Relevance of complement factor H-related 1 (*CFHR1*) genotypes in age-related macular degeneration (AMD).

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ABSTRACT

Purpose: Age-related Macular Degeneration (AMD) has a strong genetic component with a major locus at 1q31, including the complement factor H gene (*CFH*). Detailed analyses of this locus have demonstrated the existence of one SNP haplotype block, carrying the *CFH-402His* allele, which confers increased risk to AMD, and two protective SNP haplotypes, one of them carrying a deletion of the *CFHR1* and *CFHR3* genes ($\Delta_{CFHR3\text{-}CFHR1}$). The purpose of these studies was to evaluate the contribution of newly described *CFHR1* alleles to the association of the 1q31 locus with AMD.

Methods: 259 patients and 191 age-matched controls of Spanish origin were included in a transversal comparative case-control study using multivariate logistic regression analysis and ROC (Receiver Operating Characteristics) statistics to generate and to test models predictive for developing AMD.

Results: We show for the first time that a particular *CFHR1* allotype, *CFHR1*A*, is strongly associated with AMD (OR, 2.08; 95% CI, 1.59-2.73; P<0.0001) and illustrate a peculiar genotype-phenotype correlation between the *CFHR1* alleles and different diseases that may have important implications for understanding the pathophysiology of AMD. We also show that *CFHR1*A* is in strong linkage disequilibrium with the *CFH-402His* allele, which provides additional candidate variants within the major risk haplotype at 1q31 for being responsible of its association with AMD. Further, using the Spanish population as a model, we show that analysis of the *CFHR1* genotypes provide sufficient information to delineate the individual risk to develop AMD.

Conclusions: Our results support a relevant role of *CFHR1* in AMD pathogenesis.

INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of visual disability in the elderly in developed countries[1]. AMD is a multifactorial disease, influenced by age, ethnicity and a combination of environmental and genetic risk factors[2]. Genetic predisposition in AMD has been suggested based on familial segregation and twins studies. Several candidate genes with a relatively minor contribution and two major susceptibility loci (1q31, *CFH/CFHR1/CFHR3*; 10q26, *HTRA1/ARMS2*) that independently contribute to AMD disease risk have been identified by candidate region studies and whole genome association analyses[3-10].

The 10q26 locus includes two nearby genes, *ARMS2* (age-related maculopathy susceptibility 2, also known as *LOC387715*)[11] and *HTRA1* (high-temperature requirement factor A1)[11]. The most studied SNP at this locus is rs10490924, which causes the Ala69Ser amino acid substitution in *ARMS2*, a protein located in the ellipsoid, a mitochondria-concentrated part of human photoreceptor cells. The risk-associated allele *ARMS2-69Ser* is in strong linkage disequilibrium with a genetic variant resulting from a combination of a deletion and insertion (*372_815delins54) in the 3' untranslated region of the *ARMS2* mRNA, which affects the stability of the *ARMS2* mRNA and supports a role for *ARMS2* in AMD pathogenesis[11].

Several independent studies have shown that the *CFH-402His* allele (rs1061170), at the *CFH* locus in 1q31, confers a significantly increased risk to AMD with an odds ratio (OR) between 2.1 and 7.4[3, 5-7]. The association of *CFH* with AMD strengthened the implication of the complement system in the pathogenesis of AMD [5] and prompted subsequent candidate gene approaches that resulted in the identification of additional associations of complement genes with AMD[4, 9-10, 12-15]. As a whole, these genetic data reinforced the concept that complement disregulation is a major player in

AMD pathogenesis. This conclusion is supported by the functional analysis of the AMD-associated SNPs in the *CFH*[16], *CFB*[17] and *C3*[18] genes. The *CFH* Tyr402His polymorphism has also been found to have functional implications. Notably, it alters the binding specificity of factor H for different glycosaminoglycans[19-20] and decreases its binding to retinal pigment epithelial cells[21] and Bruch membrane[22]. However, the physiological relevance of these observations is still unclear.

Within the *CFH* gene, downstream of the Tyr402His polymorphism, there are three polymorphisms, a synonymous SNP in exon 11 (rs2274700) and two intronic SNPs (rs1410996 and rs7535263), showing stronger association with disease susceptibility than the *CFH-402His* variant[14-15]. These polymorphisms and the *CFH* 402His variant form part of the same *CFH* haplotype conferring increased risk to AMD (haplotype H1)[23]. Two additional haplotypes in *CFH* gene (haplotypes H2 and H4) are markedly decreased in AMD and therefore have been described associated with lower risk to AMD[13, 15, 24]. *CFH* haplotype H2 carries the Ile62 factor H variant showing increased complement regulatory activity[16], which likely confers lower risk to AMD by reducing complement activation. *CFH* haplotype H4 is also associated with decreased risk of AMD[13, 25]. Interestingly, this *CFH* haplotype is also unique because it carries a deletion of the *CFHR1* and *CFHR3* genes ($\Delta_{CFHR3\text{-}CFHR1}$)[13]. Although it has been indicated that CFHR1 and CFHR3 proteins have the potential to compete with factor H and interfere its complement regulatory activity, the potential benefit of the absence of CFHR1 and CFHR3 proteins is still puzzling as these two proteins are considered complement regulators[26]. Recently it has been proposed that complement activity is determined by a homeostatic balance between CFHR1, CFHR3 and factor H and that loss of *CFHR1* and *CFHR3*, affecting local factor H binding, may increase local factor H-mediated regulation and protecting against AMD development [27].

