Original Investigation

Single-Nucleotide Polymorphisms Associated With Age-Related Macular Degeneration and Lesion Phenotypes in the Comparison of Age-Related Macular Degeneration Treatments Trials

Maureen G. Maguire, PhD; Gui-shuang Ying, PhD; Glenn J. Jaffe, MD; Cynthia A. Toth, MD; Ebenezer Daniel, MBBS, PhD; Juan Grunwald, MD; Daniel F. Martin, MD; Stephanie A. Hagstrom, PhD; for the CATT Research Group

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IMPORTANCE Single-nucleotide polymorphisms (SNPs) associated with the *CFH*, *ARMS2*, *C3*, *LIPC*, *CFB*, and *C2* genes are associated with age-related macular degeneration (AMD); however, the association of these SNPs with angiographic features of neovascular AMD has been inconsistent in previous studies, and to date, no studies have addressed their association with features on optical coherence tomography.

OBJECTIVE To evaluate the influence of genotype of SNPs previously associated with AMD on the phenotype of neovascular lesions.

DESIGN, SETTING, AND PARTICIPANTS Participants for this cross-sectional study were recruited from the 1185 patients enrolled in the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT), a randomized clinical trial. Eligibility criteria for CATT specified that eyes have choroidal neovascularization and visual acuity between 20/25 and 20/320. A subgroup of 835 patients provided blood samples from July 2010 through September 2011 and were genotyped for the SNPs rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs2230199 (*C3*), rs10468017 (*LIPC*), rs4151667 (*CFB*), rs547154 (*C2*) using TaqMan SNP genotyping assays. Data analysis was initiated in November 2013 and completed in January 2016.

MAIN OUTCOMES AND MEASURES Pretreatment ocular characteristics on fluorescein angiography (lesion type, area of neovascularization and total lesion, retinal angiomatous proliferation) and on time-domain optical coherence tomography (presence of intraretinal, subretinal, and subretinal pigment epithelium fluid; thickness at the foveal center of the retina, subretinal fluid, and subretinal tissue complex), visual acuity, and age.

RESULTS A total of 835 (73%) of 1150 CATT patients were genotyped. Mean age decreased with the number of risk alleles for *CFH* ($P < .001$), *ARMS2* ($P < .001$), and *C3* ($P = .005$). The following results were found as the number of risk alleles increased from 0 to 1 to 2. For *CFH*, mean total thickness decreased from 476 to 476 to 434 µm ($P = .01$; adjusted for age, sex, and smoking status). For *ARMS2*, the mean area of the total lesion increased from 2.0 to 2.8 to 2.4 mm^2 ($P = .03$), the proportion with retinal angiomatous proliferation lesions increased from 8% to 10% to 12% ($P = .05$), and the proportion with intraretinal fluid increased from 72% to 71% to 82% ($P = .008$). For *C3*, the proportion with intraretinal fluid decreased from 78% to 69% to 64% ($P = .001$), and the mean retinal thickness decreased from 225 to 207 to 197 µm ($P = .02$).

CONCLUSIONS AND RELEVANCE *CFH*, *ARMS2*, and *C3* were associated with specific features of neovascularization at the time patients were enrolled in CATT. Previously identified associations of *ARMS2* and *CFH* with type of choroidal neovascularization on fluorescein angiography were not confirmed. New associations with OCT features identified in CATT need confirmation to establish whether a true association exists.

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Author Affiliations: Department of Ophthalmology, University of Pennsylvania, Philadelphia (Maguire, Ying, Daniel, Grunwald); Department of Ophthalmology, Duke University, Durham, North Carolina (Jaffe, Toth); Cole Eye Institute, Cleveland Clinic, Cleveland, Ohio (Martin, Hagstrom).

Corresponding Author: Maureen G. Maguire, PhD, Department of Ophthalmology, University of Pennsylvania, 3535 Market St, Ste 700, Philadelphia, PA 19104-3309 (maguirem@mail.med.upenn.edu).

