Genotypic Influences on Severity of Exudative Age-Related Macular Degeneration

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Purpose. Major genetic risk factors have recently been identified for age-related macular degeneration (AMD), including the *ARMS2/LOC387715* and *CFH* at-risk polymorphisms. The study was conducted to establish correlations between the AMD genotype and both the phenotype and severity of AMD.

Methods. In a prospective cohort of 1216 AMD patients, four genotypic homozygous groups were identified (n = 264): double homozygous for wild-type alleles (group 1, n = 49), homozygous for the at-risk allele of *ARMS2/LOC387715* only (group 2, n = 57), homozygous for the at-risk allele of *CFH* only (group 3, n = 106), and double homozygous for both at-risk alleles (group 4, n = 52). The phenotypic classification of exudative AMD was based on fluorescein angiography.

Results. Mean age at presentation was significantly lower in group 4 than in group 1 ($P < 0.014$). Patients in group 4 presented more often with bilateral CNV and fibrovascular scars than did patients in group 1 ($P < 0.001$ and < 0.0031 respectively) and with significantly lower visual acuity (VA) in the first affected eye than did patients in group 1 ($P < 0.02$). Patients in group 2 presented with worse VA than did patients in group 3 ($P < 0.003$). Classic CNV was more commonly associated with the at-risk allele of the *ARMS2/LOC387715* locus than with the at-risk allele of the *CFH* gene ($P < 0.026$).

Conclusions. This study demonstrates an association between the at-risk allele of the *ARMS2/LOC387715* locus and classic CNV, fibrovascular lesions, and poor VA. Individuals double homozygous for both at-risk alleles had a higher risk of being affected with a severe form of AMD at an earlier age. (*Invest Ophthalmol Vis Sci.* 2010;51:2620–2625) DOI:10.1167/iovs.09-4423

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Submitted for publication August 2, 2009; revised October 31, 2009; accepted November 19, 2009.

Disclosure: **N. Leveziel**, None; **N. Puche**, None; **F. Richard**, None; **J.E.A. Somner**, None; **J. Zerbib**, None; **S. Bastuji-Garin**, None; **S.Y. Cohen**, None; **J.-F. Korobelnik**, None; **J. Sahel**, None; **G. Soubrane**, None; **P. Benlian**, None; **E.H. Souied**, None

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In developed countries age-related macular degeneration (AMD) is the most common cause of visual loss in the elderly population.[1–4] There are two forms of the disease, exudative and atrophic, and both genetic and environmental risk factors have been identified.[5–12] Clinical features commonly described in exudative AMD, include classic neovascularization (CNV), occult CNV, predominantly classic CNV (PC), minimally classic CNV (MC), and retinal angiomatous proliferation (RAP).[13,14] The prevalence of these different exudative AMD phenotypes may vary according to ethnicity and associated environmental factors.[15,16] Two major genetic loci have recently been associated with increased risk of AMD through genome-wide scanning and candidate gene approaches. These include the well-characterized complement factor H gene (*CFH*) at 1q31 and the still debated age-related maculopathy susceptibility 2 gene (*ARMS2/LOC387715*) at 10q26.[17–25] In a previous case–control study, we analyzed some genetic factors located at the 10q26 locus (rs11200638 of *HTRA1*, rs10490924 of *LOC387715*, and rs4146894 of *PLEKHA1*) in our population. We found that *HTRA1* and *ARMS2/LOC387715* at-risk polymorphisms were in almost complete linkage disequilibrium in cases ($D' = 1.0$) and in controls ($D' = 0.98$).[26] Correlations between genetic risk factors and phenotypes of exudative AMD have to date been difficult to establish.[27–30]

In this study, we analyzed correlations between the AMD genotype and both AMD phenotype and severity of AMD, in a large cohort of patients homozygous for both the *CFH* and *ARMS2/LOC387715* at-risk alleles.

