Genotype–Phenotype Correlations for Exudative Age-Related Macular Degeneration Associated with Homozygous *HTRA1* and *CFH* Genotypes

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Purpose. Major genetic factors for age-related macular degeneration (AMD) have recently been identified as susceptibility risk factors, including polymorphisms of *HTRA1* and *CFH* genes. The purpose was to analyze the angiographic features of patients harboring homozygous genotypes for *HTRA1* and *CFH* genes in a French exudative AMD population.

Methods. Two hundred patients affected with exudative AMD were genotyped for the polymorphisms rs11200638 of the *HTRA1* gene and rs10611710 of the *CFH* gene. Four homozygous groups were extracted from the entire cohort: double homozygous for wild-type alleles of both genes (group 1), homozygous for the polymorphism of the *HTRA1* gene only (group 2), homozygous for the polymorphism of the *CFH* gene only (group 3), and double homozygous carriers for both polymorphisms (group 4). Choroidal neovascularization (CNV) was graded as classic and predominantly classic (PC), occult, minimally classic (MC), or retinal angiomatosis proliferation (RAP).

Results. Group 1 ($n = 9$) presented 44.4% classic and PC, 33.3% occult, 11.1% MC, and 11.1% RAP. Group 2 ($n = 12$) presented 50.0% classic and PC, 33.3% occult, no MC CNV and 16.7% RAP. Group 3 ($n = 28$) presented 10.7% classic and PC, 67.9% occult, 14.3% MC, and 7.1% RAP. Group 4 ($n = 17$) presented 29.4% classic and PC, 52.9% occult, 11.8% MC, and 5.9% RAP. Occult CNV or MC CNV was more frequently observed in group 3 than in group 2 (82.1% vs 33.3%; $P < 0.02$). Classic and PC CNV were more frequently observed in group 2 than in group 3 (50% vs. 10.7%; $P < 0.03$).

Conclusions. This attempt at a genotypic–angiographic correlation in an exudative AMD sample suggests an association between occult or MC CNV and the *CFH* polymorphism and between classic and PC CNV and the *HTRA1* polymorphism. (*Invest Ophthalmol Vis Sci.* 2008;49:3090–3094) DOI:10.1167/iovs.07-1540

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the elderly population in Europe and the United States.[1–4] The exudative AMD is the most rapidly progressive form, with sudden loss of central vision due to leakage or bleeding of choroidal new vessels localized beneath the macular area. Exudative AMD is phenotypically heterogeneous, including different subtypes of choroidal neovascularization (CNV), defined by angiographic criteria, such as classic CNV, predominantly classic (PC) CNV, occult CNV (OCNV), minimally classic (MC) CNV, polypoidal choroidal vasculopathy (PCV), or retinal angiomatosis proliferation (RAP).[5,6] Because AMD is a multifactorial disease including numerous genetic and environmental risk factors,[7–18] and with heterogeneous phenotypes, the correlation between any genetic risk factor and phenotype has been difficult to establish to date.[19,20] Furthermore, several genome-wide linkage studies in AMD have been published, and a large number of candidate loci for susceptibility genes have been suggested.[21–30] Recently, two major loci have been described in association with increased risk of AMD, including the *HTRA1/LOC387715* locus at 10q26 and the *CFH* gene at 1q31.[31–42] Our purpose was to analyze the angiographic features of patients with exudative AMD, harboring homozygous status for *CFH* and *HTRA1* polymorphisms.

Methods

Patients

Two hundred consecutive Caucasian patients harboring exudative AMD in one eye were prospectively recruited at the Creteil University Eye Clinic. Exudative AMD was diagnosed by the investigators (JZ, NL, GQ, EHS, GC, and GS) according to the guidelines from the international classification.[43] Inclusion criteria were age more than 55 years and unilateral exudative AMD. Exclusion criteria were (1) presence of other retinal disease (e.g., diabetic retinopathy, high myopia, or retinal dystrophies), (2) atrophic form of AMD, (3) association of atrophic and exudative forms of AMD, (4) CNV in both eyes at presentation, and (5) fibrovascular scarring that did not allow a precise grading of the phenotype. Each patient underwent complete ophthalmic examination including best corrected visual acuity measurement and fundus examination. Fluorescein angiography (FA; model 50IA camera; Topcon, Tokyo, Japan) was performed for each patient. Furthermore, indocyanine angiography (ICG; HRA, Heidelberg, Germany), and optical coherence tomography (OCT; Carl Zeiss Meditec, Inc., Oberkochen, Germany) were performed when judged necessary by investigators. For classification of the initial lesions, grading of exudative AMD was performed on the earliest FA examination available, before any treatment in any case. Grading was performed before