The features of neovascular lesions vary considerably among patients with newly diagnosed age-related macular degeneration (AMD). Some features, such as angiographic pattern of leakage and size of the lesion, are known to have a strong influence on current visual acuity (VA), prognosis for loss of vision when untreated, change in VA after laser or anti-vascular endothelial growth factor (VEGF) treatment, and development of scar or geographic atrophy after anti-VEGF treatment.[1-5]

Many single-nucleotide polymorphisms (SNPs) that confer increased risk of developing AMD have been identified; however, SNPs associated with the *CFH* (OMIM 134370), *ARMS2* (OMIM 611313), *HTRA1* (OMIM 602194), and *C3* (OMIM 120700) genes are among those most consistently associated with neovascular AMD.[6,7] Several research groups have investigated the association of these SNPs with features of neovascular lesions apparent on fundus color photography and fluorescein angiography in patients with AMD.[8-20] Most of the previous studies[8-15,18] have involved 250 or fewer patients, and the results have not been consistent. To our knowledge, there have been no previous studies of the association of these SNPs with features of neovascular AMD on optical coherence tomography (OCT). More recently, SNPs associated with the *LIPC* (OMIM 151670), *CFB* (OMIM 138470), and *C2* (OMIM 613927) genes have been associated with AMD; however, to date, association studies of these SNPs with the fluorescein angiographic and OCT features of neovascular lesions have not been conducted.[21-23]

The detailed assessments of color fundus photographs, fluorescein angiograms, and OCT scans by reading centers and genotyping of the large number of patients enrolled in the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT) allow further evaluation of the association of SNPs linked to the development of neovascular AMD with features detectable on photographs and features on OCT. Better understanding of these associations may help in determining how these SNPs affect the pathogenesis of AMD and neovascular lesions.

Key Points

Question Do single-nucleotide polymorphisms (SNPs) that lead to the development of age-related macular degeneration influence the features of neovascularization?

Findings In this cross-sectional analysis of 835 participants in the Comparison of Age-Related Macular Degeneration Treatments Trials, a greater number of risk alleles for *ARMS2* was significantly associated with larger lesions and with retinal angiomatous proliferation lesions.

Meaning Although there were modest associations for some SNPs with neovascular features, no highly predictive genotypes were identified among the 6 SNPs evaluated.

Methods

Study Population for the Clinical Trial

Details of the design and methods for CATT have been published previously.[24-27] From February 1, 2008, through December 31, 2009, a total of 1185 patients were recruited for the randomized clinical trial (clinicaltrials.gov Identifier: NCT00593450) through 43 clinical centers in the United States. Inclusion criteria were age of 50 years or older, presence in the study eye of previously untreated active choroidal neovascularization (CNV) secondary to AMD, and VA between 20/25 and 20/320 in the study eye. Active CNV was considered present when leakage on fluorescein angiography and fluid OCT were detected during central review of images. Fluid on OCT could be intraretinal (cystic edema; thickening alone was not considered evidence of fluid), subretinal, or below the retinal pigment epithelium. Neovascularization, fluid, or hemorrhage needed to be under the fovea. For the CNV to be considered secondary to AMD, at least 1 drusen greater than 63 µm needed to be present in the study eye or fellow eye, or the fellow eye needed to have CNV or geographic atrophy. Data analysis for this study was initiated in November 2013. Both the clinical trial and the substudy were approved by an institutional review board associated with each center. Participating patients provided written informed consent for the clinical trial and the substudy.

Study Procedures

During the initial visit, patients provided a medical history and were examined by a study-certified ophthalmologist. Patients underwent bilateral color stereoscopic fundus photography and fluorescein angiography that included stereoimages of the macula of the fellow eye at 2 and 10 minutes after dye injection. Study eyes were also imaged at the initial visit with OCT.

