Y402H Complement factor H polymorphism associated with exudative age-related macular degeneration in the French population

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Purpose: Identification of genetic factors for age-related macular degeneration (AMD) is of crucial importance in this common cause of blindness. A positive association between Y402H polymorphism of the complement factor H (CFH) gene and AMD has been recently reported in North American populations but not yet in European populations. The exudative form of AMD is rapidly progressive and usually associated with a severe prognosis. Our purpose was to investigate this association in a French population specifically affected with exudative AMD, in a case-control study.

Methods: Two series of unrelated exudative AMD patients, sporadic cases (n=60, mean age 74.9±5.7) and familial cases (n=81, mean age 74.0±9.4) were compared with healthy controls (n=91, mean age 74.6±6.3). The coding region of exon 9 of CFH was examined for the Y402H polymorphism by PCR-direct sequencing.

Results: The 1,279-C allele frequencies were significantly higher in exudative AMD patients than controls (0.564 compared to 0.302; p<0.0001). Genotypic distribution of the Y402H polymorphism was significantly different between sporadic cases compared to controls (χ^2=14.48 with 2 df, p<0.0007) and between familial cases compared to controls (χ^2=23.78 with 2 df, p<0.0001). The odds ratio (OR) for exudative AMD was 3.00 CI95% (1.60-5.62) for heterozygotes (CT) and 6.93 CI95% (3.11-15.46) for homozygotes (CC).

Conclusions: These results suggest the contribution of the Y402H polymorphism of the CFH gene to exudative AMD susceptibility also in the French population. This relationship with the CFH may lead to early detection and new strategies for prevention and treatment of AMD.

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the elderly population in Europe and the United States [1]. Two subgroups of AMD are classically distinguished: atrophic and exudative. The atrophic phenotype, the most common form, typically harbors a slow progressive course. In contrast, exudative AMD is certainly the most rapidly progressive form, with sudden loss of central vision (weeks or months) due to leakage or bleeding of choroidal new vessels under the macular area. The identification of risk factors is of major importance for the understanding of the origins of the disorder and for establishing strategies of prevention of AMD. While the etiology of AMD remains unclear, it is generally considered to be a multifactorial disease, with environmental and polygenic components. A genetic predisposition has been suggested, based on familial aggregation and twins studies [2,3]. Because of the extreme phenotypic heterogeneity of AMD, a monogenic model is unlikely in this disorder and thus the research for predisposing polymorphisms is of major importance. Molecular biology studies have demonstrated or suggested a role for

apoE, *ABCA4*, *hemicentin-1*, and *fibulin-5* genes in AMD [4-10]. Furthermore, linkage studies have identified several regions of interest in AMD [11-15].

Recently, three independent teams simultaneously demonstrated a significant association between the Y402H polymorphism of the complement factor H (CFH) gene and AMD in North American subjects [16-18], immediately followed by three other corroborating papers, also in North American populations [19-21]. Hitherto, this association was not yet described in European AMD populations.

Because exudative AMD generally confers a severe prognosis, we decided to focus our study on this AMD subgroup. Our purpose was to investigate this positive association in our French population specifically affected exudative AMD, in a case-control study.

METHODS

Patients: Two independent series of patients affected with exudative AMD were screened. Series F consisted of patients with a positive family history of exudative AMD. Eighty-one unrelated French patients referred to the Eye University Clinic of Creteil for unilateral or bilateral exudative AMD due to any type of choroidal neovascularization (well-defined, occult, or vascularized pigment epithelium detachment) were

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included in this series. Diagnosis of exudative AMD was performed by EHS, GC, or GS in our retinal department. Criteria for inclusion were as follows: (1) age of 55 years or older, (2) diagnosis of exudative AMD in one or both eyes, (3) no association with other retinal disease, and (4) positive family history of AMD (limited to parents, siblings or children) with at least one affected living member EHS questioned each patient about family history of macular degeneration. We subsequently collected clinical information through individual interviews of the affected relative and from the relative's referring ophthalmologist who ascertained diagnosis in relatives. Patients were included only when diagnoses of relatives were confirmed. We selected a homogeneous group of patients with a positive family history of AMD in order to increase the probability of a genetic predisposition and to evaluate the risk in patients with a positive family history. This population was previously reported and analyzed for the purpose of the analysis of the *ABCA4* gene [10,22].