Here we provide further insights into the gene variants encoded by the AMD locus at 1q31. We show that *CFHR1*A*, a recently described allele encoded by the *CFHR1* gene, is associated with AMD and in strong LD with the *CFH-402His* allele. These data add to previous results and support a relevant role of *CFHR1* in AMD pathogenesis. To further illustrate the relevance of the *CFHR1* gene we tested the value of a model of disease risk based on the *CFHR1* genotypes. We show that this model provides meaningful predictive values for the development of AMD that are comparable to those obtained with models based on SNPs at the *CFH* gene.

PATIENTS, MATERIALS AND METHODS

Patients and Controls.

Our study included 259 patients of Spanish origin with advanced AMD and a cohort of 191 age-matched controls. Cases were recruited from the Department of Ophthalmology, Clínica Universidad de Navarra, Pamplona, Spain. The general inclusion criteria for the patients with advanced AMD and controls were as follows: age of 60 years or older; absence of other retinal diseases related to coroidal neovascularization (CNV), such as angioid streaks, a nevus in the macular area, toxoplasma scars, photocoagulation scars in the posterior pole, or polypoidal choroidal vasculopathy, and less than 6 dioptres of myopia. The inclusion criteria for patients with advanced AMD included drusen and a chorioretinal macular atrophy involving central macular or signs related CNV at least in one eye (category 4 of AREDS study)[28]. The inclusion criteria for controls were absence of drusen or no more than 5 small drusen (no exceeding 65 microns), absence of retinal pigment abnormalities in the macular area, absence of chorioretinal macular atrophy or any form of CNV, (category 1 of AREDS study)[28]. All samples were obtained with informed consent in accordance with the Declaration of Helsinki and our institutional review boards.

Genotyping

Genomic DNA was extracted from peripheral blood using standard procedures or from oral swabs using QIAcube (QIAGEN, Valencia, CA). DNA samples were genotyped for seven SNPs in four of the genes previously shown to be associated with AMD (*CFH* Ile62Val, *CFH* Tyr402His, *CFH* c.2237-543A>G, *CFB* Leu9His, *CFB* Arg32Gln/Trp, *C3* Arg102Gly and *ARMS2* Ala69Ser). The genotyping was performed using multiplex PCR and primer extension methodology (ABI Snapshot, Applied Byosistem, Foster

City, CA) in a 3730 automated sequencer (Applied Byosistem, Foster City, CA). The fragments were analyzed with the Applied Biosystems GeneMapper® Software v4.0. The analysis of the $\Delta_{CFHR3\text{-}CFHR1}$ polymorphism and the genotyping of *CFHR1* was performed as described previously[29]. All the polymorphisms analyzed in this report were in Hardly-Weingberg equilibrium.

Haplotype determinations

Linkage disequilibrium analysis of the variants within *CFH* and *CFHR1* polymorphic loci was performed using the MIDAS software[30]. Haplotype frequencies in the controls and patients cohorts were estimated using the expectation maximization algorithm implemented by the SNPstats software at the Web site: http://bioinfo.iconcologia.net/snpstats/start.htm. Calculation of p values between groups was performed by Pearson's Chi-square test of association and considered significant if two sides p value <0.05. Odds ratios (OR) and 95% Confidence Intervals (CI) were also calculated.

Statistical Analysis

Statistical analyses were performed using the SPSS software for Windows version 14.0 (Chicago, IL, USA). Allele frequencies differences between cases and controls were assessed by performing a Pearson's χ^2 test of association and OR and 95% CI were calculated. *CFH*, *CFHR1*, *CFB* and *ARMS2* genotypes for each individual were included in a transversal comparative case-control study using multivariate logistic regression analysis and ROC (Receiver Operating Characteristics) statistics to generate and test models predictive for developing AMD based exclusively on the genotypes of the genes considered in the study. Cumulative risk scores (Z) were determined by the

equation $Z=\alpha+\Sigma\beta_i X_i$ where the regression coefficients α (constant) and β_i (risk score specific for each genotype X) are taken directly form the multivariate logistic regression analysis. Probabilities of developing AMD (P) were calculated as $P=e^Z/(1+e^Z)$ and were categorized in four risk groups: very low (P<25%), low (25%>P<50%), high (50%>P<75%) and very high (P>75%). A two sided p value <0.05 was considered statistically significant.

RESULTS

The *CFHR1**A allele is strongly associated with AMD.