Graders at the CATT Fundus Photography Reading Center at the University of Pennsylvania, Philadelphia, and the CATT OCT Reading Center at Duke University, Durham, North Carolina, reviewed images taken at the time of enrollment into the clinical trial. Among the features assessed from the color photographs and fluorescein angiograms were the pattern of fluorescein dye leakage (predominantly classic, minimally classic, or occult only); presence of retinal angiomatous proliferation (RAP); area of the neovascular lesion; area of the total neovascular complex, including the neovascularization and contiguous serous pigment epithelium detachment, scar, hemorrhage, and blocked fluorescence; location of the neovascularization (subfoveal or not subfoveal); presence of hemorrhage associated with the lesion; presence of blocked fluorescence; and CNV in the contralateral eye. Graders at the OCT Reading Center noted the presence of intraretinal, subretinal, and subretinal pigment epithelium fluid, retinal pigment epithelium elevation, epiretinal membrane, and subretinal hyperreflective material. In addition, graders measured the thickness at the foveal center point of the retina, subretinal fluid, and subretinal tissue complex.

A subgroup of 835 patients from private and institutional practices of retina specialists provided blood samples from July 1, 2010, through September 31, 2011. Blood samples from patients were sent to the CATT Genetics Laboratory of the Cole Eye Institute, Cleveland Clinic, Cleveland, Ohio, for DNA extraction.[28] DNA was extracted and purified from leukocytes using the Gentra Systems PUREGENE DNA Purification Kit (Qiagen). For this investigation, 8 SNPs associated with the following genes and previously associated with risk of AMD

were tested: *CFH* Y402H (rs1061170), *ARMS2* (also called *LOC387715*) A69S (rs10490924), *HTRA1* (rs11200638), *C3* R80G (rs2230199), *LIPC* (rs10468017), *CFB* (rs4151667), *C2* (rs547154), and *C2* (rs9332739). These SNPs were a subset of the SNPs included on a custom-made TaqMan OpenArray loaded with TaqMan SNP genotyping assays (Applied Biosystems) that was used for genotyping.

Statistical Analysis

The association between genotype and each phenotypical feature was assessed with linear regression (features represented as continuous variables) or logistic regression (features represented as binary variables) models. Because of nearly complete linkage disequilibrium between *ARMS2* and *HTRA1* and between *CFB* (rs4151667) and *C2* (rs9332739) in most populations, including the patients in the CATT genetics substudy, only the results for *ARMS2* and *CFB* (rs4151667) are provided. Genotype was summarized as the number of risk alleles present, where the risk allele is *C* for *CFH* and *LIPC*, *T* for *ARMS2* and *CFB*, and *G* for *C3* and *C2*. Patient age and smoking status were included in the models as covariates. Retinal thickness was analyzed as a categorical variable, in addition to as a continuous variable, because values lower than and higher than the range (120-212 µm) are associated with decreased VA due to retinal atrophy or edema.[29] Analyses that included only patients homozygous for *CFH* and *ARMS2* were conducted to compare results to a previous study.[16] Statistical computations were performed with SAS statistical software, version 9.3 (SAS Institute Inc).

The approach to adjusting *P* values for multiple comparisons depended on whether the purpose of the analysis of the feature was to confirm the results of previous studies (age, VA, lesion size, lesion type, and RAP lesion for *CFH* or *ARMS2*) or to identify new associations. No adjustment for multiple comparisons were made for the confirmatory analyses, whereas a Bonferroni correction for the 6 SNPs under analysis led to considering only $P < .008$ (or .05 per 6 SNPs) as statistically significant for newly identified associations.

Results

Age at presentation decreased with the number of risk alleles present for *CFH*, *ARMS2*, and *C3* but not for the other SNPs (**Table 1**). A higher number of risk alleles was associated with larger total area of the neovascular lesion ($P = .03$) and with the presence of RAP lesions ($P = .05$) for *ARMS2*. No other associations were found among the 6 SNPs and the features listed in Table 1. In addition, no associations were found with subfoveal location of the neovascular lesion ($P \geq .40$ for all), blocked fluorescence ($P \geq .49$), hemorrhage ($P \geq .11$), or CNV in the contralateral eye ($P \geq .89$).