Series S consisted of sporadic cases of AMD. Sixty unrelated French patients referred to the Eye University Clinic of Creteil for unilateral exudative AMD due to any type of choroidal neovascularization were included in this series. Criteria for inclusion were as follows: (1) age over 55 years, (2) diagnosis of exudative AMD in one eye and any type of drusen in fellow eye, (3) no association with other retinal disease, and (4) no positive family history of AMD.

In both series, informed consent was obtained, as required by the French bioethical legislation, in agreement with the Declaration of Helsinki for research involving human subjects. A complete ophthalmological examination including visual acuity measurement, fundus examination and fluorescein angiography (FA), using a Topcon camera (Topcon 50IA, Tokyo, Japan), was performed in both series in our retinal department. For each patient genotyped (series F and S), the diagnosis of exudative AMD was made by EHS, GC, and GS, according to the guidelines of the international classification.

TABLE 1. PHENOTYPE AND GENOTYPE FREQUENCIES

Parameter	Series F (familial)	Series S (sporadic)	Total exudative AMD	Controls
Sample size	81	60	141	91
Male (%)	26 (32.1%)	28 (46.7%)	54 (38.3%)	38 (41.8%)
Age (±SD)	74.0 ± 9.4	74.9 ± 5.7	74.3 ± 8.0	74.6 ± 6.3
Genotypes frequencies				
CC	28 (0.346)	20 (0.333)	48 (0.340)	11 (0.121)
CT	38 (0.469)	25 (0.417)	63 (0.447)	33 (0.363)
TT	15 (0.185)	15 (0.250)	30 (0.213)	47 (0.516)
p value	<0.0001	<0.0007	<0.0001	
Allele frequencies				
C	47 (0.580)	33 (0.542)	80 (0.564)	27 (0.302)
T	34 (0.420)	27 (0.458)	61 (0.436)	64 (0.698)
p value	<0.0001	<0.0001	<0.0001	
Genotype frequencies under HWE				
CC	17.62	27.25	44.85	8.30
CT	29.79	39.46	69.35	38.37
TT	12.59	14.29	26.80	44.33
χ-sq statistic	1.55	0.11	1.18	1.79

Exudative AMD groups are compared to controls. There was no statistical difference between the genotype and allele frequencies in Series F and Series S. The p values are for the comparison of patients to controls within each group. Additionally, the genotype frequencies are estimated under the assumption of a Hardy Weinberg Equilibrium (HWE). The χ^2 statistics are for the comparison of the HWE genotype frequencies to the observed genotype frequencies. No statistical difference is seen.

Controls: Patients were compared to healthy French controls issued from our local department for clinical research (UFRC). The cohort of UFRC was composed of healthy aged-individuals without visual impairment (Table 1). All enrolled individuals underwent a fundus examination of both eyes in order to ascertain the absence of any type of drusen, of geographic atrophy, or exudative AMD. Fundus photographs were performed in all control cases, using a Topcon camera (Topcon 50IA, Tokyo, Japan). Informed consent was obtained, as required by the French bioethical legislation, in agreement with the Declaration of Helsinki for research involving human subjects. DNA of this population was extracted from circulating white blood cells, from venous blood, and collected in our local center for clinical research in agreement with the French legislation. All patients and control groups were Caucasians, recruited in the same geographic area, reducing the possible bias resulting from different ancestral background.

Genomic factor H DNA sequencing: Genomic DNA was amplified using primer sequences and conditions previously described [23]. We genotyped the single-nucleotide polymorphisms (Y402H; rs 1061170) located in exon 9 of *CFH* by PCR-directed sequencing using the following primer sequences: 5'-GAG TGT TTA TTA CAG TAA AAT TTC-3' (forward) and 5'-GAA AAT CAC AGG AGA AAT A-3' (reverse).

PCR products were purified using the multiscreen plates according to the manufacturer's instructions (Millipore, Molsheim, France). Direct DNA sequencing of the purified PCR products was then carried out by the dye terminator cycle sequencing method (Applied Biosystems, Courtaboeuf, France) using a 96 capillary sequencer 3700. Sequence track analysis was performed using the Sequencher software (Applied Biosystems).