We have recently described a novel polymorphism of the *CFHR1* gene with two alleles, *CFHR1**A and *CFHR1**B, encoding CFHR1 proteins that present different degree of similarity to factor H (Figure 1). The CFHR1*B allotype, with greater sequence similarity to factor H is strongly associated with atypical hemolytic uremic syndrome (aHUS), perhaps suggesting that increased competition between CFHR1*B and factor H decreases protection of cellular surfaces against complement damage[29]. To study whether this novel *CFHR1* polymorphism also influences predisposition to AMD we genotyped our AMD cohort and the matched control population for the *CFHR1* allotypes. In addition, we included in these studies the analysis of other polymorphisms that had been previously associated with AMD[3-10, 13] (see Materials and Methods). The allelic frequencies of each of these polymorphisms were determined and compared between the two groups (Table 1). In agreement with previous studies we have found strong positive associations between the alleles *CFH-402His*, *CFH-c.2237-543G* and *ARMS2-69Ser* and AMD, and a strong protective association of *CFH-62Ile*, *CFB-9His* and *CFB-32Gln/Trp* with AMD. *C3-102Gly*, previously found associated with AMD, shows a similar positive trend in our population that was not statistically significant. Interestingly, the three *CFHR1* alleles show very distinct associations with AMD. A very strong and significant association was found between AMD and the *CFHR1* allotype *CFHR1*A* (OR= 2.08, 95% CI 1.59-2.73), whereas no significant differences in the frequency of the *CFHR1*B* allele were found between AMD patients and controls (0.34 *vs* 0.38). As previously reported, the third *CFHR1* allele $\Delta_{CFHR3\text{-}CFHR1}$ shows a very significant protective effect for AMD (OR= 0.34, 95% CI 0.23-0.50) (Table 1). The associations between the six *CFHR1* genotypes and AMD are depicted in Table 2.

*CFHR1*A* show a very strong positive association with AMD, but only when this allele is in homozygosis (OR= 3.08, 95% CI 1.92-4.92). Interestingly, the positive association of the *CFHR1*A* allele with AMD disappear in the *CFHR1*A-CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ heterozygote carriers. In fact, the frequency of these heterozygotes is significantly decreased in AMD indicating that this genotype protects against AMD (OR=0.40, 95% CI 0.24-0.69). Therefore, the *CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ allele has a dominant effect over the *CFHR1*A* allele. The *CFHR1* genotype data shown in Table 2 confirms the neutral role of the *CFHR1*B* allele in AMD. In agreement with previous reports, the *CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ homozygote genotype has a strong AMD protective effect (OR= 0.48, 95% CI 0.17-1.37), although it is not statistically significant in our study due to the reduced size of our control and patient cohorts.

CFHR1*A is in strong linkage disequilibrium with _CFH_ 402His.

Strong linkage disequilibrium across the *CFH-CFHR3-CFHR1* genes has been described earlier[24, 27]. Consistently with these findings we found strong linkage disequilibrium between SNPs in the *CFH* gene and the *CFHR1* alleles in both the AMD and control populations (Figure 2), which further illustrate that the *CFH* haplotypes associated with AMD extend away from the *CFH* gene to include the *CFHR1* alleles. Comparison of the frequencies for these extended haplotypes (including the *CFH*, *CFHR3* and *CFHR1* genes) between the AMD and the control cohorts demonstrated three mayor extended haplotypes associated with risk or protection to AMD that correlate well with the *CFHR1* alleles. Accordingly, *CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ occurs predominantly on an extended haplotype that includes the AMD protective *CFH* haplotype H4, *CFHR1*A* is in strong linkage disequilibrium with the AMD risk allele *CFH-402His* and occurs predominantly in the AMD risk *CFH* haplotype H1, and

*CFHR1*B* distributes almost equally between two extended haplotypes, one including the protective *CFH* haplotype H2 and another including the neutral *CFH* haplotype H3 (Figure 2). It is also interesting that the *CFH-402His* and *CFHR1**A alleles shows much stronger linkage disequilibrium in controls than in AMD patients (0.90 vs 0.78) (Table 3). This could be related to the slight increase of some minoritary haplotypes carrying *CFHR1**A without *CFH-402His* in the AMD cohort (Figure 3). If confirmed, these data would emphasize the relevance of *CFHR1* for the association of this extended *CFH* haplotype with AMD.

Usefulness of the *CFHR1* genotypes in the calculation of AMD risk scores and probability of AMD development.

To evaluate the relevance of the *CFHR1* genotypes in assessing the predictability of AMD development, we compared two alternative models including different sets of polymorphisms associated with AMD in the Spanish population. Model 1, considers the *CFH* Tyr402His genotypes together with those of the *CFB* Leu9His, *CFB* Arg32Gln/Trp, *ARMS2* Ala69Ser and $\Delta_{CFHR3\text{-}CFHR1}$ polymorphisms. Model 2, is based on the genotypes at the triallelic locus *CFHR1* in addition to the genotypes of the *CFB* Leu9His, *CFB* Arg32Gln/Trp and *ARMS2* Ala69Ser polymorphisms. We have used multivariate logistic regression analysis and ROC statistics for the calculation of the AMD risk scores and the probability of AMD development using these two models (Table 4; Figure 3). Regression coefficients (risk scores) for all the genotypes were determined comparing their frequencies in cases and controls for the two models (Table 4). To assess the cumulative AMD risk scores (Z) for each individual we simply added the risk scores (α and β regression coefficients) obtained in the multivariate logistic regression analysis (Table 4) for each of the genotypes carried by the individual. The

ROC curve analysis showed no significant differences between both models (AUC values: 0.79 *vs* 0.78) in assessing the predictability of AMD (Figure 3). Furthermore, at the appropriate cumulative risk score cut-offs, both models yielded virtually identical sensitivities and specificities of approximately 70%. Therefore, the *CFHR1* genotypes provide as meaningful predictive values for AMD as the *CFH* Tyr402His and $\Delta_{CFHR3\text{-}CFHR1}$ genotypes.