When the associations of OCT characteristics, other than thickness measurements, with the genotype of the 6 SNPS were examined, the only associations were with the presence of intraretinal fluid (**Table 2**). Eyes of patients with a higher number of risk alleles were more likely to have intraretinal fluid for *ARMS2* ($P = .008$), whereas those with a lower number of risk alleles for *C3* were more likely to have intraretinal fluid ($P = .001$). There were no other associations among the 6 SNPs and the features listed in Table 2. In addition, there were no associations with the presence of epiretinal membranes ($P \geq .26$) or vitreomacular attachment ($P \geq .29$).

The associations of thickness of the retina, subretinal fluid, and subretinal tissue complex and the total thickness (sum of thickness of retina, subretinal fluid, and subretinal tissue complex) with the genotype for the 6 SNPs are given in **Table 3**. Mean retinal thickness decreased with a higher number of risk alleles ($P = .02$) for *C3*. The mean total thickness decreased with a higher number of risk alleles for *CFH* ($P = .01$). No other associations were found among the 6 SNPs and the other thickness measurements.

The results of analyses, including only the 228 patients homozygous for risk alleles from *CFH* and *ARMS2,* may be compared to the study by Leveziel et al[15] for the features that were defined similarly in their study and CATT (**Table 4**). Patients homozygous for the risk allele for both SNPs were a mean of 6 years younger at study entry than patients homozygous for the wild-type allele for both SNPs ($P < .001$). No associations among the 4 groups or between the groups homozygous for both SNPs were identified for baseline VA, lesion type, presence of RAP lesion, or bilateral CNV.

Discussion

In CATT, the analysis of genotype for *CFH*, *ARMS2*, and *C3* confirmed some previously identified associations with angiographic features of neovascular AMD, did not support some other associations, and yielded new associations with features of neovascular AMD on OCT. No associations with *LIPC, CFB*, or *C2* were identified. Results from previous studies[10,11,15,18,20] identified patients with a higher number of risk alleles for *ARMS2* or the closely associated SNP *HTRA1* as having a larger area of the total area of the neovascular lesion. In CATT, the mean total area of CNV was larger when risk alleles were present ($P = .03$), confirming the previous results. However, the magnitude of the effect on the lesion area was modest, with the mean area of 2.42 mm^2 for patients with 2 *ARMS2* risk alleles and 1.99 mm^2 for patients with no risk alleles (Table 1).

The association of lesion type on fluorescein angiography with genotype for *CFH, ARMS2,* and/or *HTRA1* has been examined in at least 9 previous studies with between 84 and 264 patients with neovascular AMD in the analyses.[8-10,12-16,18] *CFH* has been reported as not being associated with classic or occult CNV in 5 studies,[10,13-15,18] associated with classic CNV in 2 studies,[8,9] and associated with occult CNV in 1 study,[12] although *ARMS2* has been reported as being associated with occult CNV in 2 studies[12,18] and classic CNV in 2 studies.[8,16] In CATT with 835 patients, no association was detected with either SNP for classic or occult CNV.

Although the association of RAP lesions with *CFH* and *ARMS2* has been examined in a number of studies, a low number (<40) of patients with RAP lesions among the patients with neovascular AMD has limited the statistical power in several of the analyses.[12,14,16,17,] In a larger study, Caramoy

Table 1. Association of Genotype With Baseline Age, Visual Acuity, and Features of CNV on Color Photography and Fluorescein Angiography