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genetic testing separately by each investigator and classified in the following categories: classic or PC CNV, occult CNV, MC CNV, PCV, or RAP. To check the grading, all angiographies were finally submitted to a senior examiner (EHS or GS) for a grading masked from genetic testing. RAP was defined as an anastomosis connecting the chorioretinal circulation to the retinal circulation, commonly associated with a localized intraretinal hemorrhage and hard exudates surrounding the anastomosis. Diagnosis of RAP was made on the basis of FA. When RAP was diagnosed on FA, ICG and OCT where always performed.[44] Diagnosis of vascularized pigment epithelial detachment (PED) was performed on the basis of FA. Both ICG and OCT were performed each time PED was diagnosed on FA to corroborate the diagnosis. Only vascularized PEDs were considered in these exudative AMD eyes. Classification of PEDs as a separate phenotype would result in too small subgroups, and thus drusenoid PED and other types were not considered.

In addition, a short questionnaire was completed for each patient, including personal and familial history of AMD, tobacco use, and weight and size, to calculate the body mass index (BMI).

Informed consent was obtained, as required by the French bioethical legislation, in agreement with the Declaration of Helsinki for research involving human subjects, and in agreement with our local ethics committee.

Genotyping Methods

Genomic DNA was extracted from blood leukocytes by a nonphenolic solvent method with a DNA isolation kit (Puregene; Gentra Systems, Minneapolis, MN).

Genotyping of the rs11200638 *HTRA1* SNP and the Y402H *CFH* SNP was performed using PCR amplification and direct sequencing. Briefly, we genotyped the SNP (Y402H; rs1061170) located in exon 9 of *CFH* by PCR-directed sequencing, with the following primer sequences: 5′-GAGTGTTTATTACAGTAAAATTTC-3′ (forward) and 5′-GAAAATCACAGGAGAAATA-3′ (reverse), as previously described.[45] We genotyped the rs11200638 SNP using the following primer sequences: 5′-ATGCCACCCACAACAACTTT-3′ (forward) and 5′-CGCGTCCTTCAAACTAATGG-3′ (reverse). PCR conditions have also been previously described.[41] 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 performed by the dye terminator cycle sequencing method (Applied Biosystems [ABI], Courtaboeuf, France), with a 96-capillary sequencer (model 3700; ABI). Sequence track analysis was performed on computer (Sequencher software; ABI).

Heterozygous individuals, submitted to a codominant effect, were excluded from the analysis. Homozygous patients were selected, to determine the phenotype of pure genotypes. From the entire cohort, we extracted patients homozygous for *CFH* and *HTRA1* polymorphisms, defining four different groups.

Group 1 consisted of patients double homozygous for the wild-type (wtwt) allele of the *HTRA1* and *CFH* genes (*CFH* wtwt/*HTRA1* wtwt). Group 2 consisted of patients homozygous for the polymorphism (pp) of the *HTRA1* gene, and homozygous for the wild allele of the *CFH* gene (*CFH* wtwt/*HTRA1* pp). Group 3 consisted of patients homozygous for the polymorphism of the *CFH* gene, and homozygous for the wild-type allele of the *HTRA1* gene (*CFH* pp/*HTRA1* wtwt). Group 4 consisted of patients double homozygous for polymorphisms of both the *HTRA1* and *CFH* genes (*CFH* pp/*HTRA1* pp).

Statistical Analysis

The four groups were compared for different variables: the categorical variables (all binary) were studied with the Fisher exact test or χ^2 test, as appropriate, and the two quantitative variables (age and BMI) were studied with the nonparametric Kruskal-Wallis rank test. Logistic regression models were used to estimate the adjusted odds ratio (OR) with the 95% confidence interval (95% CI). Adjustment variables were age and sex. The Bonferroni correction was applied to multiple comparisons. A difference was said to be significant if it reached $P < 0.05$.