Methods

One thousand two hundred sixteen patients with various clinical forms of AMD and age-related maculopathy (ARM) were prospectively recruited in four clinical centers between 2006 and 2008. The AMD cases were diagnosed by the investigators according to international classification guidelines. We enrolled a large number of exudative forms of AMD because patients with neovascular AMD are more commonly referred to specialized retina departments than atrophic AMD or ARM. All these patients were genotyped for different at-risk alleles, including *ARMS2/LOC387715* and *CFH* Y402H. Patients were selected according to their genotype, not according to their form of AMD or ARM. They were included in the study if they presented initially with either unilateral or bilateral age-related maculopathy and exudative or atrophic forms of AMD, either alone or in association with fibrovascular scarring observed at the initial examination. Exclusion criteria were the presence of other retinal diseases (e.g., diabetic retinopathy, high myopia, or retinal dystrophies), the association of geographic atrophy and exudative forms of AMD in one or both eyes, and media opacities that would not allow a precise evaluation of the fundus. The first eye was defined as the first eye presenting with AMD or ARM, or if both eyes presented simultaneously with AMD or ARM as the eye presenting with the worse visual acuity. A complete ophthalmic examination was

performed on each patient including best corrected visual acuity measurement by ETDRS chart, a fundus examination, color photographs, and fluorescein angiography (FA; model 50IA camera; Topcon, Tokyo, Japan). Furthermore, indocyanine angiography (ICG; HRA; Heidelberg Engineering, Heidelberg, Germany), and optical coherence tomography (OCT; Carl Zeiss Meditec, Inc., Oberkochen, Germany) were performed when judged necessary by investigators.

During the first visit, AMD phenotypes in both eyes were analyzed independently by each investigator (EHS, GS, NL, NP) before treatment and genetic testing, according to color photographs and FA at presentation. When investigators disagreed on a particular clinical feature, the patient was excluded from further analysis. Soft drusen, hard drusen, temporal drusen, pseudoreticular drusen, pigmentary alterations, and atrophic lesions with or without central sparing, were analyzed, based on color photographs and fluorescein angiography. Because the presence of exudative features can mask soft or hard drusen and RPE changes, analysis of drusen and RPE changes were performed only in eyes without exudative AMD or fibrovascular scars. Exudative forms of AMD were classified by phenotype as classic CNV (classic), occult CNV (occult), predominantly classic CNV (PC), minimally classic CNV (MC), or retinal angiomatosis proliferation (RAP) or as a fibrovascular scar. Although RAP is a different clinical entity from classic exudative AMD forms, we included cases of RAP in our study because it is observed in aged populations and can be considered an atypical form of exudative AMD. RAP was diagnosed on the basis of FA and ICG and defined as an anastomosis between chorioretinal and retinal circulations, commonly associated with a localized intraretinal hemorrhage frequently surrounded by macular edema and hard exudates. Because idiopathic polypoidal choroidal vasculopathy is an atypical and unusual form of exudative AMD in the Caucasian population, this clinical form was not analyzed in our study.

Criteria for severity of the disease were best corrected VA of 0.1 or worse, a fibrovascular lesion, and involvement of both eyes.

For the subgroup analysis of clinical features, the first group was used as the reference group, against which each at-risk genotype group (groups 2, 3, and 4) was evaluated.

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

Genotyping Methods

Genomic DNA was extracted from blood leukocytes by phenol chloroform and precipitated by ethanol as previously described. Genotyping of *ARMS2/LOC387715* (rs10490924) and of the Y402H *CFH* SNP (rs1061170) was performed by polymerase chain reaction and allelic discrimination using reagents and conditions from SNP genotyping assays (*Taq*man; Applied Biosystems, Inc. [ABI], Courtaboeuf, France). The solution of primers and probes (0.0625 μL) and 2.5 μL of 2× genotyping master mix with ROX (ABI) was made up to 5 μL with 20 ng genomic DNA. PCR reaction (40 cycles) and allelic discrimination were performed on 384-well microtiter plates with a qPCR System (7900 HT; ABI). For each SNP, three pairs of DNA samples exhibiting representative genotypes ascertained by DNA sequencing were used as internal controls, and the results were obtained from duplicate samples of test DNA.

