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Comprehensive Analysis of CFH and LOC387715/ARMS2/HTRA1 Variants with respect to Phenotype in Advanced Age Related Macular Degeneration

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Abstract

Purpose—To examine the interaction of genotypic variation of 16 single nucleotide polymorphisms (SNPs) in the Complement Factor H (*CFH*) and *LOC387715/ARMS2/HTRA1* loci with clinical characteristics of age-related macular degeneration (AMD).

Design—Retrospective cohort study

Methods—Eighty-four patients with neovascular AMD were genotyped using direct sequencing or Sequenom iPLEX technology. Fisher's exact test, Cochran-Mantel-Haenszel statistics, and Mann-Whitney tests were used to assess the effect of each SNP with respect to the following phenotypic manifestations: age at diagnosis, sex, affected eye, study and fellow eye visual acuity at diagnosis and at last follow-up, study eye best acuity during follow-up, presence of large drusen and retinal pigment epithelium (RPE) hyperpigmentation in study and fellow eye, choroidal neovascularization (CNV) angiographic subtype (classic vs. occult), CNV size, presence of wet AMD in fellow eye, presence of dry AMD in fellow eye, and smoking history.

Results—Only SNPs in the *LOC387715/ARMS2/HTRA1* (10q26) region were associated with disease phenotypes. The polymorphisms rs10664316 and rs1049331 were associated with decreased risk of poor visual acuity during follow-up and at diagnosis; rs2672598 and rs2293870 were associated with decreased risk of RPE hyperpigmentation; rs10664316 was associated with decreased risk of RPE hyperpigmentation with large drusen in the study eye, but increased risk of large drusen in the fellow eye; rs11200638 was associated with increased risk of larger CNV; rs10490924 and rs11200638 were associated with younger age of diagnosis.

Conclusions—Several polymorphisms examined in the *LOC387715/ARMS2/HTRA1* locus, but none in the *CFH* region, correlated with specific phenotypic attributes of AMD.

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INTRODUCTION

Knowledge regarding the genetic basis of age-related macular degeneration (AMD) has advanced significantly in the past few years. Multiple groups have confirmed the complement factor H (*CFH*) gene and its associated polymorphisms on human chromosome 1 as risk factors for AMD in diverse populations.[1–8] More recently, single nucleotide polymorphisms (SNPs) in the age-related maculopathy susceptibility 2/HtrA serine peptidase 1 gene(s) (*LOC387715/ARMS2/HTRA1*) on chromosome 10 have also shown strong associations with both dry and wet AMD. However, the clinical applications of our ability to identify genetic predisposition to AMD has yet to be clarified.

Several groups have evaluated the associations of specific genotypes with clinical features in AMD. Some groups have reported an association of the high-risk allele of the complement factor H (*CFH*) Y402H polymorphism (rs1061170) with predominantly classic choroidal neovascularization (CNV) [9–12], but others have noted the opposite correlation.[13] Larger CNV lesion size has been associated with the low-risk allele at *CFH* rs1061170.[14] Some correlations have also been noted with respect to treatment response. Both the low- and high-risk genotypes of the *CFH* Y402H polymorphism have been reported as predisposing factors to vision loss after photodynamic therapy (PDT).[12, 15] Recently, the high-risk *CFH* Y402H genotype has been shown to be associated with lower rates of visual improvement after treatment with bevacizumab[14].

There are only a few reports demonstrating the correlation of genotype at the chromosome 10q26 locus with respect to AMD phenotype. The risk genotype of the *LOC387715/ARMS2* A69S polymorphism (rs10490924) was reported to be associated with larger CNV lesion size and younger age of onset.[14, 16] The risk allele of the SNP rs11200638 in *HTRA1* has been associated with bilaterality, classic CNV, and increased CNV size.[13, 17, 18]

Therefore, prior studies demonstrate some conflicting results and have been limited to studying the phenotypic correlations for mainly 3 SNPs – rs1061170 in *CFH*, rs10490924 in *LOC387715/ARMS2*, and rs11200638 in *HTRA1*. This study was designed to investigate the correlation of 10 SNPs in the CFH gene (1q32) and 6 SNPs in the 10q26 region with a number of phenotypic characteristics in a population of patients with neovascular AMD.

METHODS

The protocol was reviewed and approved by the institutional review board at the Massachusetts Eye and Ear Infirmary, Boston, MA, and conforms to the tenets of the Declaration of Helsinki. Eligible patients were enrolled in this study after they gave informed consent in person, through the mail, or over the telephone. All patients were interviewed utilizing a standardized questionnaire and donated 10 to 50 mL of venous blood.