Results

Genotypic statuses for both polymorphisms within the entire initial cohort are presented in Table 1. From the initial cohort of 200 patients with AMD presenting exudative AMD in one eye, 66 patients presented a homozygous status and were included in one of the four groups. It is notable that 8 patients affected with PCV were excluded from the initial cohort. Among the 8 patients with PCV, 2 were observed in group 3, reducing the number of patients from 30 to 28. No PCV was observed in groups 1, 2, or 4. At the diagnosis of CNV, the mean age was 72.8 years (±8.8 SD) in group 1, 71.6 (±7.8) in group 2, 71.1 (±8.0) in group 3, and 69.2 (±7.4) in group 4. Mean age at diagnosis, sex ratio, tobacco use, and BMI did not significantly differ in the four groups (Table 2).

The second grading (senior examiner) was in agreement with the first grading in 54 of 66 cases. In the 12 remaining cases, the final diagnosis was established by EHS and GS together.

Among the 66 patients with exudative AMD, 35 (53.0%) presented occult CNV only, 18 (27.3%) presented classic and PC CNV, 7 (10.6%) presented MC CNV, and 6 (9.1%) presented RAP.

Analysis of CNV revealed different angiographic features in the four groups: Group 1 ($n = 9$) presented three (33.3%) occult CNV, four (44.4%) classic and PC CNV, one (11.1%) MC CNV, and one (11.1%) RAP. Group 2 ($n = 12$) presented four (33.3%) occult CNV, six (50.0%) classic and PC CNV, no MC CNV, and two (16.7%) RAP. Group 3 ($n = 28$) presented 19 (67.9%) occult CNV, 3 (10.7%) classic and PC CNV, 4 (14.3%) MC CNV, and 2 (7.1%) RAP. Group 4 ($n = 17$) presented nine (52.9%) occult CNV, five (29.4%) classic and PC CNV, two (11.8%) MC CNV, and one (5.9%) RAP.

A comparison between groups 2 and 3 demonstrated that occult CNV or MC CNV was more frequent in group 3 than in group 2 (82.1% vs. 33.3%; $P < 0.02$) and that classic and PC CNV were more frequent in group 2 than group 3 (50.0% vs. 10.7%; $P < 0.03$).

Table 1. Genotype of the Entire Initial Cohort of Exudative AMD Patients

rs11200638 (*HTRAI*) \ rs10611710 (*CFH*)	TT (wtwt)	CT	CC (pp)
GG (wtwt)	9 (22.5%) (Group 1)	20 (22.7%)	30 (41.6%) (Group 3)
GA	19 (47.5%)	49 (55.6%)	25 (34.7%)
AA (pp)	12 (30%) (Group 2)	19 (21.5%)	17 (23.6%) (Group 4)

Both polymorphisms were analyzed: rs10611710 for the *CFH* gene (Y402H) and rs11200638 for the *HTRA1* gene. The four groups were defined by homozygous status for each polymorphism. The C allele rs10611710 of *CFH* SNP and the A allele of rs11200638 *HTRA1* are the at-risk alleles.

Table 2. Distribution of Exudative AMD Phenotypes in the Four Genetic Subgroups

	Group 1 *CFH* wtwt *HTRA1* wtwt	Group 2 *CFH* wtwt *HTRA1* pp	Group 3 *CFH* pp *HTRA1* wtwt	Group 4 *CFH* pp *HTRA1* pp	*P*	
					All Groups	Groups 2 vs. 3
n	9	12	28	17		
Females, *n* (%)	7 (77.8)	9 (75.0)	17 (60.7)	10 (58.8)	NS	NS
Age at diagnosis, y, mean (SD)	72.8 (8.8)	71.7 (8.2)	71.6 (7.8)	69.2 (7.4)	NS	NS
BMI, mean (SD)	26.7 (4.2)	24.9 (2.8)	25.4 (3.8)	28.2 (5.1)	NS	NS
Tobacco use (%)	1 (14.3)	3 (27.3)	9 (40.9)	6 (35.3)	NS	NS
C + PC (%)	4 (44.4)	6 (50.0)	3 (10.7)	5 (29.4)	0.057	0.0244
Occult + MC (%)	4 (44.4)	4 (33.3)	23 (82.1)	11 (64.7)	0.0292	0.015
Occult (%)	3 (33.3)	4 (33.3)	19 (67.9)	9 (52.9)	NS	0.15
MC (%)	1 (11.1)	0 (0)	4 (14.3)	2 (11.8)	NS	NS
RAP (%)	1 (11.1)	2 (16.7)	2 (7.1)	1 (5.9)	NS	NS

Exudative AMD was classified as occult CNV, minimally classic CNV (MC), classic or predominantly classic CNV (C + PC), retinal angiomatosis proliferation (RAP). NS, not significant.