For quality-control purposes, reference genotypes for each SNP were obtained by direct sequencing of 20 randomly selected PCR amplified DNA samples. Target sequences surrounding each SNP were amplified by PCR with the following primer pairs: 5′-GTG GAG AAG GAG CCA GTG AC-3′ (forward) and 5′-CAG TGT CAG GTG GTG CTG AG-3′ (reverse) for SNP rs10490924 of *ARMS2/LOC387715*; 5′-GAG TGT TTA TTA CAG TAA AAT TTC-3′ (forward) and 5′-GAA AAT CAC AGG AGA AAT A-3′ for SNP rs1061170 of the Y402H *CFH* SNP. Amplification cycles ($n = 35$) consisted of a denaturation step at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Direct DNA sequencing of the purified PCR products was performed by the dye-terminator cycle sequencing method on a 96-capillary sequencer (model 3700; ABI). Sequence track analysis was performed with the sequencer software (Genecodes; ABI).

Selection of Patients

From the cohort of 1216 patients, those who were homozygous for *CFH* and *ARMS2/LOC387715* at-risk alleles were selected. Patients heterozygous for the at-risk alleles were excluded to avoid assessment of any co-dominant effect on phenotype. This left four groups from which to determine the effect of genotype on phenotype: group 1, patients double homozygous for the wild-type (wtwt) alleles of the *ARMS2/LOC387715* and *CFH* polymorphisms (*CFH* wtwt/*ARMS2/LOC387715* wtwt); group 2, patients homozygous for (pp) the at-risk allele of the *ARMS2/LOC387715* polymorphism and homozygous for the wild allele of the *CFH* polymorphism (*CFH* wtwt/*ARMS2/LOC387715* pp); group 3, patients homozygous for the at-risk allele of the *CFH* polymorphism and homozygous for the wild-type allele of the *ARMS2/LOC387715* polymorphism (*CFH* pp/*ARMS2/LOC387715* wtwt) and group 4, patients double homozygous for the at-risk alleles of both the *ARMS2/LOC387715* and *CFH* polymorphisms (*CFH* pp/*ARMS2/LOC387715* pp).

Statistical Analysis

The four groups were compared for different variables: the categorical variables (all binary) were studied with χ^2 test or Fisher's exact test, as appropriate, and the quantitative variables were studied with general linear models. Logistic regression models were used to estimate the adjusted odds ratio (OR) with a 95% confidence interval (95% CI). Adjustment variables were age and sex. A difference was said to be significant if it reached $P < 0.05$.

Results

Initially, 1216 patients were included in the study (407 men and 809 women; mean age at diagnosis, 79 ± 8.1 years). Genotypic data for both genes within the entire initial cohort are presented in Table 1.

From the entire cohort, 264 patients were selected according to their genotype (79 men and 185 women; mean age, 79.7 ± 6.8 years). Among these patients, 49 were in group 1, 57 were in group 2, 106 were in group 3, and 52 were in group 4. Patients double homozygous for both at-risk alleles (group 4) were significantly younger at initial presentation than were the patients who were double homozygous for the wt alleles of *CFH* and *ARMS2/LOC387715* (group 1; 76.9 ± 7 years vs. 79.8 ± 9.2 years; $P < 0.014$). The nonoverlapping phenotypes in the worse eye were compared with each other for age at first presentation. Patients with fibroglial forms of AMD were sig-

Table 1. Genotypic Data from the Entire Initial Cohort of AMD Patients

rs10490924 (*LOC387715*)	rs10611710 (*CFH*)		
	TT (wtwt)	**CT (wt,p)**	**CC (pp)**
GG (wtwt)	49 (4.0%) (group 1)	211 (17.3%)	106 (8.7%) (group 3)
GT (wt,p)	134 (11.0%)	289 (23.8%)	170 (14%)
TT (pp)	57 (4.6%) (group 2)	140 (11.5%)	52 (4.2%) (group 4)

Both polymorphisms were analyzed: rs10611710 for the *CFH* gene (Y402H) and rs10490924 for the *ARMS2/LOC387715*. The four groups analyzed in this study were defined by homozygous status for each polymorphism. The C allele of rs10611710 of *CFH* and the T allele of rs10490924 of *LOC387715* are the at-risk alleles.

