Growth of Geographic Atrophy on Fundus Autofluorescence and Polymorphisms of CFH, CFB, C3, FHR1-3, and ARMS2 in Age-Related Macular Degeneration

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Importance
Identification of the genetic risk factors that contribute to geographic atrophy (GA) could lead to advancements in interventional trials and/or therapeutic approaches for combating vision loss.

Objective
To investigate whether single-nucleotide polymorphisms (SNPs) are associated with the presence and progression of established GA in age-related macular degeneration (AMD).

Design, Setting, and Participants
Prospective, controlled, multicenter study of 154 patients with GA/AMD and 141 age-matched control participants at 8 Spanish hospitals.

Main Outcomes and Measures
Samples of DNA were collected to analyze SNPs within AMD-related genes (CFH, CFB, C3, FHR1-3, and ARMS2). Fundus autofluorescence imaging was used to evaluate GA progression during a 2-year period in 73 patients with GA/AMD. Finally, logistic regression was used to analyze the associations of SNPs, age, body mass index, and cigarette smoking with the rate of progression and relative growth of GA.

Results
This case-control analysis revealed a significant (P < .05) association between the presence of GA and SNPs within CFH, ARMS2, and FHR1-3. Moreover, logistic regression analysis identified significant associations of the rate of progression with genetic polymorphisms (CFH-402His [P = .04] and CFH-62Ile [P = .04]) and demographic factors (sex [P = .02] and age [P = .02]), whereas relative growth was associated with 1 polymorphism (CFB-32Gln [P = .04]).

Conclusions and Relevance
Taken together, our findings confirm that genetic risk factors related to the presence of GA are not identical to those associated with GA progression. In fact, we demonstrate that gene variants of CFH and CFB, as well as demographic risk factors, confer significant risk for GA progression (both rate of progression and relative growth) within a Spanish population.

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A
ger-related macular degeneration (AMD) is the leading cause of vision loss and blindness in people aged 65 years or older in the developed world, accounting for half of all new cases of blindness.\(^1-3\) The pathological hallmark of AMD is the presence of drusen in the macula. Drusen are deposits of insoluble extracellular protein and lipid aggregates that progressively form near the retinal pigment epithelium (RPE).\(^1-4\) This process results in maculopathy, which can progress to degeneration and vision loss through 2 distinct forms of advanced disease. In the neovascular or “wet” form of AMD, choroidal neovascularization (CNV) occurs in which newly formed immature blood vessels grow toward the outer retina from the underlying choroid.\(^1\) These new blood vessels may leak fluid below or within the retina, blurring or distorting central vision. The other form of late-stage AMD is the “dry” form of the disease, which is geographic atrophy (GA). It is characterized by scattered or confluent areas of degenerated RPE cells and photoreceptors, which normally rely on the RPE for trophic support.\(^7-10\) While intravitreal delivery of antibodies against vascular endothelial growth factor has successfully reversed vision loss in some patients with neovascular AMD, GA remains untreatable. This absence of therapeutic options for combating pure GA is of fundamental interest as most advanced cases of AMD involve GA.\(^2,9-12\)

Age-related macular degeneration is a complex condition that stems from both genetic and environmental factors. Therefore, previous studies have investigated the ocular, systemic, and environmental risk factors that are associated with AMD prevalence, incidence, and progression. In these studies, sex, age, cigarette smoking, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), arterial hypertension, diet, family history, and retinal features (ie, drusen or pigment changes) showed strong associations with advanced forms of AMD.\(^1,6-9,13-17\)

Several recent studies have demonstrated the close association between AMD and specific variants in genes related to the complement system. In particular, complement factor H (encoded by the CFH gene), CFH-related genes (FHR1 and FHR3), complement factor B (CFB), and C3 have all been associated with AMD. In addition, LOC387715/ARMS2 has been postulated to be the second most highly AMD-associated gene, independent of CFH, and has been linked to both forms of the advanced disease (wet and atrophic).\(^19-21\) In addition, various other proposed genes (eg, ABCA4, ELOVL4, VLDLR, TLR4, HMCN1, and FBLN5) have been linked to certain types of AMD; however, these associations lack definitive evidence.\(^18,22\) The most frequent genetic variants associated with AMD are single-nucleotide polymorphisms (SNPs). Indeed, some SNPs are related to protective effects, whereas others have been linked to increased risk for AMD. According to recently published literature, the main SNPs identified to confer AMD risk include rs1061170 (corresponding to Y402H in CFH), rs10490924 (A69S in ARMS2), rs9332739 (E318D in C2), and rs641153 or rs4151667 (R32Q or L9H in CFB, respectively).\(^19-25\)