SNP[a] and Genotype	Total No. of Patients	Mean (SE)				No. (%) of Lesions			
		Age, y	Visual Acuity, Letters	CNV Area, mm^2	Total Area of CNV Lesion, mm^2	Predominantly Classic	Minimally Classic	Occult Only	RAP
CFH									
CC	270	76.8 (0.5)	63.0 (0.7)	1.63 (0.11)	2.58 (0.19)	64 (23.7)	45 (16.7)	154 (57.0)	17 (6.3)
TC	392	78.8 (0.4)	60.2 (0.7)	1.82 (0.09)	2.51 (0.12)	75 (19.1)	63 (16.1)	245 (62.5)	41 (10.5)
TT	173	80.4 (0.5)	60.9 (1.0)	1.60 (0.14)	2.21 (0.17)	36 (20.8)	28 (16.2)	106 (61.3)	22 (12.7)
P value[b]	...	<.001	.20	.97	.18	.53	.66	.32	.07
ARMS2									
TT	170	76.5 (0.5)	60.9 (1.0)	1.56 (0.13)	2.42 (0.17)	34 (20.0)	22 (12.9)	110 (64.7)	20 (11.8)
GT	399	78.9 (0.4)	60.8 (0.7)	1.87 (0.10)	2.81 (0.15)	88 (22.1)	63 (15.8)	241 (60.4)	40 (10.0)
GG	266	79.1 (0.5)	62.1 (0.8)	1.57 (0.09)	1.99 (0.11)	53 (19.9)	51 (19.2)	154 (57.9)	20 (7.5)
P value[b]	...	<.001	.08	.82	.03	.94	.13	.21	.05
C3									
GG	56	78.1 (0.9)	62.9 (1.9)	1.53 (0.23)	3.16 (0.52)	8 (14.3)	12 (21.4)	36 (64.3)	8 (14.3)
CG	318	77.7 (0.4)	61.9 (0.7)	1.79 (0.11)	2.49 (0.14)	62 (19.5)	56 (17.6)	194 (61.0)	24 (7.5)
CC	461	79.0 (0.4)	60.6 (0.6)	1.67 (0.08)	2.38 (0.12)	105 (22.8)	68 (14.8)	275 (59.7)	48 (10.4)
P value[b]	...	.005	.23	.67	.09	.12	.12	.58	.90
LIPC									
CC	441	78.2 (0.4)	61.5 (0.6)	1.64 (0.08)	2.34 (0.12)	92 (20.9)	71 (16.1)	266 (60.3)	42 (9.5)
CT	345	78.8 (0.4)	60.9 (0.7)	1.77 (0.10)	2.61 (0.15)	72 (20.9)	57 (16.5)	210 (60.9)	36 (10.4)
TT	48	78.5 (1.0)	61.5 (1.9)	1.94 (0.32)	2.66 (0.37)	11 (22.9)	7 (14.6)	29 (60.4)	2 (4.2)
P value[b]	...	.39	.84	.16	.13	.83	.91	.88	.65
CFB									
AA	2	82.0 (2.0)	55.0 (22.0)	0.41 (.)	4.72 (4.32)	0	1 (50.0)	1 (50.0)	0 (0.0)
AT	47	80.2 (1.2)	59.8 (2.0)	1.61 (0.26)	2.55 (0.34)	4 (8.5)	10 (21.3)	29 (61.7)	2 (4.3)
TT	786	78.4 (0.3)	61.4 (0.5)	1.72 (0.07)	2.46 (0.09)	171 (21.8)	125 (15.9)	475 (60.4)	78 (9.9)
P value[b]	...	.07	.47	.56	.47	.04	.21	.99	.16
C2									
GG	680	78.4 (0.3)	61.6 (0.5)	1.68 (0.07)	2.47 (0.10)	136 (20.0)	110 (16.2)	416 (61.2)	66 (9.7)
GT	149	78.8 (0.6)	59.5 (1.1)	1.86 (0.15)	2.51 (0.18)	39 (26.2)	24 (16.1)	85 (57.0)	12 (8.1)
TT	4	78.8 (5.4)	68.5 (4.1)	1.40 (0.44)	1.76 (0.76)	0	1 (25.0)	3 (75.0)	2 (50.0)
P value[b]	...	.51	.22	.36	.99	.20	.94	.51	.81

Abbreviations: ellipses, data not applicable; CNV, choroidal neovascularization; RAP, retinal angiomatous proliferation; SNP, single-nucleotide polymorphism.