Table 2. Nonoverlapping Categories of the Phenotype of the Worse Eye at the First Examination in the Different Groups and Median Age of Each Group

Phenotype	Group 1 *CFH* wtwt LOC wtwt	Group 2 *CFH* wtwt LOC pp	Group 3 *CFH* pp LOC wtwt	Group 4 *CFH* pp LOC pp	Total	Mean Age at Inclusion (Range)
ARM	3	3	14	2	21	78.5 (52–89)
Exudative form	33	33	62	27	155	81 (56–98)
Atrophic form	4	3	9	2	18	80 (59–89)
Fibroglial scar	9	18	21	21	68	82 (58–95)

nificantly older than those with age-related maculopathy (82 years vs. 78.5 years, respectively; $P = 0.0445$). A nonsignificant trend was also observed between exudative and atrophic forms compared with age-related maculopathy ($P = 0.11$ and 0.34, respectively). The nonoverlapping phenotypes in the worse eye in the four different groups and the median age at first presentation of each group are presented in Table 2. The demographic data and clinical features at initial presentation are presented in Table 3.

Analysis of the clinical features observed in the macular area in the four groups of patients combined showed that 22.4% and 3.4% of patients presented with a fibrovascular scar in one or both eyes, respectively; 70.2% presented with soft drusen in one or both eyes; 44.2% presented with occult CNV in one or both eyes; 25.6% presented with classic CNV in one or both eyes; and 11.6% presented with RAP in one or both eyes. These data are summarized in Table 4.

The subgroup analysis indicated that fibrovascular scars were more frequently observed in patients homozygous for both at-risk alleles (group 4) and in patients homozygous for the *ARMS2/LOC387715* at-risk allele (group 2), with odds ratios of 3.2 ([95% CI, 1.2–8.2], $P < 0.003$) and 2.1 ([95% CI, 0.8–5.4], $P < 0.003$), respectively. Both soft drusen and occult CNV were significantly associated with groups 2, 3, and 4 ($P < 0.0001$ and < 0.043, respectively). The complete data concerning all phenotypic criteria are summarized in Table 5.

The association between each clinical feature and the at-risk alleles was also analyzed. The occurrence of a fibrovascular scar at initial presentation was associated with the *ARMS2/LOC387715* at-risk allele (OR, 2.7 [95% CI, 1.5–4.8], $P < 0.0012$). Soft drusen, occult CNV, and pigment clumping were all significantly associated with the *CFH* at-risk allele, (OR, 4.5 [95% CI, 2.4–8.4], $P < 0.0001$; OR, 2.1 [95% CI, 1.2–3.8], $P < 0.013$; and OR, 3.2 [95% CI, 1.4–7.4], $P < 0.006$, respectively). Complete data are presented in Table 6.

The BCVA at initial presentation also correlated significantly with the genotype. Concerning the first affected eye, the patients in group 4 initially presented with worse VA than did the patients in group 1 (0.2 ± 0.19 vs. 0.3 ± 0.26; $P < 0.02$). For the first affected eye, the difference between the patients homozygous for the *ARMS2/LOC387715* at-risk allele only (group 2) and those homozygous for the *CFH* at-risk allele only (group 3) was also significant for presenting VA (0.22 ± 0.19 for group 2 and 0.33 ± 0.25 for group 3; $P < 0.003$). No significant difference was observed between the different groups for VA in the second eye. Furthermore, at initial presentation, group 2 had a higher percentage of patients with VA of 0.1 or less in the first eye than did group 3 (41.5% vs. 23.3%, $P = 0.025$). The prevalence of bilateral CNV was higher in group 4 than in group 1 (82.7% vs. 32.3%; $P < 0.001$). These VA data are presented in Table 7.