A comparison between the *CFH* pp (groups 3 and 4) and *CFH* wtwt (groups 1 and 2) genotypes demonstrated that occult or MC CNV were more frequent in the *CFH* pp groups (75.6% vs. 38.1%, $P < 0.007$; Table 3). Moreover, classic CNV and PC CNV were more frequent in the *CFH* wtwt groups than in the *CFH* pp groups (47.6% vs. 17.8%, $P < 0.03$).

A comparison between the *HTRA1* pp (groups 2 and 4) and *HTRA1* wtwt (groups 1 and 3) genotypes demonstrated that classic and PC CNV were more frequent in the *HTRA1* pp groups (37.9 vs. 18;9%), but the difference was not significant (Table 3).

Occult or MC CNV was associated with the homozygous Y402H *CFH* polymorphism (OR, 6.4 [95% CI: 1.7–23.7], $P < 0.02$). Classic and PC CNV were more frequently associated with the homozygous *HTRA1* polymorphism but not significantly (OR, 2.4 [95% CI: 0.7–8.0]). These results are summarized in Table 4.

Discussion

Exudative AMD is a multifactorial disorder characterized by both clinical and genetic heterogeneity. Using on FA, ICGA, and OCT, we attempted to establish a genotype–phenotype correlation between two major at-risk polymorphisms involved in AMD (rs11200638 of *HTRA1* and rs10611710 of *CFH*) and subtypes of exudative AMD. From the initial cohort of 200 patients presenting exudative AMD in one eye only, we selected patients homozygous for wild-type, or for one or both at-risk polymorphisms, with the purpose of selecting four genetically homogenous subgroups.

Few studies have focused on the phenotypic classification of exudative AMD, including vascularized PED, PCV, and RAP.[46–49] All these studies confirmed very heterogeneous phenotypes in exudative AMD, and the proportion of each phenotype may vary according to ethnicity. In our entire cohort ($n = 200$), we observed classic and PC CNV in 22% ($n = 44$) of cases, MC in 10.5% ($n = 21$), occult in 52.5% ($n = 105$), RAP in 11% ($n = 22$), and PCV in 4% ($n = 8$). Because polypoidal vasculopathy harbors a distinct clinical presentation and ethnic background, all PCV cases were excluded from the analysis. However, this did not affect the significance of statistical analysis.

In our series of homozygous patients, we observed 26.5% classic and PC CNV, 10.3% MC CNV, 51.5% occult CNV, 8.8 of RAP, and 2.9% PCV. Our findings are in agreement with those of Cohen et al.,[47] who recently observed in the French exudative AMD population classic and PC CNV in 23% of cases, MC in 8.3%, occult in 56.6%, and RAP in 15.1%.

Despite relatively small series of exudative AMD cases in each subgroup, statistical analysis revealed significant phenotype–genotype correlations. Patients harboring the homozygous Y402H *CFH* polymorphism only (group 3) were significantly more frequently associated with occult or MC CNV than were patients harboring the homozygous *HTRA1* polymorphism only (group 2; respectively, 82.1% vs. 33.3%; $P < 0.02$). On the other hand, patients harboring the homozygous *HTRA1*

Table 3. Phenotypic Features of Exudative AMD in Patients Homozygous for *CFH* and *HTRA1* Polymorphisms

	CFH wtwt	*CFH* pp	*P*	*HTRA1* wtwt	*HTRA1* pp	*P*
n	21	45		37	29	
Females, *n* (%)	16 (76.2)	27 (60.0)	NS	24 (64.9)	19 (65.5)	NS
Age at diagnosis y, mean (SD)	72.1 (8.2)	70.7 (7.7)	NS	71.9 (7.9)	70.2 (7.7)	NS
BMI, mean (SD)	25.6 (3.4)	26.7 (4.5)	NS	25.8 (3.8)	26.9 (4.6)	NS
Tobacco use (%)	4 (22.2)	15 (38.5)	NS	10 (34.5)	9 (32.1)	NS
C + PC (%)	10 (47.6)	8 (17.8)	0.0224	7 (18.9)	11 (37.9)	0.18
Occult + MC (%)	8 (38.1)	34 (75.6)	0.0064	27 (73.0)	15 (51.7)	0.15
Occult (%)	7 (33.3)	28 (62.2)	0.057	22 (59.5)	13 (44.8)	NS
MC (%)	1 (4.8)	6 (13.3)	NS	5 (13.5)	2 (6.9)	NS
RAP (%)	3 (14.3)	3 (6.7)	NS	3 (8.1)	3 (10.3)	NS