Statistical analysis: Hardy-Weinberg equilibrium was tested using a χ^2 test. The whole group of exudative AMD was compared to the control group. Because the Y402H polymorphism could contribute to familial or sporadic AMD, we repeated the analysis separately for patients with or without positive family history. Differences between groups were assessed by analysis of variance (ANOVA) or χ^2 test when appropriate. We estimated odds ratio (OR) with 95% confidence intervals (95% CI) for AMD risk, crude, or after adjusting for age and gender by using a multivariate logistic regression. ORs were calculated separately for the CT and the CC genotypes of the Y402H polymorphism considering TT homozygotes as the reference, but a general test of association was performed. To compare the dominant and codominant models and to select the best-fitting genetic model, we used Akaike Information Criterion (AIC) because the two models were not

			OR for CT heterozygotes (95% CI)	OR for CC homogygotes (95% CI)
Study	AMD	Controls		
Klein [16]	96	50	4.6 (2.0 –11)	7.4 (3.0 –19)
Edwards [17]	400	202	2.7 (1.9 – 3.9)*	
Haines [18]	495	185	2.45 (1.41– 4.25)	3.33 (1.79– 6.20)
Total exudative AMD subgroup	24	24	3.45 (1.72– 6.92)	5.57 (2.52–12.27)
Hageman [19]:				
Cohort 1 (Iowa)	403	131	2.82 (2.11– 3.78)*	
Cohort 2 (Columbia)	549	272	2.25 (1.79– 2.75)*	
Haplotype 1 (including C)	549	272	2.46 (1.95– 3.11)	3.51 (2.13– 5.78)
Zareparsi [20]	616	275	2.44 (2.08– 2.83)	5.93 (4.33– 8.02)
Conley [21]	130	120	3.46 (2.33– 5.13)*	
Exudative atrophic	57	120	4.76 (2.87– 7.91)*	
This study:				
Series F (familial)	81	91	3.54 (1.66– 7.56)	8.08 (3.24–20.14)
Series S (sporadic)	60	91	2.47 (1.12– 5.45)	5.71 (2.23–14.63)
Total	141	91	3.00 (1.60– 5.62)	6.93 (3.11–15.46)

TABLE 2. ODDS RATIO FOR TYR402HIS

This table summarizes the odds ratio for heterozygous or homozygous carriers of the Tyr402His carriers in previous studies and in our current study. Our results are adjusted for age and sex ratio. Asterisk indicates odds ratio for individuals harboring one or both alleles with the polymorphism (C carriers: CC and CT).

nested. The model with the lowest AIC reflects the best balance of goodness-of-fit and parsimony. Statistical interactions between Y402H genotype and covariates were systematically explored. Significance levels were set at p<0.05. Statistical analyses were performed with the SAS software release 8.02 (SAS institute Inc., Cary, NC).

RESULTS

No statistical difference was observed between exudative AMD patients and healthy controls groups for age or gender (Table 1). Mean age was 74.0±9.4 in series F, 74.9±5.7 in series S, 74.3±8.0 in the collective series F and S exudative AMD patients, and 74.6±6.3 in controls. Gender distribution was 26/81 (32.1%) males in series F, 28/60 (46.7%) males in series S, 54/141 (38.3%) males in total exudative AMD patients, and 38/91 (41.8%) males in controls.

The distributions of Y402H genotype and allele frequencies in different groups are shown in Table 1. In all groups, genotype distributions were in Hardy-Weinberg equilibrium and were similar in men and women. Exudative AMD patients had significantly higher C allele frequencies than controls (χ^2=30.5 with 1 df, p<0.0001). Genotypic distribution of the Y402H polymorphism was significantly different between cases and controls (χ^2=26.8 with 2 df, p<0.0001): CC homozygotes (OR 6.8, CI95% 3.0-15.2) and CT heterozygotes (OR 3.0, CI95% 1.6-5.6) were at increased risk of AMD compared with TT homozygotes subjects. Similar results were obtained after adjustment for age and sex (p<0.0001): for CT heterozygotes OR=3.0 (CI95% 1.6-5.6), and for CC homozygotes OR=6.9 (CI95% 3.1-15.6). The AIC of the codominant model (AIC=291.013) was lower than the AIC of the dominant model (AIC=295.534), suggesting that the codominant model was the best-fitting genetic model. No significant interaction between the Y402H polymorphism and gender or age was detected. Genotypic distribution of the Y402H polymorphism was significantly different between familial cases and controls (χ^2=23.78 with 2 df, p<0.0001): CC homozygotes (OR 8.0, CI95% 3.2-19.8), and CT heterozygotes (OR 3.6, CI95% 1.7-7.6) were more frequent among familial cases than among controls. Similar results were obtained for sporadic exudative AMD cases (χ^2=14,48 with 2 df, p<0.0007): CT heterozygotes (OR 2.4, CI95% 1.1-5.2), and CC homozygotes (OR 5.7, CI95% 2.2-14.6) were at increased risk of sporadic AMD. Association with this polymorphism tends to be stronger for unrelated patients with a positive family history of AMD, even if no significant difference was observed between sporadic and familial exudative AMD.