Patient Population

Patients with neovascular AMD included for the current study were originally recruited from the Retina Service of the Massachusetts Eye and Ear Infirmary from June 2001 until June 2006 for an ongoing genetic analysis of extremely discordant sibling pairs. Details of the recruitment and of the clinical description of the patients have been previously published.[1] In brief, all patients had the neovascular form of AMD in the study eye. The study eye was defined as the eye with the neovascular form of AMD, defined by fibrosis, subretinal hemorrhage, or fluorescein angiographic presence of choroidal neovascularization documented at the time of or before study enrollment.[19–21]. For patients with bilateral neovascular disease, the eye with more complete clinical information (in most cases, the eye with the more recent diagnosis of CNV) was chosen as the study eye. Disease status of every participant was confirmed by at

least 2 of the investigators (I.K.K. and J.W.M.) by evaluation of fundus photographs and fluorescein angiograms. Although each patient with AMD was required to have a sibling of at least the same age without AMD for our previous publications, this current study only involved the subset of index patients (i.e. those with neovascular AMD) on whom adequate clinical information was available for phenotypic analysis.

Genotyping Analysis

All SNPs, including insertions and deletions, analyzed in this cohort had been previously found to be associated with neovascular AMD risk in a much larger cohort of extremely discordant sibpairs. Specifically, all individuals had been previously genotyped for *CFH* (rs800292, rs35507625, rs572515, rs1061147, rs7529589, rs482934, rs1061170, rs12038333, rs2274700, and rs203674) and *LOC387715/ARMS2/HTRA1*(rs10490924, rs10664316, rs11200638, rs2672598, rs1049331, and rs2293870) variants by direct sequencing for all but three *CFH* SNPs (rs7529589, rs12038333, rs203674) where the Sequenom iPLEX technology was utilized.[1, 22, 23]

Phenotype Data

A retrospective chart review of 84 patients was conducted to ascertain disease details. Age at diagnosis, sex, affected eye, follow-up duration (defined as time from diagnosis to most recent clinic visit), study and fellow eye visual acuity at diagnosis and at last follow-up, study eye best acuity during follow-up (best visual acuity between diagnosis and most recent clinic visit), presence of large drusen (≥125um) and retinal pigment epithelium (RPE) hyperpigmentation in study and fellow eye, choroidal neovascularization (CNV) angiographic subtype (classic vs. occult), CNV size (< 4 disc areas (DA) versus ≥ 4 DA), treatment for CNV, presence of wet AMD in fellow eye, presence of dry AMD in fellow eye, and smoking history were recorded when available.

Statistical Analysis

Initially, Fisher's exact test was used to evaluate the effect of each SNP with the phenotypic characteristics as described above using EpiCalc 2000 (Brixton Health, http://www.brixtonhealth.com/epicalc.html). Cochran-Mantel-Haenszel statistics (SAS 9.1 [http://www.sas.com]) were used to estimate the odds ratios, 95% confidence intervals and corresponding *P* values associated with the phenotypic variables as described above. Potential risk factors were initially evaluated one at a time while controlling for age and sex. For all SNPs, the allele associated with an *increased* risk of neovascular AMD (determined from the larger sibpair cohort that included unaffected patients)[1, 22] was tested, regardless of the allele frequency in this neovascular only cohort. Specifically, the SNP was examined in the following manner: at least one risk allele versus no risk alleles. Genotype and allele frequencies for all variants examined were calculated (Table 1).

Ages and visual acuities (after conversion to logMAR) were further analyzed as continuous variables using Mann-Whitney tests. The mean age and mean visual acuities were then stratified according to genotype and tested using a Student's T test. All test results were considered statistically significant if $P < 0.05$. Because it is well established that cigarette smoking is a significant environmental AMD risk factor, we stratified our cohort according to smoking exposure to further examine those factors that appeared to be significant in the initial analysis ($P < 0.05$). Smoking exposure was measured in pack-years as previously described[1]. Briefly, a pack-year was defined as 1 pack of cigarettes per day for 1 year, with 1 pack defined as 20 cigarettes. For our statistical analysis, the reference cut-off for smoking was defined as greater than or equal to 10 pack-years versus less than 10 pack-years. A patient was considered to have "ever smoked" if they had smoked greater than 100 or more cigarettes in his or her lifetime.