[a] The risk allele is *C* for *CFH* and *LIPC*, *T* for *ARMS2* and *CFB*, and *G* for *C3* and *C2*.

[b] Adjusted by age (continuous), sex, and smoking status (never, quit, and current).

et al[19] reported that among 108 patients with RAP and 258 patients with CNV without RAP, the proportion of patients with RAP decreased when the *CFH* risk alleles were present (43% for 0 risk alleles, 28% for 1 risk allele, and 27% for 2 risk alleles; *P* = .03). In addition, Wegschieder et al[9] and Seitsonsen et al[14] reported that among patients with CNV, the percentage with RAP lesions was less when risk alleles for *CFH* were present. The percentage of patients with RAP lesions in CATT also decreased with more *CFH* risk alleles present (13% for 0 risk alleles, 10% for 1 risk allele, and 6% for 2 risk alleles; *P* = .07) (Table 1), consistent with these 3 previous studies.[9,14,19]

Fewer genotype-phenotype studies have been conducted for *ARMS2* and RAP lesions; 2 studies[12,16] with fewer than 30 patients with RAP had no association with *ARMS2*. In a study reported by Hayashi et al[17] that involved 36 patients with RAP and 408 patients with CNV but neither RAP nor polypoidal choroidal vasculopathy, RAP was present in 31 (14.5%) of 214 patients having the *TT* genotype, 3 (1.9%) of 158 having 1 risk allele, and 2 (2.9%) of 69 having no risk alleles (*P* < .0001). Consistent with this result, the proportion with RAP was similar (10%-12%) in patients with 1 or 2 risk alleles and lower (7.5%) in patients with no risk alleles (*P* = .05) (Table 1).

Although previous researchers have addressed the association of *CFH*, *ARMS2*, and *C3* with features of neovascular AMD on fluorescein angiography, they have not addressed the association with features on OCT. Although intraretinal fluid was present in most eyes at baseline, the proportion of eyes with intraretinal fluid increased with the number of risk alleles for *ARMS2* (*P* = .008) and decreased with the number of risk alleles for *C3* (*P* = .001) (Table 2). The associations for intraretinal fluid were reflected in the retinal thickness measurements; however, the associations were not as strong for *ARMS2* (*P* = .09) or *C3* (*P* = .02) (Table 3). The mean total thickness of the retina, subretinal fluid, and subretinal tissue complex decreased with the number of risk alleles for *CFH* (*P* = .01). None of the other associations with the presence of subretinal fluid, subretinal pigment epithelium fluid, retinal pigment epithelium elevation, subretinal hyperreflective

Table 2. Association of Genotype With Presence of Fluid and Other Features on Optical Coherence Tomography