Discussion

In this study, we attempted to establish correlations between AMD phenotype and both major genetic susceptibil-

Table 3. Demographic Data and Clinical Features at Initial Presentation in the Four Groups of Patients Selected According to Their Genotypes

	Group 1 *CFH* wtwt LOC wtwt	Group 2 *CFH* wtwt LOC pp	Group 3 *CFH* pp LOC wtwt	Group 4 *CFH* pp LOC pp	*P* All Groups	*P* Group 2 vs. Group 3
n	49	57	106	52		
Men, *n* (%)	14 (28.5)	18 (31.5)	31 (29.2)	16 (30.7)	<0.84	<0.41
Age at diagnosis, y (SD)	79.8 (9.2)	80.6 (5.8)	80.6 (7.2)	76.9 (7.0)	<0.014	<0.98
Soft drusen, *n/N* (%)	23/47 (48.9)	35/54 (64.8)	80/98 (81.6)	43/49 (87.8)	<0.0001	<0.03
Classic+PC, *n/N* (%)	16/41 (39.0)	16/43 (37.2)	28/95 (29.5)	13/38 (34.2)	<0.68	<0.37
Occult, *n/N* (%)	12/40 (30.0)	21/45 (46.7)	48/97 (49.5)	25/40 (62.5)	<0.034	<0.76
Classic, *n/N* (%)	14/41 (34.2)	15/43 (34.9)	24/95 (25.3)	10/38 (26.3)	<0.57	<0.026
Occult+MC, *n/N* (%)	16/39 (41.0)	25/47 (53.2)	50/97 (51.6)	31/45 (68.9)	<0.08	<0.86
MC, *n/N* (%)	5/38 (13.2)	6/44 (13.6)	5/92 (5.4)	7/39 (18.0)	<0.11*	<0.18
PC, *n/N* (%)	3/34 (8.8)	4/92 (4.4)	2/42 (4.8)	3/34 (5.1)	<0.77*	<1.0
RAP, *n/N* (%)	5/47 (10.6)	5/52 (9.6)	12/99 (12.1)	5/48 (10.4)	NS	NS
Atrophy with central sparing, *n/N* (%)	8/46 (17.4)	7/49 (14.3)	16/95 (16.8)	7/45 (15.6)	<0.98	<0.70
Atrophy without central sparing, *n/N* (%)	3/44 (6.8)	2/47 (4.3)	7/93 (7.5)	2/41 (4.7)	<0.91*	<0.72*
Fibrovascular scar, *n/N* (%)	9/49 (18.4)	18/55 (32.7)	21/109 (19.3)	21/52 (40.4)	<0.013	<0.06

NS, nonsignificant.

* Fisher's exact test.

TABLE 4. Analysis of the Clinical Features Observed in the Cohort of Patients Selected for At-Risk Allele Homozygosity

	No Eyes	1 Eye	2 Eyes
Hard drusen	120 (54.3%)	20 (9.1%)	81 (36.6%)
Soft drusen	67 (29.8%)	23 (10.2%)	135 (60.0%)
Occult	116 (55.8%)	62 (29.8%)	30 (14.4%)
Classic	154 (74.4%)	51 (24.6%)	2 (1.0%)
Occult+MC	106 (51.2%)	63 (30.4%)	38 (18.4%)
Classic+PC	144 (69.6%)	59 (28.5%)	4 (1.9%)
RAP	183 (88.4%)	21 (10.1%)	3 (1.5%)
Atrophy with central sparing	197 (86.4%)	14 (6.1%)	17 (7.5%)
Atrophy without central sparing	213 (94.3%)	5 (2.2%)	8 (3.5%)
Fibrovascular scar	196 (74.2%)	59 (22.4%)	9 (3.4%)

Data are expressed as the number of patients with the clinical feature (% of the group).