Figure 3b shows a plot of the individual probabilities (P) of developing AMD, calculated from the cumulative AMD risks scores for model 2 (see Materials and Methods). In general, cases had higher probabilities than controls. To illustrate this better we categorized the probabilities in four groups of risk: very low (VL; P<25%), low (L; 25%>P<50%), high (H; 50%>P<75%) and very high (VH; P>75%). As expected, the controls with no sign of AMD tend to cluster in the low risk groups, whereas our AMD cases fell predominantly into the high-risk groups (Figure 3c).

AMD shows a prevalence of 25% in the general Spanish population older than 80 years[31]. We have used this figure and the frequency distribution of cases and controls in the four risk categories to simulate the frequencies of AMD cases within each risk category in the general population and thus to provide an estimation of the relative risk to have AMD by 80 years-old of the individuals in each category (Figure 4). Under this model an individual in the very low-risk (VL) category has a 10-fold lower relative risk for AMD that one in the very high-risk (VH) category. The overall population prevalence of 25% was exceeded only in the two highest-risk groups (H and VH), with an estimated AMD prevalence rising to 61% for those in the VH risk group. This risk group represents approximately 18% of the general population over 80 years-old.

DISCUSSION

Here we report that *CFHR1*A*, a recently described allele in the *CFHR1* gene, is over-represented in AMD patients (OR, 2.08; 95% CI, 1.59-2.73; P<0.0001) and included in the risk haplotype carrying the *CFH-402His* allele. The identification of *CFHR1*A* as a risk factor for AMD is consistent with the strong AMD protective effect associated with the deletion of this gene and suggests a relevant role for *CFHR1* in AMD pathogenesis. Moreover, our findings illustrate a distinct association of the *CFHR1* alleles with AMD and other diseases, like atypical Hemolytic Uremic Syndrome, which may provide insights into AMD pathogenesis. Further, we demonstrate the value of *CFHR1* genotyping in prediction of AMD risk.

AMD has a strong genetic component with a major susceptibility locus encompassing the *CFH*, *CFHR3* and *CFHR1* genes within the Regulators of Complement Activation (RCA) gene cluster in 1q31. Strong linkage disequilibrium at this genomic region limit the genetic variability to a small set of extended *CFH* haplotypes, which includes one haplotype conferring increased risk and two haplotypes that protect from AMD. *CFH-402His*, *CFH-62Ile* and $\Delta_{CFHR3\text{-}CFHR1}$, respectively, are considered the genetic variations within each of these extended *CFH* haplotype responsible for the association with AMD[23]. The *CFH* Tyr402His polymorphism, in particular, has been extensively studied from a functional point of view. These studies indicated that this amino acid substitution alters the binding specificity of factor H for different glycosaminoglycans[19-20] and decreases its binding to retinal pigment epithelial cells[21] and Bruch membrane[22]. However, the physiological relevance of these observations is still unclear. In contrast, the factor H 62Ile variant, showing increased complement regulatory activity, likely confers lower risk to AMD by reducing complement activation[16].

In close proximity to *CFH,* within the extended *CFH* haplotypes, are *CFHR1* and *CFHR3*, two genes coding for proteins showing sequence and structural homology to factor H (Figure 1). CFHR3 and CFHR1 are both complement regulators that compete with factor H for binding to C3b. CFHR1 acts downstream of factor H and inhibits the C5-convertase and the lytic pathway, while CFHR3 acts as a cofactor for factor I in inactivating C3b[26-27]. Based on the reported functional activities of CFHR1 and CFHR3 it seems paradoxical that the loss of these two complement regulators strongly protects against AMD. On the other side, it has been consistently found that $\Delta_{CFHR3\text{-}CFHR1}$ is an independent protective factor for AMD, which suggest that CFHR3 and/or CFHR1 protein function confers risk to AMD.

The concept that CFHR1 confers risk to AMD is further supported by our findings that *CFHR1* encodes two major alleles, *CFHR1*A* and *CFHR1*B* alleles with different degree of similarity with factor H, which associated differentially with disease[29]. We have shown earlier that homozygosity for the *CFHR1*B* allotype, with greater sequence similarity to factor H, is strongly associated with atypical hemolytic uremic syndrome (aHUS) (OR=2.54; 95% CI 1.36-4.73; P=0.0032) and suggested that increased competition between CFHR1*B and factor H decreases protection by factor H of cellular surfaces against complement damage[29]. Here we show that CFHR1*A, which is neutral in aHUS, is strongly associated with AMD in homozygosis (OR=3.08; 95% CI 1.92-4.92; P<0.0001). Interestingly, the association of both *CFHR1*B* with aHUS and *CFHR1*A* with AMD seems to be dose dependent, as they disappear if only one allele is preset (*CFHR1*B/CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ or *CFHR1*A/CFHR1*$\Delta_{CFHR3\text{-}CFHR1}$ heterozygotes). This indicates that the *CFHR1*$\Delta_{CFHR3\text{-}CFHR}$ allele is protective in both aHUS and AMD, despite homozygotes *CFHR1*$\Delta_{CFHR3\text{-}CFHR}$ are increased in aHUS due to the association with anti factor H autoantibodies[26-27]. A last relevant observation from

our association studies is that *CFHR1*A/CFHR1*B* heterozygotes are not increased in AMD and aHUS. If we assume that CFHR1 proteins associate with disease because of competition with factor H, these data suggests that the CFHR1*A and CFHR1*B allele may compete distinct functional activities in factor H that are essential in the pathogenesis of one or other disorder. Detailed functional analyses of the CFHR1*A and CFHR1*B allele would be needed to determine whether this hypothesis is correct.