SNP[a] and Genotype	Total No. of Patients	No. (%) of Patients				
		Intraretinal Fluid	Subretinal Fluid	Sub-RPE Fluid	RPE Elevation	Subretinal Hyperreflective Material
CFH						
CC	270	184 (68.1)	224 (83.0)	134 (49.6)	235 (87.0)	207 (76.7)
TC	392	304 (77.6)	324 (82.7)	197 (50.3)	334 (85.2)	297 (75.8)
TT	173	128 (74.0)	136 (78.6)	80 (46.2)	139 (80.3)	126 (72.8)
P value[b]	...	.36	.58	.16	.05	.27
ARMS2						
TT	170	139 (81.8)	136 (80.0)	83 (48.8)	149 (87.6)	130 (76.5)
GT	399	285 (71.4)	329 (82.5)	198 (49.6)	335 (84.0)	305 (76.4)
GG	266	192 (72.2)	219 (82.3)	130 (48.9)	224 (84.2)	195 (73.3)
P value[b]	...	.008	.28	.72	.23	.23
C3						
GG	56	36 (64.3)	48 (85.7)	29 (51.8)	50 (89.3)	41 (73.2)
CG	318	219 (68.9)	266 (83.6)	165 (51.9)	275 (86.5)	238 (74.8)
CC	461	361 (78.3)	370 (80.3)	217 (47.1)	383 (83.1)	351 (76.1)
P value[b]	...	.001	.50	.08	.07	.50
LIPC						
CC	441	324 (73.5)	359 (81.4)	216 (49.0)	372 (84.4)	332 (75.3)
CT	345	251 (72.8)	284 (82.3)	175 (50.7)	290 (84.1)	260 (75.4)
TT	48	40 (83.3)	40 (83.3)	20 (41.7)	45 (93.8)	37 (77.1)
P value[b]	...	.54	.80	.98	.23	.99
CFB						
AA	2	2 (100.0)	2 (100.0)	1 (50.0)	2 (100.0)	2 (100.0)
AT	47	30 (63.8)	39 (83.0)	23 (48.9)	39 (83.0)	38 (80.9)
TT	786	584 (74.3)	643 (81.8)	387 (49.2)	667 (84.9)	590 (75.1)
P value[b]	...	.14	.40	.90	.90	.36
C2						
GG	680	492 (72.4)	553 (81.3)	343 (50.4)	578 (85.0)	510 (75.0)
GT	149	120 (80.5)	127 (85.2)	67 (45.0)	126 (84.6)	115 (77.2)
TT	4	3 (75.0)	2 (50.0)	1 (25.0)	3 (75.0)	3 (75.0)
P value[b]	...	.06	.58	.15	.79	.55

Abbreviations: ellipses, data not applicable; RPE, retinal pigment epithelium; SNP, single-nucleotide polymorphism.

[a] The risk allele is *C* for *CFH* and *LIPC*, *T* for *ARMS2* and *CFB*, and *G* for *C3* and *C2*.

[b] Adjusted by age (continuous), sex, and smoking status (never, quit, and current).

material, epiretinal membrane, vitreomacular attachment, or thickness of the subretinal fluid or subretinal tissue complex were statistically significant after application of the Bonferroni correction to account for analysis of the 6 SNPs.

There are limitations and advantages to the analyses of the CATT data. Only images at 1 time, the time of enrollment into CATT, are available for characterization of the dynamic process of neovascularization. Although prompt referral was the general practice during the recruitment phase of CATT, undoubtedly there was variation in time since the development of the lesion that would be expected to have an effect on lesion size, fluid, thickness of the retina, or angiographic pattern. However, there is no reason to believe that patients with a particular genotype were referred earlier or later than other patients to induce artificial associations with these features. The SNPs examined were limited to a subset of the SNPs that are associated with the prevalence of AMD. Given the wide variability in the AMD phenotype and in the types of neovascular lesions, it is reasonable to hypothesize that the SNPs that lead to initiation of AMD might influence the nature of neovascular lesions. Selecting specific SNPs for association studies because of such hypotheses does not require adopting the very high thresholds for strength of associations needed to protect genome-wide association studies from identifying false associations, although genome-wide association studies have the ability to identify SNPs that affect the nature of neovascular lesions via any number of pathways. Finally, the associations identified are modest; neovascular lesions did not segregate neatly by any of the genotypes examined.

Conclusions

The analyses of the CATT data for genotype-phenotype associations for features of neovascular AMD confirmed the association of larger lesions in patients with risk alleles for

Table 3. Association of Genotype With Thickness at the Foveal Center on Optical Coherence Tomography