Occult CNV + MC was significantly associated with the homozygous at-risk allele of *CFH* ($P = 0.008$). There was a nonsignificant trend for an association of C + PC CNV with homozygous at-risk allele of *HTRA1* ($P = 0.07$). C + PC was significantly less frequently observed with the homozygous at-risk allele of *CFH* ($P = 0.01$). NS, not significant.

TABLE 4. OR for Association between Subtypes of CNV and Homozygous Polymorphisms for the *CFH* or *HTRA1* Genes

	HTRA1 OR (95% CI)	*P*	*CFH* OR (95% CI)	*P*
C + PC	2.4 (0.7–8.0)	NS	0.2 (0.1–0.8)	0.0406
Occults + MC	0.4 (0.1–1.2)	NS	6.4 (1.7–23.7)	0.0112
Occults	0.5 (0.2–1.5)	NS	3.4 (1.0–11.8)	0.0994
MC	0.7 (0.1–4.2)	NS	4.4 (0.4–43.7)	NS

ORs are calculated after adjustment for age and sex. NS, not significant.

polymorphism only (group 2) were significantly more frequently associated with classic and PC CNV than were patients harboring the homozygous Y402H *CFH* polymorphism only (group 3; respectively, 50% vs. 10;7%; $P < 0.03$). Globally, occult or MC CNV were significantly associated with the polymorphism Y402H of the *CFH* gene ($P < 0.007$), and classic and PC CNV were not significantly associated with homozygosity for the at-risk allele of the *HTRA1* gene ($P = 0.18$). However, these results have to be interpreted cautiously; in fact, the power to detect a significant OR of 2.4 after Bonferroni correction was 34% for *HTRA1*.

Recently, investigators in two studies tried to establish genotype–phenotype correlations between the Y402H polymorphism of the *CFH* gene and exudative AMD in Austria and the United States, respectively.[19,20] Both studies concluded that there was association between at-risk allele C of the Y402H *CFH* polymorphism, and PC CNV. For homozygous at-risk allele carriers, PC (with occult CNV) lesions were found in 2.4% of exudative AMD cases in the first study[19] and in 47% in the second study.[20] Although a similar conclusion, both studies showed major heterogeneous phenotypic repartition. Furthermore, despite its major role as a susceptibility risk factor for AMD, the status for *HTRA1* was not considered in these studies. Environmental factors (nutrition, smoking) that play a major role in AMD should also be considered in larger cohort studies.

To simplify the phenotypic classification, we selected patients with exudative AMD without atrophy. Because exudative AMD is extremely heterogeneous, and discordant phenotypes can be observed in bilateral cases, we selected individuals affected with exudative AMD in one eye only.

To simplify the genotypic classification, we decided to analyze only one polymorphism on each locus. For the *CFH* gene, the Y402H polymorphism appears as one of the most commonly described in association with AMD to date.[32–36] The question of the best polymorphism on locus 10q was raised. However, the *HTRA1* and *LOC387715* polymorphism are strongly associated by complete linkage disequilibrium, and we decided to analyze the *HTRA1* polymorphism.[50]

From these data, perspectives could be to evaluate prospectively the occurrence of CNV in the fellow eye according to the genotype. Furthermore, the analysis of the response to anti-VEGF therapies according to the genotype would be of great interest. These findings could also contribute to enlarging our knowledge of the physiopathologic processes involved in AMD. However, the results in this small series have to be interpreted with caution, considering that discordant phenotypes have been observed in bilateral exudative AMD. Although we present evidence of differences in the distribution of CNV types with different genotypes, it is also clear that a particular genotype is not associated with a single form of CNV.

In conclusion, this attempt at genotype–phenotype correlation based on angiographic features in a population with exudative AMD suggests an association between occult CNV and the *CFH* gene. Classic and PC CNV do not appear to be the main phenotype associated with the Y402H *CFH* polymorphism ($P = 0.01$).

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