Despite these uncertainties, our genetic analysis of *CFHR1* in AMD suggests that models based on *CFHR1* genotypes should provide meaningful predictive values for the development of AMD. The results presented here probe this suggestion to be correct. In fact, our model of disease risk based on the *CFHR1, CFB* and *ARMS2* genotypes works well in assessing the predictability of AMD (Figure 3) with AUC values of 0.78 and sensitivities and specificities of approximately 70%. These are significant values for a model based exclusively on genetic markers. Relatives of AMD patients and individuals with very early-stage disease signs may benefit from knowing their genetic risk as this may anticipate diagnosis and potential treatments.

Conclusions and limitations of the study.

We show here that the *CFHR1*A* allele is strongly associated with AMD. These novel data and previous studies in our laboratory[29] demonstrate a differential association of the two major *CFHR1* allotypes, *CFHR1*A* and *CFHR1*B*, with AMD and aHUS, respectively, which suggests that *CFHR1* play a relevant role in these pathologies. In fact, and despite *CFHR1*A* is in strong linkage disequilibrium with the *CFH-402His* allele, our data support that *CFHR1*A* is a strong candidate within the mayor risk haplotype at 1q32 for being responsible for the association with AMD. However, our study has limitations. It includes a relatively small case-control cohort, which prevent us

to determine whether the *CFHR1*A* variant remains significantly associated with AMD after removing the *CFH-402His*-carrying haplotypes. Additional studies and further understanding of the functional activities of CFHR1, including the potential differences between the *CFHR1*A* and *CFHR1*B* allotypes, may help to determine whether the *CFHR1* genotypes are more relevant to AMD than the *CFH* Tyr402His variant. Meanwhile, we have tested the usefulness of *CFHR1* to generate models of disease risk and demonstrated that the *CFHR1* genotypes provide meaningful predictive values for the development of AMD.

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Table 1. Polymorphisms in the *CFH, CFB, C3, CFHR1* and *ARMS2* genes associated with AMD.

Gene (SNP)	Allele	Allele frequencies		p_value	OR (95% CI)
		Controls (n=191)	AMD (n=259)		
CFH (rs88292)	c.62Ile	0.25	0.14	<0.0001	0.48 (0.34-0.67)
CFH (rs1061170)	c.402His	0.29	0.49	<0.0001	2.27 (1,71-3.00)
CFH (rs1410996)	c.2237-543G	0.55	0.76	<0.0001	3.96 (2.98-5.30)
CFB (rs4151667)	c.9His	0.04	0.02	0.0087	0.34 (0.14-0.79)
CFB (rs12614; rs64115; rs641153)	c.32Gln/Trp	0.26	0.19	0.0093	0.66 (0.48-0.90)
ARMS2 (rs10490924)	c.69Ala	0.8	0.55	<0.0001	0.30 (0.22-0.41)
CFHR1	*CFHR1*A*	0.39	0.57	<0.0001	2.08 (1.59-2.73)
	*CFHR1*B*	0.38	0.34	0.176	0.83 (0.63-1.09)
	$\Delta_{CFHR1\text{-}CFHR3}$	0.23	0.09	<0.0001	0.34 (0.23-0.50)
C3 (rs4151667)	c.102Gly	0.2	0.24	0.094	1.32 (0.95-1.82)

The nomenclature of the polymorphisms is referred to the transcription start site (+1) of each gene. Alleles at the *CFHR1* gene are as described in the text. The P_value is the result of a two-sided Pearson's Chi-square test of association for the comparison of the allelic frequencies between the AMD and control groups.

TABLE 2. *CFHR1* genotypes associated with AMD

	Controls (n=191)		AMD (n=259)			
Genotype	**n**	**frecuency**	**n**	**frequency**	**P_value**	**OR (95%CI)**
*CFHR1*A / CFHR1*A*	29	0.15	92	0.36	<0.0001	3.08 (1.92-4.92)
*CFHR1*B / CFHR1*B*	32	0.17	38	0.15	0.55	0.85 (0.51-1.43)
$\Delta_{CFHR1\text{-}CFHR3}/\Delta_{CFHR1\text{-}CFHR3}$	9	0.05	6	0.02	0.16	0.48 (0.17-1.37)
*CFHR1*A* / $\Delta_{CFHR1\text{-}CFHR3}$	40	0.21	25	0.1	0.0008	0.40 (0.24-0.69)
*CFHR1*B* / $\Delta_{CFHR1\text{-}CFHR3}$	30	0.16	11	0.04	<0.0001	0.39 (0.12-0.49)
*CFHR1*A / CFHR1*B*	51	0.27	87	0.34	0.12	1.39 (0.92-2.09)