RESULTS

Genotype information for 16 SNPs was available for all 84 patients with neovascular age-related macular degeneration. A summary of patient information is shown in Table 2. Our patient population consisted of 45 females and 39 males, with a mean age at diagnosis of 72.5 years and a mean follow-up time of 3.58 years. Of the 84 subjects, 61 (73%) had a history of smoking and 49 (58%) had smoked 10 or more pack-years. The nucleotide base change (common allele and minor allele) for each SNP investigated was previously determined by the unaffected patients from a larger cohort of sibpairs in order to determine what was representative of the general population.[22, 23] The corresponding genotype frequencies for the cohort of neovascular subjects in the present study are summarized in Table 1.

The results of statistical analysis are summarized in Table 3 and Table 4. Patients who carried at least one copy of the common allele at *LOC387715/ARMS2* rs10664316, an "AT" insertion in intron 1, were protected by 7.0-fold and 7.6-fold from having visual acuity worse than 20/200 in the study eye at the time of initial diagnosis and at any time during the follow-up period, respectively ($P = 0.003$; Table 3). Similarly, patients who had at least one copy of the minor allele in exon 1 of *HTRA1 r*s1049331, were protected by 4.5-fold and 5.6-fold from having visual acuity worse than 20/200 in the study eye at the time of diagnosis and at any time during the follow-up period, respectively ($P = 0.007$ and $P = 0.02$; Table 3). When we stratified logMAR visual acuity according to genotype, (i.e. TT or TC versus CC, Table 4), the minor allele at *HTRA1* rs1049331, was associated with better visual acuity during the follow-up period using both the Student's T-Test and the Mann-Whitney test ($P = 0.0082$ and $P = 0.0048$, respectively).

The common allele of *LOC387715/ARMS2* rs10664316 was shown to be associated with increased rates of large drusen in the fellow eye ($P = 0.023$) but lower rates of RPE hyperpigmentation and large drusen in the study eye ($P = 0.027$), even after excluding those study eyes with a history of laser treatment, which may have resulted in reactive hyperpigmentation ($P = 0.005$). *HTRA1* SNPs rs2672598 and rs2293870 were also shown to be associated with a decreased risk of RPE hyperpigmentation in the study eye ($P = 0.01$ and $P = 0.02$, respectively). The HTRA1 promoter SNP rs11200638 was associated with an increased risk of CNV size ≥ 4 DA ($P = 0.02$) and younger age at diagnosis ($P = 0.0145$). Similarly, rs10490924 in *LOC387715/ARMS2* was associated with younger age at diagnosis ($P = 0.0076$). All significant results were independent of smoking history. Nonsignificant polymorphisms can be found in Table 6. When we stratified the significantly associated findings shown in Table 3 according to smoking history, the association of *LOC387715/ARMS2* rs10664316 became more significant, with lower rates of RPE hyperpigmentation and large drusen in the study eye in those subjects who smoked 10 or more pack-years ($P = 0.000046$), even after excluding those study eyes with a history of laser treatment ($P = 0.000013$, data not shown).

DISCUSSION

The genotype-phenotype analysis presented here represents the most comprehensive study to date with respect to the number of genotypic variations evaluated. In our cohort of neovascular AMD patients, only genotypes at the chromosome 10q26 locus correlated with clinical features of disease, whereas no correlations were detected for the ten complement factor H variations. Predictably, alleles for the two SNPs in the 10q26 region most consistently shown to increase risk of AMD (rs10490924 and rs11200638) were associated with younger age of diagnosis of neovascular AMD in this study as well as others. Certainly one of the most common phenotypic manifestations of genetic predisposition to disease is earlier age of onset, and therefore these

findings serve to confirm the validity of variations in the *LOC387715/ARMS2/HTRA1* region as genetic risk factors for AMD.