However, there is little information concerning the relationship between genetic risk factors and disease progression in patients with established advanced AMD, specifically in relation to those with GA where there have been controversial results.\(^6,9\) Therefore, the purpose of our study was to determine whether genotype is associated with the presence and progression of established GA, which was determined by rate of growth of the GA area. Specifically, we have assessed the relationship between GA and previously identified AMD-associated variants of genes (CFH, CFB, C3, FHR1, FHR3, and ARMS2/HTRA).

Methods

Patients

Our study included 154 patients with GA/AMD (Age-Related Eye Disease Study [AREDS] category 4) and 141 age-matched control participants (AREDS category 1)\(^26\) from 8 Spanish hospitals (from the Spanish Multicenter Group on AMD and Red Temática de Investigación Cooperativa en Salud). Exclusion criteria for this study (for both patients with GA/AMD and control participants) included the following: age younger than 55 years; the presence of other CNV-related retinal diseases (eg, angioid streaks, nevus in the macular area, toxoplasmosis scars, photocoagulation scars in the posterior pole, or polypoidal choroidal vasculopathy); history of retinal surgery; retinal disease in the studied eye (ie, diabetic retinopathy or hereditary retinal dystrophies); and more than 6 diopters of myopia. Inclusion criteria for patients with GA/AMD included the following: eyes with unifocal or multifocal drusen and chorioretinal macular atrophy involving the central macula in at least 1 eye (AREDS category 4). For control participants, inclusion criteria were the following: absence of drusen or no more than 5 small drusen (≤65 μm); absence of retinal pigment abnormalities in the macular area; and absence of chorioretinal macular atrophy or any other form of CNV (AREDS category 1). The control group was analyzed only in relation to genetic associations with the presence of GA. Samples were obtained from each participant after proper explanation of the nature and possible consequences of the study. All participants provided written informed consent in accordance with the Declaration of Helsinki, and the study was approved by the local ethics committees from all centers involved in the study as well as our institutional review boards. Patients and control participants completed questionnaires regarding age, history of smoking, weight, and BMI.

Genotyping

We surveyed the available literature on genetic associations in AMD and selected SNPs that showed the most significant associations. Genomic DNA was extracted from oral swabs using QIACube (Qiagen). Samples of DNA were genotyped for 9 SNPs in 5 previously identified AMD-associated genes: CFH Ile62Val, CFH Tyr402His, CFH c.2237-543A>G, ΔFHR1-3, CFB Leu9His, CFB Arg32Gln/Trp, C3 Arg102Gly, and ARMS2 Ala66Ser. All of these SNPs are independently related, but some of them (in CFH, FHR1-3, and CFB) are associated as risk or protective haplotypes.\(^9\)

Genotyping was performed using multiplex polymerase chain reaction combined with the primer extension method
GA Progression

A trained retinal specialist examined both eyes of each patient using slitlamp biomicroscopy. After anterior segment evaluation, the pupil of the study eye was dilated using eyedrops containing tropicamide, 0.1%, and phenylephrine hydrochloride, 10%, to allow fundus examination. Fundus photographs and fundus autofluorescence (FAF) images were taken in patients with advanced GA at 0 months (baseline) and 24 months (Figure). Notably, trained graders from each center were responsible for obtaining the photographs through a detailed protocol. Only 1 eye per individual was selected for progression analysis. For the analysis, we considered only those eyes that had been examined at least twice (follow-up ≥2 years) and yielded images of sufficient quality to allow accurate determination of atrophy size. Following screening, 73 patients (73 eyes) with GA fulfilled the inclusion criteria. Based on conventional fundus photographs, GA was defined as 1 or more sharply demarcated areas (>175 μm) within the macula with an apparent absence of RPE cells. For our study, the macular area was defined as a 3000-μm-diameter circle centered at the fovea, which was generally circular in shape and displayed underlying choroidal blood vessels (as visualized by stereoscopic color fundus photography). Central GA, which denotes GA involving the center of the fovea, was diagnosed through retinal vascular configuration and pigment alteration on fundus photographs.9,27