TABLE 3. Linkage disequilibrium analysis between *CFH* SNPs and *CFHR1* alleles

CONTROLS

CFH (rs88292) **c.62Val**	D'=0,77 r2=0,08	D'=0,83 r2=0,29	**A**	D'=0,52 r2=0,06
			B	D'=-0,62 r2=0,21
			Δ_{R3R1}	D'=-0,81 r2=0,07
	CFH (rs106117) **c.402His**	D'=0,91 r2=0,28	**A**	D'=0,90 r2=0,53
			B	D'=-0,82 r2=0,17
			Δ_{R3R1}	D'=-1 r2=0,12
		CFH (rs1410996) **c.2237-543G**	**A**	D'=0,83 r2=0,36
			B	D'=-0,12 r2=0,01
			Δ_{R3R1}	D'=-0,89 r2=0,29

CFHR1

AMD

CFH (rs88292) **c.62Val**	D'=0,95 r2=0,14	D'=0,92 r2=0,45	**A**	D'=0,64 r2=0,09
			B	D'=-0,55 r2=0,10
			Δ_{R3R1}	D'=-0,01 r2=0,00
	CFH (rs106117) **c.402His**	D'=0,86 r2=0,22	**A**	D'=0,78 r2=0,43
			B	D'=-0,76 r2=0,28
			Δ_{R3R1}	D'=-0,77 r2=0,51
		CFH (rs1410996) **c.2237-543G**	**A**	D'=0,63 r2=0,17
			B	D'=-0,13 r2=0,01
			Δ_{R3R1}	D'=-0,97 r2=0,23

CFHR1

Table 4. Logistic regression models.

Gen (polymorphism)	Genotype	Model 1 (*402His; $\Delta_{CFHR3-CFHR1}$*)			Model 2 (*CFHR1*)		
		Regression coefficient (β)	P_value	OR (95%CI)	Regression coefficient (β)	P_value	OR (95%CI)
CFB (L9H)	L/L	0			0		
	L/H	-1.114	0.018	0.32 (0.12-0.82)	-1.084	0.023	0.34 (0.13-0.86)
CFB (R32Q/W)	R/R	0.632	0.252	1.88 (0.64-5.54)	0.552	0.317	1.73 (0.60-5.11)
	R/Q or W	-0.284	0.613	0.75 (0.25-2.26)	-0.359	0.524	0.70 (0.23-2.10)
	Q or W/Q or W	0			0		
ARMS2 (A69S)	A/A	0			0		
	A/S	0.960	<0.001	2.61 (1.64-4.16)	0.963	<0.001	2.62 (1.65-4.15)
	S/S	2.148	<0.001	8.57 (3.89-18.8)	2.223	<0.001	9.23 (4.19-20.3)
CFH (Y402H)	Y/Y	0			-	-	-
	Y/H	0.585	0.017	1.79 (1.11-2.90)	-	-	-
	H/H	1.178	0.001	3.25 (1.60-6.61)	-	-	-
$\Delta_{CFHR3-CFHR1}$	Del/Del	-1.577	0.025	0.21 (0.05-0.80)	-	-	-
	Del/wt	-1.029	<0.001	0.36 (0.21-0.60)	-	-	-
	wt/wt	0			-	-	-
CFHR1 (A/B/$\Delta_{CFHR3-CFHR1}$)	A/A	-	-	-	0.992	0.004	2.69 (1.37-5.31)
	B/B	-	-	-	0		
	Del/Del	-	-	-	-1.041	0.102	0.35 (0.10-1.23)
	A/B	-	-	-	0.525	0.111	1.69 (0.88-3.23)
	A/Del	-	-	-	-0.538	0.157	0.58 (0.28-1.23)
	B/Del	-	-	-	-1.012	0.026	0.36 (0.15-0.89)
	Constant (α)	-0.678			-0.658		

FIGURE LEGENDS

Figure 1. Structural similarities between the *CFHR1* alleles and factor H

Schematic representation of the structural organization of factor H and the CFHR1*A and CFHR1*B. Short consensus repeats (SCRs) are represented by circles and are numbered from the N-ternimal end. Homologous SCRs are aligned and the amino acid differences with factor H indicated for SCR-3, -4 and -5 of CFHR1*A and CFHR1*B. SCR 1 and 2 of CFHR1 show only a 41% homology with factor H. The homologous position to factor H 402His in both CFHR1*A and CFHR1*B is 100Ser.

Figure 2: *CFH-CFHR3-CFHR1* haplotypes and their association with AMD.

Figure shows the exon structure of *CFH*, *CFHR3* and *CFHR1* showing the location of the polymorphism included in these studies. Exon 10, characyteristic of the alternative spliced protein CFHL1 is depicted in green. Haplotype frequencies in the control and patient cohorts were estimated using the EM algorithm (Expectation Maximization algorithm) implemented by the SNPStats software available on-line at (http://bioinfo.iconcologia.net/SNPstats). *CFH* haplotypes with a frequency >1% are shown. The frequency of each *CFH* haplotype was compared between the controls and the AMD cohorts and the p-values and the OR were calculated. Risk haplotypes are shaded in red whilst protective haplotypes are shaded in green. p-values were derived using Pearson's Chi-square test of association. Odds ratio (OR) and 95% confidence intervals (CI) are shown. The nucleotide and amino acid numbering are referred to the translation start site (A in ATG is +1; Met is +1) as recommended by the Human Genome Variation Society (HGVS).