The other finding that appears consistent across several studies is the association between lesion size and risk alleles of SNPs in *LOC387715/HTRA1*.[14, 18] Those alleles in this region that increase risk of AMD also appear to predispose to larger CNV lesions. Specifically, in this study *HTRA1* rs11200638 was associated with larger CNV size. Brantley and colleagues noted an association between the common (low-risk) allele of the *CFH* Y402H polymorphism and larger lesion size, but interpreted this finding as suggestive that the *CFH* polymorphism did not influence lesion size. Similarly, we did not find any relationship between *CFH* polymorphisms and lesion size in this cohort. Although others have found associations between lesion composition and genotype, these findings have not been consistent. Our data reveal no correlations between genotype and lesion subtype. One reason we may not have found associations between clinical manifestations of neovascular AMD and variants in CFH (1q32) could be due to the stronger overall association between variants on the 10q26 region and neovascular AMD risk.[23–25]

Interestingly, associations between genotype and clinical features considered predictive of progression to neovascular AMD were weak and/or inconsistent. We did find that the major allele of rs10664316 in *LOC387715* (which is associated with increased risk of AMD)[22, 26] is associated with increased incidence of large drusen in the fellow eye in the current study. However, it was also found to be associated with *decreased* incidence of RPE hyperpigmentation and large drusen in the eye with CNV. We also observed inverse correlations between other polymorphisms and the clinical risk factor of RPE hyperpigmentation in the study eye. However, the clinical significance of large drusen and RPE hyperpigmentation in an eye with established CNV is unclear, and it is possible that these types of findings become less visible after the development of CNV. Alternatively, it could be that for those patients with protective genotypes in the 10q26 region that do develop neovascular AMD, other factors, genetic or environmental, play a predominant role in CNV development. These other factors may also play a greater influence in creating the pathologic changes leading to the biomicroscopic findings such as RPE hyperpigmentation, which have previously been recognized as markers of high risk for CNV development. Similar inverse correlations were observed between vision and 10q26 region polymorphisms, suggesting that those factors that lead to neovascularization in the setting of protective genotypes may result in more aggressive disease.

Although analysis of this cohort has identified significant associations between genotypes and phenotypes, both false negatives and false positives (Type I and Type II errors) may have occurred due to an admittedly small sample size leading to a small number of observations for each particular clinical manifestation examined. The relatively small patient cohort represents a select population from the Massachusetts Eye and Ear Infirmary Retina Service, which in itself could have introduced selection bias; however, there is no reason to believe that this bias would skew the results in a particular direction. The stringency of our inclusion criteria and statistical methodologies, combined with the corroboration of our data by several recent studies, speaks to the validity of our results. While this study provides further evidence for the complicated interactions between genetic variation and macular degeneration disease state, future multicenter studies involving larger patient populations are necessary to substantiate current findings and discover new interactions. Continued critical examination of phenotypes and treatment outcomes in the context of expanding knowledge regarding the genetic contributors to AMD is imperative for clinical breakthroughs.

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Table 1

Single nucleotide polymorphisms analyzed in cohort of 84 neovascular age-related macular degeneration patients

Gene	SNP	Base Change†	Freq. of Homozygous Common Allele	Freq. of Heterozygous Allele	Freq. of Homozygous Rare Allele
CFH	rs800292*	G>A	0.80	0.18	0.02
CFH	rs35507625*	insTT	0.80	0.17	0.02
CFH	rs572515	G>A	0.20	0.44	0.36
CFH	rs1061147	C>A	0.20	0.44	0.36
CFH	rs7529589	C>T	0.20	0.42	0.37
CFH	rs482934*	T>G	0.46	0.37	0.17
CFH	rs1061170	C>T	0.35	0.42	0.22
CFH	rs12038333	A>G	0.20	0.43	0.37
CFH	rs2274700*	G>A	0.69	0.24	0.07
CFH	rs203674	T>G	0.14	0.44	0.42
LOC387715/ARMS2	rs10490924	G>T	0.31	0.35	0.34
LOC387715/ARMS2	rs10664316*	delAT	0.60	0.27	0.13
LOC387715/ARMS2	rs11200638	G>A	0.29	0.39	0.32
HTRA1	rs2672598*	C>T	0.48	0.41	0.11
HTRA1	rs1049331	C>T	0.31	0.37	0.32
			GT	CT	GG
HTRA1	rs2293870	G/T/C	0.29	0.13	0.21
			CC	CG	TT
HTRA1	rs2293870	G/T/C	0.01	0.06	0.30

Abbreviations: SNP, Single Nucleotide Polymorphism; Freq, frequency; CFH, complement factor H gene; ARMS2, age-related maculopathy susceptibility 2 gene; HTRA1, HtrA serine peptidase 1 gene.

*Previously reported as associated with decreased risk of neovascular AMD in a larger cohort of discordant sibpairs that included these patients; therefore the common allele was used as the risk allele.

†As determined by previous studies[1,22,23]. Base change is written common allele > minor allele.