DISCUSSION

In *CFH*, the nonsynonymous variant of exon 9 c.1279 (T->C) results in a tyrosine to histidine change at codon 402. Recently, six North American teams observed a higher risk of AMD for individuals carrying the common Y402H polymorphism (Table 2) [16-21]. Considering the concordance of previous studies for Y402H, we investigated specifically this particular polymorphism.

In this study, we confirm the higher relative frequency of the Y402H polymorphism of *CFH* in two groups of unrelated patients affected with exudative AMD, the most severe form of macular degeneration. Odds ratios are shown in Table 2. It is notable that our results issued from the French population are in concordance with previously reported results issued from North American populations. This could be partially explained by the fact that we included Caucasian individuals. A trend for higher risk in cases with positive family history was noted but was not statistically significant when compared with sporadic cases. From our results we could speculate that for an individual with positive familial history of AMD, being homozygotes (CC) for the Y402H polymorphism approximately confers a eight fold higher risk of being affected with exudative AMD. Prevention of exudative AMD is a major goal in this blinding disorder, and several clinical studies investigated this topic [24]. Thus, detection of individuals at risk could lead to strategies of management and early prevention of the disease.

Our data suggest that the Y402H polymorphism of CFH either predisposes exudative AMD or is in linkage disequilibrium with a predisposing genetic factor in both the sporadic and familial series. One criticism of this case-control association study could be that analysis is limited to one specific polymorphism, which does not investigate linkage disequilibrium [17]. Regarding the previous publications, linkage disequilibrium is likely in this region. However, case-control analysis of single polymorphism is a common approach for polygenic disorders such as AMD [4,6,9,10,20,21]. This positive association suggests that the variant Y402H, or polymorphisms at the *CFH* locus in linkage with the SNP at codon 402, may have an effect on the protein function and may thus play a role in the development of AMD, but it does not prove that the Y402H polymorphism is pathogenic by itself. It, however, raises the question of the relationship between the CFH and AMD.

From an immunological point of view, factor H is a crucial negative regulator of the alternative pathway (AP) of complement activation. Factor H is a 150 kDa serum protein consisting of 20 short consensus repeats (SCRs) each composed of 60 aa. Regions of Factor H responsible for binding glycosaminoglycans such as heparin have previously been mapped to SCRs 7, 12-14, and 20, whereas C-reactive protein (CRP)-binding sites have been localized to SCRs 7-11. The Y402H polymorphism occurs within the seventh SCR domains, which binds heparin and CRP. Therefore, it is likely that this codon change might partially affect the regulation of the inflammatory process in the retina. The impairment of the regulation leads to the excessive liberation of different cleavage fragments such as C3a and C5a, and to the formation of the C5b9 complex which in turn are able to induce endothelial cell activation and release of growth factors such as vascular endothelial growth factor (VEGF) or β-fibroblast growth factor (β-FGF). Interestingly, the implication of C5b9 has been successfully demonstrated in a mouse model of laser-induced choroidal neovascularization [25].

From an ophthalmological point of view, several lines of evidence support a role for inflammation in AMD. Anatomic studies provided initial suggestion for the role of inflammation in choroidal neovascularization (CNV) formation in AMD [26]. Anderson et al. [27] hypothesized that accumulation of neutral fats derived from degenerate retinal pigment epithelium (RPE) cells, sequestered between the RPE basal lamina and Bruch's membrane, constitutes a chronic inflammatory stimulus, and a potential nucleation site for drusen formation. It has been suggested that focal concentration of these materials may produce a powerful chemotactic stimulus for leukocytes, possibly acting via a complement cascade [28]. Immunolocalization studies have shown factor H throughout the choroid, the subRPE space, Bruch membrane and in drusen of the macular area of AMD eyes and abundant transcript for factor H in the RPE and choroid were found by RT-PCR assays [19]. To corroborate the role of inflammation on AMD, it is noteworthy that increased CRP and interleukin-6 serum levels have been observed in AMD patients [29]. Finally, it is notable that antiinflammatory treatments are recently emerging for exudative AMD [30].

In conclusion, we confirm the increase risk for individuals carrying the His402 allele in a European population of exudative AMD patients. Detection of this predisposing gene polymorphism in AMD may lead to early intervention and new strategies for prevention of macular degeneration. Control of inflammation may become an additional goal in treating exudative macular degeneration.

ACKNOWLEDGEMENTS

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