ity factors identified in exudative AMD.[17–25,31,32] To simplify the analysis and to avoid the bias of the co-dominant effect, heterozygous patients were excluded, and the study focused only on the Y402H polymorphism of *CFH* and on the A69S polymorphism of *ARMS2/LOC387715*. Although a strong association between the Y402H polymorphism of *CFH* and AMD has been widely established,[33] it is still unclear whether this risk effect is solely due to this variant because of a strong linkage disequilibrium (LD) between this variant and three other downstream variants of the *CFH* gene.[20] With regard to *ARMS2/LOC387715*, it is again unclear whether the causative gene is the *LOC387715* or the *HTRA1* gene, because of a strong LD between their respective rs10490924 and rs11200638 polymorphisms.[26,34,35] Because a strong LD was observed between these two polymorphisms, the choice of the rs10490924 of *ARMS2/LOC387715* rather than the rs11200638 of *HTRA1* in this study would be unlikely to modify the results.[26] The genotypic selection was based on two genetic factors that could explain up to 60% of AMD cases, with population-attributable risks for *CFH* Y402H ranging from 43% to 68% and from 36% to 57% for *ARMS2/LOC387715* A69S.[36,37] When considering the entire initial cohort, the allelic frequencies of *CFH* and *ARMS2/LOC387715* calculated from the data in Table 1 were 0.53 and 0.45, respectively. These results are similar to those previously published in Caucasian populations.[23,26,36–39]

Other genes such as complement factor B, *C2*, *C3*, *SCARB1*, and *APOE* are also implicated in AMD.[40,41] However, due to their low allelic frequencies, the inclusion of these genes alongside both *CFH* and *ARMS2/LOC387715* in this study would not have allowed sufficient power to detect an effect. The prevalence of the different clinical forms of AMD was similar to other observations in Caucasian populations.[42,43] Subgroup analysis of clinical features showed that classic CNV was preferentially associated with the *ARMS2/LOC387715* at-risk allele, whereas occult CNV was preferentially associated with the *CFH* at-risk allele (Table 3). These results are consistent with those reported in a previous study focusing on unilateral forms of exudative AMD.[29]

Soft drusen and pigment clumping are pathologic processes associated with local inflammation and immune-mediated processes.[44,45] Because *CFH* is a key regulator of the alternative pathway of the complement cascade, it may be that a mild dysfunction in this regulator element induced by the variant Y402H of *CFH* is associated with more local inflammation and the development of the observed pathologic processes.[46] In a study based on the cohort of the Blue Mountains Eye Study (BMES), a significant association has been established between bilateral early AMD lesions, particularly bilateral soft drusen and pigmentary abnormalities (OR, respectively, of 2.8 [95% CI, 1–8.1] and 1.7 [95% CI, 1–2.8]).[47] Although our cohort was different from the BMES cohort, a significant association was also established between pigmentary abnormalities and soft drusen and the *CFH* at-risk allele (Table 6). However, it must be emphasized that drusen and RPE changes can be masked by exudative features related to CNV, or fibrovascular scarring. In these cases, the presence of drusen or RPE changes could have been missed, inducing a bias into the results. To avoid this bias, the eyes presenting with exudative features or fibrovascular scars were excluded from the analysis of drusen or RPE changes.

At initial presentation, fibrovascular scars that represent the terminal phase of exudative AMD were observed in 22.4% and in 3.4% of patients in our genotypically selected group in one or both eyes respectively. A fibrovascular scar was observed in 18.4% of patients in group 1 and in 40.4% of patients in group 4. This significant difference between both groups could be explained by the fact that homozygosity for both major at-risk alleles analyzed in this study is associated with advanced forms

TABLE 5. Subgroup Analysis of Clinical Features Observed at Initial Presentation in the First Eye