In our study, we used FAF imaging to measure areas of atrophy. Pixels were automatically converted into millimeters, taking into account the original image resolution and focusing during acquisition. The total area of unifocal or multifocal GA was measured by outlining dark atrophic regions using image analysis software (Adobe Photoshop CS5.2; Adobe Systems Inc). The rate of progression (RP) of GA was determined by subtracting the baseline lesion area (at 0 months) from the area at 24 months and dividing by the time of follow-up (2 years). In addition, relative growth (RG) during the 2-year period was calculated by subtracting the baseline lesion area from the area at 24 months and then dividing by the baseline area.

Statistical Analysis

The continuous patient variables (RP, RG, BMI, and age) were separated into 2 groups based on the medians (ie, group 1, median or less; group 2, greater than median). Therefore, RP and RG were dichotomized as either 0 (low progression or growth) or 1 (high progression or growth). The RP intervals were 0 to 0.72 and 0.77 to 11.06 mm²/y, whereas the RG intervals were 0.01 to 0.40 and 0.41 to 27.46 times that of the initial area. The BMI values were also separated into 2 groups (ranging from 14.4-26.7 and 26.8-36.9), which were coded as 0 and 1, respectively. Similarly, 2 age groups were created (ages 53-76 and 77-95 years; coded as 0 and 1, respectively). For sex, women were coded as 1 and men as 0. The SNPs were also coded as 0 or 1 based on whether they were considered protector or risk alleles, respectively.

These dichotomized variables were analyzed via binary regression analysis to test the association of genetic, demographic, and environmental factors with GA/AMD. A multivariable logistic regression model was developed and adjusted for significant covariates to estimate the adjusted odds ratios (AORs) and 95% confidence intervals for risk factors. The significance level was P < .05 (2-tailed). For the analysis of GA progression, only 73 patients with GA (who fulfilled the inclusion criteria) were included. We used a univariate general linear model analysis with RP and RG set as continuous variables to assess the significance of the studied factors as well as the interaction between SNPs within the same gene (ie, CFH-402His and CFH-62Ile for CFH; CFB-32Gln, CFB-32Trp, and CFB-9His for CFB). All tests were performed using SPSS version 20 statistical software (SPSS Inc).
Results

General Characteristics
We did not identify any meaningful differences in sex, age, hypercholesterolemia, or BMI between patients with GA/AMD and control participants (Table 1). However, the rates of arterial hypertension and tobacco smoking differed significantly between the 2 groups (P = .04 and P = .03, respectively).

Allelic Frequencies
As shown in Table 2, we observed strong positive associations of CFH-402His, CFH-c.2237-543G, and ARMS2-69Ser with GA/AMD (P < .001). Furthermore, we identified strong protective associations of CFH-621le (P < .001), CFB-9His (P = .009), ΔFHR1-3 (P < .001), and CFB-32Gln/Trp (P = .009) with GA/AMD. In addition, C2-102Gly was previously found to be associated with AMD and displayed a similar positive trend in our population. However, this finding did not reach statistical significance (P = .09), which could be attributed to our small sample size.

