *C. pneumoniae* were not performed because of the lack of significance.

Discussion

The *CFH Y402 gene polymorphism* is a major risk factor for AMD. Several studies have confirmed an association of the *CFH* gene, an inhibitor of the alternative complement activation pathway to the risk of AMD.

In CFH, the non-synonymous variant of exon 9 c. 1279 (T→C) results in a tyrosine-to-histidine change at codon 402.[3–9]

This study was set up to investigate two major aspects in the aetiology of developing AMD.

The primary aim was to investigate the prevalence of the *CFH Y402 polymorphism* in patients with AMD compared with a healthy control group in the Austrian population.

Here, we confirm the higher frequency of the *Y402 polymorphism* of CFH in AMD patients. The observed ORs were similar to those previously reported for North American and European populations.

The analysis of the genotype distribution among different subtypes of AMD showed no statistical difference. Interestingly, in eyes with occult CNV, homozygosity and heterozygosity were found nearly equally. Homozygosity was more prevalent in eyes with occult CNV in comparison to those with RAP, predominantly classic and minimal classic CNVs. Subgroup analysis by Wegscheider and colleagues[29] showed similar results, suggesting that the CFH Y402 polymorphism to be a major risk factor for all types of exudative AMD, but homozygosity was more prevalent in predominantly classic CNV than in other forms of CNV.

The secondary aim of this study was to examine an association between exposure to *C. pneumoniae* and risk of AMD in patients with CFH polymorphism.

CFH is an inhibitor of the alternative pathway of the complement activation. Some epidemiological studies have found associations between the risk of AMD and serum levels of C-reactive protein (CRP), a systemic marker of inflammation.[30]

The complement system must be activated. By far, the most common triggers of the alternative pathway are microbes. Therefore, certain microbial infections have been postulated to be triggers for AMD.[7]

C. pneumoniae has been considered to be a trigger for the complement activation in AMD.[17,18]

C. pneumoniae is an obligate intracellular pathogen responsible for up to 20 percent of cases of community-aquired pneumoniae. Most infections are mild or asymptomatic. The organism is thought to be disseminated through the bloodstream and has a particular attraction for vascular tissue, where it may establish a chronic infection. As there is evidence that chronic inflammation may be involved in the development of AMD,[19,20] a few studies to date have linked *C. pneumoniae* to AMD; however, the outcomes have been very differing. Two studies showed serological association between *C. pneumoniae* infection and AMD,[26,31] but consisted only of a small number of patients. *C. pneumoniae* has also been identified in surgically excised subretinal neovascular membranes,[24] and AMD progression has been linked to exposure to *C. pneumoniae*.[25] In contrast, two other studies showed no association between *C. pneumoniae* and AMD, neither could *C. pneumoniae* be identified in surgically excised subretinal neovascular membranes[23] nor in a recent serological study.[21]

Regarding a positive *C. pneumoniae*-specific IgG, our study shows no statistical significance between AMD patients and healthy controls ($P = 0.192$), Figure 1.

Our data do not support the hypothesis of the involvement of *C. pneumoniae* in the pathogenesis of AMD.

In conclusion, we confirm the increased risk for developing AMD in individuals carrying the His402 allele in the Austrian population. An association between *C. pneumoniae* and the risk of developing AMD could not be confirmed.

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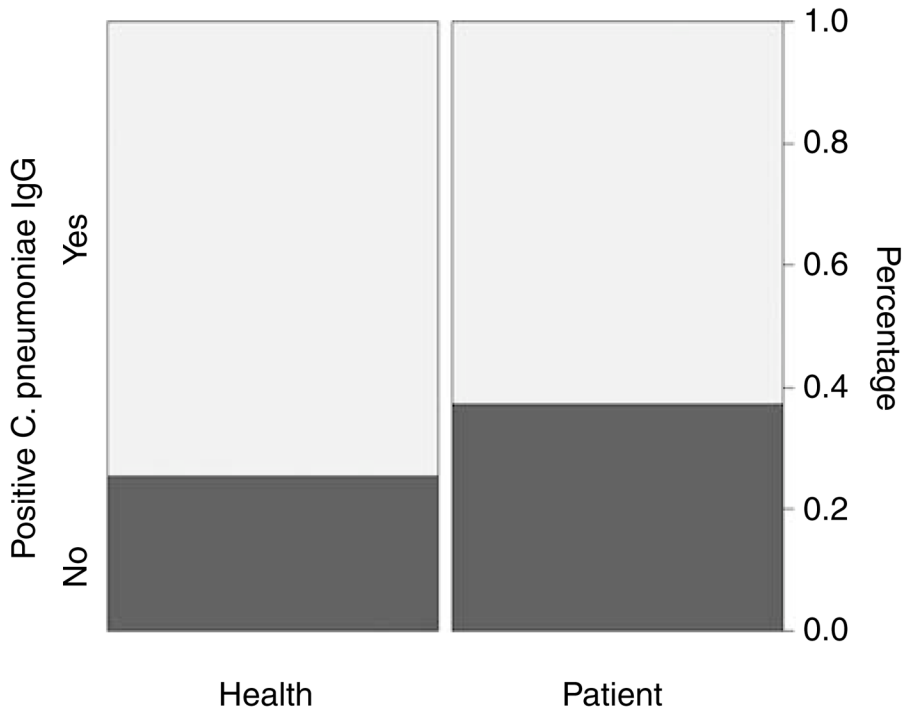

Figure 1.
Chlamydia pneumoniae IgG in AMD patients and healthy controls.

Table 1

Genotype distribution in both groups

Complement factor H Y402H genotype	Patients with AMD (*n* = 75)	Controls (*n* =75)	*P*-value
TT	22.7% (*n* = 17)	48.0% (*n* =36)	—
TC	53.3% (*n* = 40)	38.7% (*n* =29)	0.005
CC	24.0% (*n* = 18)	13.3% (*n* =10)	0.0066

AMD = age-related macular degeneration.

Table 2

Genotype distribution in non-exudative and exudative AMD patients

	Hetero	Homo	Normal
Dry	5	1	3
Wet	35	17	14

AMD = age-related macular degeneration.

Table 3

Genotype distribution in different subtypes of AMD

AMD	CFH polymorphism		
	Normal	Hetero	Homo
Drusen	2 (28.6%YY)	4 (57.1%YH)	1 (14.3%HH)
GA	1 (50.0%YY)	1 (50.0%YH)	0 (0%HH)
Predominantely classic CNV	2 (12.5%YY)	10 (62.5%YH)	4 (25.0%HH)
Minimally classic CNV	3 (27.3%YY)	7 (63.3%YH)	1 (9.0%HH)
Occult CNV	6 (19.4%YY)	13 (41.9%YH)	12 (38.7%HH)
RAP	3 (37.5%YY)	5 (62.5%YH)	0 (0%HH)

CNV = choroidal neovascularisation; GA = geographic atrophy; RAP = retinal angiomatous proliferation.