Table 2

Baseline demographic and clinical data of neovascular age-related macular degeneration cohort

Total included patients	84
Female	45/84 (53.6%)
Male	39/84 (46.4%)
Average age (years ± SD)	72.5 ± 7.8
Patients with eventual bilateral disease	49/78 (63%)
Predominantly classic CNV in study eye	34/71 (48%)
Lesion size greater than 4 DA	12
Lesion size less than 4 DA	21
Non-predominantly classic CNV in study eye	37
Lesion size greater than 4 DA	23
Lesion size less than 4 DA	14
Patients with AMD treatment	64/78 (82%)
PDT Treatment	47
Macugen	6
Lucentis	11
IVTA	7
Laser	19
Proton beam	5
Smokers (ever/never)	61/84 (73%)
Smokers (≥10 pack-years)	49/84 (58%)

Abbreviations: SD, Standard Deviation; DA, Disc Area; PDT, Photodynamic Therapy; IVTA, Intravitreal triamcinolone acetonide injection

Table 3

Single nucleotide polymorphisms significantly associated with neovascular age-related macular degeneration phenotypes

Phenotype	Gene	SNP	Likelihood of Demonstrating Phenotype	Odds Ratio (95% C.I.)	*P* value
Best acuity worse than 20/200	LOC387715/ ARMS2	rs10664316	Decreased	0.131 (0.032–0.529)	0.003
	HTRA1	rs1049331	Decreased	0.178 (0.050–0.640)	0.007
Diagnosis acuity worse than 20/200	LOC387715/ ARMS2	rs10664316	Decreased	0.142 (0.036–0.557)	0.003
	HTRA1	rs1049331	Decreased	0.223 (0.065–0.773)	0.017
RPE hyperpigmentation	HTRA1	rs2672598	Decreased	0.098 (0.015–0.673)	0.014
	HTRA1	rs2293870	Decreased	0.210 (0.057–0.772)	0.019
RPE hyperpigmentation and large drusen	LOC387715/ ARMS2	rs10664316	Decreased	0.211 (0.052–0.845)	0.027
RPE hyperpigmentation and large drusen (previous laser group excluded)	LOC387715/ ARMS2	rs10664316	Decreased	0.091 (0.015–0.532)	0.005
Large drusen in fellow eye	LOC387715/ ARMS2	rs10664316	Increased	15.625 (3.745–63.740)	0.023
Large CNV (≥ 4 DA)	HTRA1	rs11200638	Increased	3.368 (1.185–9.531)	0.023

Abbreviations: SNP, Single Nucleotide Polymorphism; CI, Confidence Interval; RPE, Retinal Pigment Epithelium; DA, Disc Area; ARMS2, Age-related maculopathy susceptibility 2 gene; HTRA1, HtrA serine peptidase 1 gene.

Note: All findings our in the study eye unless otherwise noted

** For all SNPs, the allele associated with an increased risk of neovascular AMD (determined from the larger sibpair cohort that included unaffected patients) was tested.

Table 4

Single nucleotide polymorphisms associated with neovascular age-related macular degeneration phenotypes analyzed as continuous variables (age and logMAR visual acuity)

Phenotype	Gene	SNP	Genotype	Mean Value in Cohort	T-test *p* value*	Mann Whitney *p* value
Age at Diagnosis	LOC387715/ ARMS2	rs10490924	TT or TG	71.07 years	0.0101	0.0076
			GG	75.73 years		
	HTRA1	rs11200638	AA or AG	71.42 years	0.0183	0.0145
			GG	75.56 years		
Study Eye Best Acuity	HTRA1	rs1049331	TT or TC	20/76	0.0082	0.0048
			CC	20/144		

Abbreviations: SNP, Single Nucleotide Polymorphism; CI, Confidence Interval; ARMS2, Age-related maculopathy susceptibility 2 gene; HTRA1, HtrA serine peptidase 1 gene.

*T-test performed comparing those patients above and below the mean value.

Table 5

Single nucleotide polymorphisms not significantly associated with neovascular age-related macular degeneration phenotypes

Gene	SNP
CFH	rs800292
CFH	rs35507625
CFH	rs572515
CFH	rs1061147
CFH	rs7529589
CFH	rs482934
CFH	rs1061170
CFH	rs12038333
CFH	rs2274700
CFH	rs203674

Abbreviations: SNP, Single Nucleotide Polymorphism, CFH, Complement Factor H gene.