	Group 1 *CFH* wtwt LOC wtwt	Group 2 *CFH* wtwt LOC pp	Group 3 *CFH* pp LOC wtwt	Group 4 *CFH* pp LOC pp	*P*
Soft drusen	1	2.0 (0.9–4.6)	5.0 (2.3–10.8)	7.5 (2.6–21.3)	<0.0001
Pseudoreticular drusen	1	1.1 (0.3–3.6)	0.8 (0.2–2.3)	1.0 (0.3–3.5)	0.93
Hard drusen	1	0.8 (0.3–1.8)	1.5 (0.7–3.0)	1.3 (0.6–3.0)	0.37
Temporal drusen	1	3.4 (1.2–9.8)	2.8 (1.0–7.4)	4.2 (1.4–12.1)	<0.06
Pigment clumping	1	0.9 (0.4–2.3)	0.5 (0.2–1.3)	0.8 (0.3–2.0)	0.47
Pigment mottling	1	1.2 (0.5–3.0)	1.3 (0.6–2.8)	2.0 (0.8–5.0)	<0.43
Hypopigmentation	1	2.4 (0.8–6.8)	1.8 (0.7–1.7)	3.7 (1.3–10.4)	<0.09
Occult	1	2.2 (0.9–5.5)	2.5 (1.1–5.5)	3.8 (1.5–9.7)	<0.043
Occult+MC	1	1.7 (0.7–4.1)	1.6 (0.8–3.5)	3.3 (1.3–8.0)	<0.09
Classic	1	1.0 (0.4–2.4)	0.6 (0.3–1.4)	0.8 (0.3–2.0)	<0.57
Classic+PC	1	0.9 (0.3–2.1)	0.6 (0.3–1.3)	0.9 (0.4–2.3)	<0.58
Atrophy with central sparing	1	0.8 (0.3–2.4)	0.9 (0.4–2.4)	1.0 (0.3–2.7)	<0.99
Atrophy without central sparing	1	0.6 (0.13–3.7)	1.1 (0.3–4.4)	0.8 (0.1–5.4)	<0.90
Fibrovascular scar	1	2.1 (0.8–5.4)	1.0 (0.4–2.5)	3.2 (1.2–8.2)	<0.0031

Data for groups 2–4 are OR (95% CI). Group 1 is the reference group for the analysis. Fibrovascular scars were more frequently observed in groups 2 and 4. Soft drusen and occult CNV were more commonly observed in the groups 2, 3 and 4. No significant association could be established between other clinical features and genotypes.

Table 6. Associations between Clinical Features and Homozygosity for Each of the At-Risk Alleles of the *CFH* Y402H and ARMS2/LOC387715 At-Risk Polymorphisms

	CFH	*P*	*LOC387715*	*P*	$P_{interaction}$
Soft drusen	4.5 (2.4–8.4)	<0.0001	1.8 (0.9–3.4)	0.07	0.67
Pseudoreticular drusen	0.8 (0.4–1.9)	<0.64	1.2 (0.5–2.3)	0.73	0.82
Hard drusen	1.5 (0.9–2.7)	<0.12	0.9 (0.5–1.5)	0.58	0.80
Temporal drusen	1.8 (0.9–3.4)	<0.07	2.0 (1.1–3.8)	<0.03	0.23
Pigment mottling	1.5 (0.8–2.0)	<0.23	1.4 (0.8–2.6)	<0.24	<0.59
Pigment clumping	3.2 (1.4–7.4)	<0.006	1.7 (0.8–3.6)	<0.15	—
Hypopigmentation	1.7 (0.9–3.2)	<0.12	2.1 (1.1–4.0)	<0.02	0.80
Occult	2.1 (1.2–3.8)	<0.013	1.8 (1.0–3.2)	<0.06	0.56
Occult+MC	1.7 (1.0–3.1)	<0.06	1.9 (1.1–3.3)	<0.031	<0.79
Classic	0.7 (0.4–1.3)	<0.22	1.1 (0.6–2.1)	<0.80	<0.70
Classic+PC	0.8 (0.4–1.4)	<0.37	1.1 (0.6–2.1)	<0.68	<0.39
Atrophy with central sparing	1.0 (0.5–2.1)	<0.94	0.9 (0.4–1.9)	<0.82	<0.74
Atrophy without central sparing	1.2 (0.4–3.8)	<0.80	0.7 (0.2–2.3)	<0.54	<0.84
Fibrovascular scar	1.3 (0.7–2.3)	<0.45	2.7 (1.5–4.8)	0.0012	0.56