GA Progression
For the 73 patients with GA, we calculated a mean RP value of 1.31 to 1.67 mm²/y and a mean RG of 1.62 to 2.20 times that of the initial area. We used univariate general linear model analysis with RP and RG set as continuous variables to assess the effects of the studied factors as well as interactions between SNPs within the same gene. Our results indicated associations of sex (P = .001), tobacco smoking (P = .001), BMI (P = .003), CFH-402His (P = .03), CFH-621le (P = .01), and CFB-9His (P = .04) with RP. Notably, we did not identify interactions occurring between SNPs from the same gene (CFH-402His and CFH-621le for CFH; CFB-32Gln, CFB-32Trp, and CFB-9His for CFB). As described in Methods, we also used binary logistic regression to evaluate the effect of several factors (demographic or environmental [sex, age, smoking, BMI] and genetic [SNPs]) on RP and RG (Table 3). This analysis revealed associations between RP and the following polymorphisms: the risk allele corresponding to CFH-402His (P = .04; AOR = 7.31 [95% CI, 1.30-41.80]) and the protective allele corresponding to CFH-621le (P = .04; AOR = 0.12 [95% CI, 0.01-0.98]). In our analysis of demographic factors, there were risk associations of RP with female sex (P = .02; AOR = 7.31 [95% CI, 1.30-41.80]) and advanced age (P = .02; AOR = 8.71 [95% CI, 1.40-54.10]). Also, the C2-102Gly polymorphism, which corresponds to a risk allele, was found to be at the limit of significance (P = .06; AOR = 6.29 [95% CI, 0.90-44.40]) (Table 3). In the RG analysis, we identified an association between RG and the protective allele corresponding to CFB-32Gln (P = .04; AOR = 0.23 [95% CI, 0.06-1.00]). Also, in this same analysis, the risk-related C2-102Gly polymorphism was found to be at the limit of significance (P = .06; AOR = 5.00 [95% CI, 0.90-25.60]) (Table 3).
Discussion

Specific AMD susceptibility genes were previously demonstrated to predict progression from intermediate to advanced AMD. For this reason, we hypothesized that it might be plausible for these genes (or other genes) to be associated with the progression of already established GA to GA. Therefore, the objective of this study was to determine whether known AMD-associated genetic variants might be related to the presence and/or growth rate of GA. Our findings have revealed that the genetic risk factors linked to the presence of GA are not the same as those associated with disease progression. In fact, we identified only 1 risk allele (corresponding to \( CFH-402H \)) and 2 protective alleles (corresponding to \( CFH-62Ile \) and \( CFB-32Gln \)) that were associated with both the presence and progression of GA in established lesions. Additionally, sex and age were also related to GA progression.

To our knowledge, this is the first study in a Spanish population to prospectively evaluate the association between the frequencies of 9 SNPs (within 5 genes: \( CFH \), \( FHR1-3 \), \( CFB \), \( C3 \), and \( ARMS2 \)) and the presence and progression of GA. Our case-control analysis revealed strong positive risk associations between 3 polymorphisms (ie, \( CFH-402H \), \( CFH-c.2237-543G \), and \( ARMS2-69Ser \)) and GA/AMD. Moreover, several strong protective associations were observed with GA/AMD, including \( CFH-62Ile \), \( CFB-9His \), \( AFHR1-3 \), and \( CFB-32Gln/Trp \). Our findings are in agreement with results from previous studies in other populations. Furthermore, these findings support the notion that SNP analysis might constitute a valid method of meaningfully predicting GA development and progression in AMD.

To verify the association of specific genetic, demographic, and environmental factors with GA progression, we used univariate general linear model analysis. Our results did not demonstrate the existence of interactions occurring between SNPs from the same gene. This finding may result from the low number of patients included in this study because several of these SNPs present both risk and protective haplotypes in AMD.

In addition, we used logistic regression to specifically evaluate the effect of individual factors on RP and RG. This analysis revealed an association between RP and 2 distinct polymorphisms, including the risk-related \( CFH-402H \) and the protective \( CFH-62Ile \). We also observed associations between certain demographic factors (ie, female sex and age) and RP. In contrast, analysis of RG revealed only 1 association, which was related to the protective \( CFB-32Gln \) polymorphism. Moreover, risk-related \( C3-102Gly \) showed a tendency toward significance in both of our regression analyses (RP and RG). Although the findings on \( C3 \) in this study did not reach statistical significance (for case-control or progression), it is the only gene that showed a tendency to be significant in both of the progression parameters analyzed. This result may suggest that the \( C3 \) SNP plays an important role in GA progression, but more patient studies will be needed to confirm this hypothesis.

The progression rate observed in our investigation is similar to that of previous studies of patients with GA/AMD, 1.31 to 1.67 mm²/y and 1.3 to 2.8 mm²/y, respectively.