Figure 3: Model of disease risk based on *CFHR1*, *CFB* and *ARMS2* genotypes

A) Comparison of disease risk models. Model 1 (based on genotypes of the *CFH* Tyr402His, $\Delta_{CFHR3\text{-}CFHR1}$, *CFB* Leu9His, *CFB* Arg32Gln/Trp and *ARMS2* Ala69Ser polymorphisms). Model 2 (based on genotypes of the *CFHR1*, *CFB* Leu9His, *CFB* Arg32Gln/Trp and *ARMS2* Ala69Ser polymorphisms). The ROC curve analysis showed no significant differences between both models (AUC values: 0.79 *vs* 0.78) in assessing the predictability of AMD. Optimal risk cut-offs provide sensitivities and specificities of approximately 70% for both models.

B) Dot diagram for the distribution of cases and controls for model 2. Risk categories were established as described in Materials and Methods.

C) Distribution of Spanish AMD cases and controls according to their frequencies in the risk groups. Risk categories were established as described in Materials and Methods.

Figure 4: Distribution of AMD cases and healthy individuals in the >80y old Spanish population according to their predicted distribution in the risk groups and on a prevalence of AMD of 25%.

Percentages of AMD (black) cases and healthy individuals (white) within each group are indicated.

Figure 1.

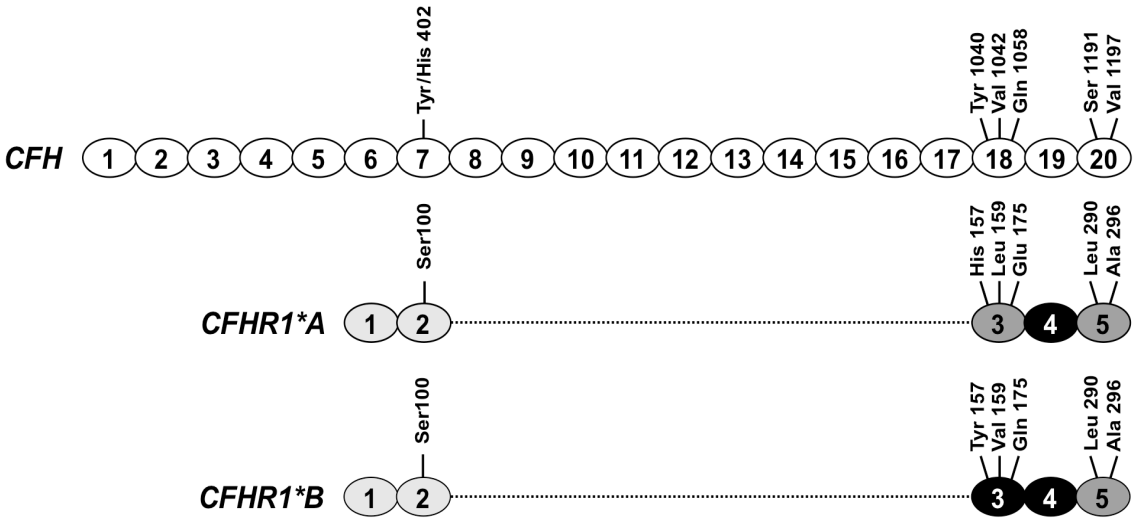
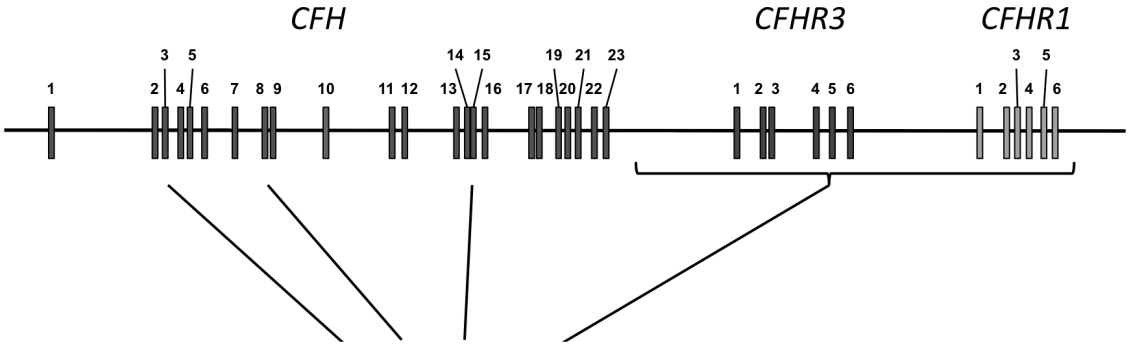
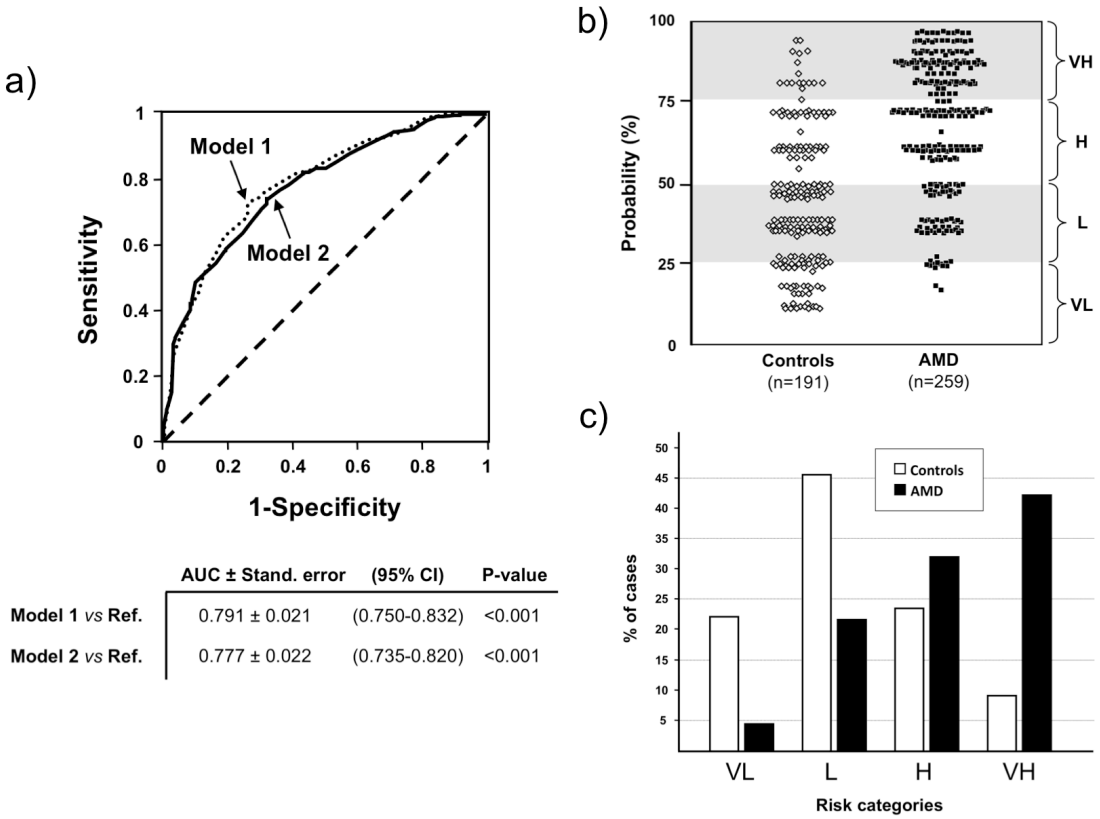