Data are OR (95% CI). A significant association was noted between soft drusen, pigment clumping and occult CNV with the at-risk allele of the *CFH* gene, whereas a significant association was also noted between fibrovascular scar, temporal drusen, hypopigmentation and occult+MC CNV with the at-risk allele of the ARMS2/LOC387715 at-risk polymorphism. OR were calculated after adjustment for age and sex. MC, minimally classic CNV; PC, predominantly classic CNV.

of AMD, as previously demonstrated in different studies.[47,48] Furthermore, fibrovascular scars and worse visual acuity were more frequently observed in patients homozygous for the *ARMS2/LOC387715* at-risk allele. A similar finding was recently reported by Gotoh et al.[30] who demonstrated that, in Asians, more severe forms of exudative AMD were observed in patients homozygous for the *HTRA1* at-risk allele.

It is noteworthy that patients homozygous for the *ARMS2/LOC387715* at-risk allele presented with severe forms of AMD, such as fibrovascular forms, and a low visual acuity. Patients homozygous for the *ARMS2/LOC387715* at-risk allele also had a higher percentage of VA of 0.1 or less in the first eye at presentation than patients homozygous for the *CFH* at-risk allele (respectively, 41.5% vs. 23.3%, $P = 0.025$). Although not significant, patients with both at-risk alleles of *CFH* and *ARMS2/LOC387715* seem to have a higher percentage of VA at 0.1 or less in the first and the second eye at presentation than patients homozygous for both wt alleles (respectively, 51% vs. 35.7%, $P = 0.199$). Furthermore, patients carrying both *CFH* and *ARMS2/LOC387715* at-risk alleles presented with the disease at a younger age than patients homozygous for both wt alleles (76.9 ± 7 years vs. 79.8 ± 9.2 years; $P < 0.014$) and presented more frequently with bilateral CNV than did patients homozygous for both wt alleles ($P < 0.001$). This result suggests that patients carrying both at-risk alleles develop the disease earlier, implying an association between age at inclusion, visual acuity and these AMD susceptibility genes. This observation is supported by a previous study conducted on a smaller group of patients ($n = 68$) with unilateral exudative AMD with a similar trend observed between age at presentation and genotype, although it did not achieve significance, probably because of insufficient statistical power.[29]

Table 7. Severity Criteria Including VA Data and Bilateral CNV at Presentation between the Different Groups at Initial Examination

	Group 1	Group 4	*P*
Group 1 vs. group 4 (SD)			
BCVA first eye (±SD)	0.3 (0.26)	0.2 (0.19)	<0.02
BCVA second eye (±SD)	0.50 (0.27)	0.49 (0.29)	<0.5
First eye VA ≤0.1, *n/N* (%)*	15/42 (35.7)	24/47 (51.0)	0.199†
Second eye VA ≤0.1, *n/N* (%)*	4/42 (9.5)	6/47 (12.7)	0.743†
Bilateral CNV, *n/N* (%)	11/34 (32.3)	24/29 (82.7)	<0.001†
	Group 2	**Group 3**	***P***
Group 2 vs. group 3 (SD)			
BCVA first eye (±SD)	0.22 (0.19)	0.33 (0.25)	<0.003
BCVA second eye (±SD)	0.52 (0.28)	0.58 (0.27)	<0.18
First eye VA ≤0.1, *n/N* (%)*	22/53 (41.5)	21/90 (23.3)	0.025†
Second eye VA ≤0.1, *n/N* (%)*	6/53 (11.3)	4/90 (4.4)	0.173†
Bilateral CNV, *n/N* (%)	17/38 (44.7)	36/67 (53.7)	<0.5†

* Percentage of patients with a VA of 0.1 (20/200) or less at initial presentation.

† Fisher's exact test.

In conclusion, our analysis suggests a correlation between severe phenotypes and double homozygous status for both at-risk alleles. This study also confirms the association between classic CNV and the *ARMS2/LOC387715* at-risk allele. Further studies are needed to validate these results, which in the long-term may influence the monitoring and the treatment of exudative AMD.

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