However, none of these studies identified associations of demographic or environmental risk factors (eg, age, sex, BMI, smoking, or hypertension) with GA, which could possibly account for the observed intra-individual differences.

Ocular features have been reported to have predictive value for future GA growth. In fact, larger initial lesion size and multifocal GA (in contrast to unifocal GA) appear to be factors favoring enhanced GA growth rates.

Another factor associated with the RP of GA is the presence of abnormal baseline FAF patterns. Areas with increased FAF signal and excessive RPE lipofuscin load precede de novo development of GA lesions or the enlargement of preexisting atrophic patches. Therefore, FAF can also be used to predict progression of GA in AMD.

However, there is currently little information regarding the associations between genotype and GA growth in patients with established GA. Nevertheless, 2 studies have reported that specific genes (\( CFH \), \( ARMS2/ HTRA1 \), and \( C3 \) ) increased the risk of progression not only from intermediate drusen to large drusen but also from large drusen to GA and CNV. In addition, a case-control study observed an association of the \( T \) allele of rs1800325 in \( ABCA1 \) with decreased risk of intermediate drusen, large drusen, GA, and CNV. In contrast with our results, another recent study following up 99 individuals with bilateral GA during a mean of 3 years found an association between variants of \( CFH (Y402H) \), \( ARMS2 (A69S) \), and \( C3 (R102G) \) and the presence of GA; however, they did not identify any correlation of these polymorphisms with GA progression.

### Table 3. Genetic and Demographic Effects of Geographic Atrophy Progression Measured by Rate of Progression and Relative Growth Groups for Binomial Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Binomial Logistic Regression</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>P Value</td>
</tr>
<tr>
<td><strong>RP data</strong></td>
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<tr>
<td>( CFH-402H )</td>
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</tr>
<tr>
<td>( CFH-62Ile )</td>
<td>-2.908</td>
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<tr>
<td>( C3-102Gly )</td>
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<td>.06</td>
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<td>.02</td>
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<tr>
<td><strong>Age</strong></td>
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<td>.02</td>
</tr>
<tr>
<td><strong>RG data</strong></td>
<td></td>
<td></td>
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<tr>
<td>( CFB-32Gln )</td>
<td>-1.435</td>
<td>.04</td>
</tr>
<tr>
<td>( C3-102Gly )</td>
<td>1.607</td>
<td>.06</td>
</tr>
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</table>

Abbreviations: AOR, adjusted odds ratio; RG, relative growth; RP, rate of progression.

* All parameters were classified into 2 categories.

* P values are the result of a 2-tailed logistic regression.

* P < .05.
Although we have obtained very promising results, this study has some limitations. In particular, this analysis involved a small number of eyes, which diminishes our power for identifying relationships between SNP frequencies and the presence and progression of GA/AMD. Furthermore, while 154 patients with GA/AMD were enrolled for genetic analysis at the beginning of this study, only 72 patients (47.4%) completed the longitudinal analysis of GA progression. This discrepancy reflects the fact that not all of the centers involved in the study had access to an FAF measuring device.

Similar to other trials, we measured the progression of GA using FAF imaging. This method is particularly useful for following and predicting GA progression because it yields a quantitative measurement of GA through detection and quantification of atrophic areas on the fundus. Thus, the use of this technique at different times allows for efficient calculation of atrophy enlargement rates.

The identification of predictive factors (eg, genetic traits) for atrophy progression not only increases our understanding of the underlying pathophysiological mechanisms involved in AMD but also has the potential to reduce the time necessary for interventional clinical trials in patients with GA. Moreover, distinguishing patients with high-risk genotypes might allow for the implementation of preventive measures (eg, healthy lifestyle habits) to diminish the development of GA and AMD. The discovery of predictive factors for GA could also lead to novel therapeutic strategies for AMD.

Conclusions

Our study conducted in a Spanish population of patients with AMD has yielded significant and original findings suggesting that not all AMD-associated SNPs (in CFH, CFB, C3, FHR1-3, and ARMS2/HTRA1) contribute to the progression of GA. In fact, we have demonstrated that only SNPs in specific genes (CFH and CFB) and certain demographic factors (sex and age) are involved in the development and progression of GA.

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REFERENCES

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