CFH
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Tyr/His 402
Tyr 1040
Val 1042
Gln 1058
Ser 1191
Val 1197
CFHR1*A
1 2 3 4 5
Ser100
His 157
Leu 159
Glu 175
Leu 290
Ala 296
CFHR1*B
1 2 3 4 5
Ser100
Tyr 157
Val 159
Gln 175
Leu 290
Ala 296

Figure 2.



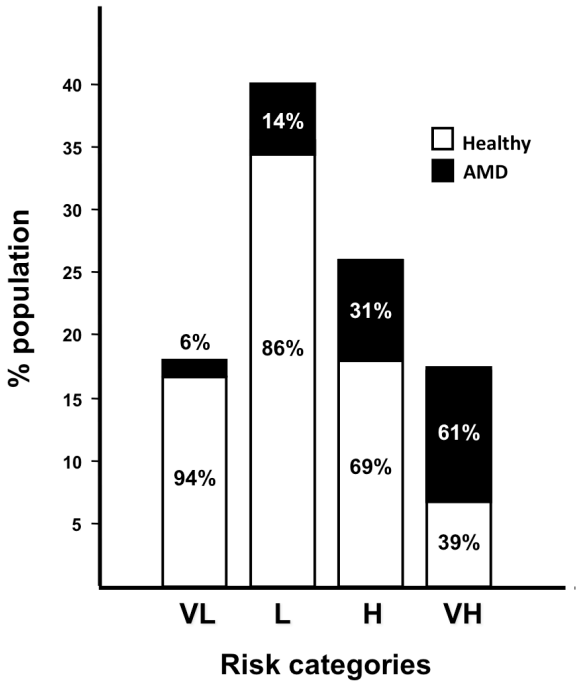
	c.184G/A V62I (rs800292)	c.1204T/C Y402H (rs1061170)	c.2237-543A/G (rs1410996)	*CFHR1*	Control (n=191)	AMD (n=259)		
					Freq	Freq	OR (95% CI)	P value
H1	G	C	G	*CFHR1*A*	0,26	0.43	2.12 (1.59-2.82)	$< 10^{-4}$
H2a	A	T	A	*CFHR1*B*	0.19	0.10	0.46 (0.31-0.67)	$< 10^{-4}$
H2b	A	T	A	*CFHR1*A*	0.02	0.02	-	NS
H2c	A	T	A	$\Delta_{CFHR3\text{-}CFHR1}$	0.02	0.01	-	NS
H3a	G	T	G	*CFHR1*B*	0.17	0.19	-	NS
H3b	G	T	G	*CFHR1*A*	0.08	0.09	-	NS
H4	G	T	A	$\Delta_{CFHR3\text{-}CFHR1}$	0.20	0.06	0.26 (0.17-0.40)	$< 10^{-4}$

Figure 3.



	AUC ± Stand. error	(95% CI)	P-value
Model 1 *vs* **Ref.**	0.791 ± 0.021	(0.750-0.832)	<0.001
Model 2 *vs* **Ref.**	0.777 ± 0.022	(0.735-0.820)	<0.001

Figure 4.


40
35
30
25
20
15
10
5
% population
14%
Healthy
AMD
31%
6%
86%
61%
94%
69%
39%
VL
L
H
VH
Risk categories