SNP[a] and Genotype	Total No. of Patients	Retinal Thickness, Mean (SE), μm	No. (%) of Patient by Retinal Thickness, μm			Mean (SE), μm		
			<120	120-212	>212	Subretinal Fluid	Subretinal Tissue Complex	Total Thickness
CFH								
CC	270	207 (6.0)	34 (12.6)	146 (54.1)	89 (33.0)	29.3 (4.1)	198 (9.8)	434 (10.2)
TC	392	224 (5.5)	40 (10.2)	197 (50.3)	152 (38.8)	32.1 (3.6)	219 (9.4)	476 (9.7)
TT	173	212 (8.0)	17 (9.8)	104 (60.1)	52 (30.1)	38.9 (6.0)	225 (14.8)	476 (16.6)
P value[b]	...	.71	.41	.24	.54	.16	.05	.01
ARMS2								
TT	170	226 (8.9)	18 (10.6)	86 (50.6)	65 (38.2)	31.9 (5.0)	221 (15.1)	478 (16.0)
GT	399	214 (5.1)	42 (10.5)	217 (54.4)	138 (34.6)	37.4 (4.1)	219 (9.2)	470 (9.6)
GG	266	213 (6.3)	31 (11.7)	144 (54.1)	90 (33.8)	26.0 (3.6)	201 (10.0)	440 (10.8)
P value[b]	...	.09	.54	.36	.19	.27	.29	.020
C3								
GG	56	197 (11.0)	6 (10.7)	33 (58.9)	17 (30.4)	54.5 (13.1)	239 (24.6)	490 (24.6)
CG	318	207 (5.5)	38 (11.9)	182 (57.2)	97 (30.5)	31.9 (3.7)	213 (10.1)	451 (10.9)
CC	461	225 (5.2)	47 (10.2)	232 (50.3)	179 (38.8)	30.5 (3.3)	211 (8.4)	466 (8.8)
P value[b]	...	.02	.66	.08	.04	.11	.49	.96
LIPC								
CC	441	217 (5.1)	56 (12.7)	230 (52.2)	153 (34.7)	33.7 (3.6)	207 (8.5)	458 (9.0)
CT	345	214 (5.5)	31 (9.0)	191 (55.4)	121 (35.1)	31.4 (3.7)	224 (9.9)	470 (10.6)
TT	48	220 (14.4)	4 (8.3)	25 (52.1)	19 (39.6)	32.5 (9.4)	192 (24.7)	445 (22.8)
P value[b]	...	.85	.11	.50	.69	.68	.56	.77
CFB								
AA	2	184 (46.8)	0 (0.0)	1 (50.0)	1 (50.0)	9.17 (9.17)	293 (222)	487 (166)
AT	47	228 (15.6)	2 (4.3)	32 (68.1)	13 (27.7)	37.8 (11.5)	214 (28.3)	480 (29.5)
TT	786	215 (3.7)	89 (11.3)	414 (52.7)	279 (35.5)	32.4 (2.5)	213 (6.4)	461 (6.8)
P value[b]	...	.66	.14	.06	.34	.83	.70	.49
C2								
GG	680	213 (4.0)	81 (11.9)	364 (53.5)	231 (34.0)	31.7 (2.7)	217 (7.0)	461 (7.4)
GT	149	229 (8.7)	10 (6.7)	79 (53.0)	60 (40.3)	35.8 (6.1)	202 (13.7)	467 (15.2)
TT	4	247 (71.5)	0 (0.0)	2 (50.0)	2 (50.0)	0.00 (0.00)	192 (95.0)	439 (66.9)
P value[b]	...	.09	.06	.93	.14	.80	.41	.81

Abbreviations: ellipses, data not applicable; SNP, single-nucleotide polymorphism.

[a] The risk allele is *C* for *CFH* and *LIPC*, *T* for *ARMS2* and *CFB*, and *G* for *C3* and *C2*.

[b] Adjusted by age (continuous), sex, and smoking status (never, quit, and current).

Table 4. Comparison of CNV Characteristics in the 4 Groups From the Combination of *CFH* and *ARMS2*

Characteristic	*CFH/ARMS2*[a]				*P* Value[b]	
	TT/GG (Group 1) (n = 51)	TT/TT (Group 2) (n = 34)	CC/GG (Group 3) (n = 94)	CC/TT (Group 4) (n = 49)	All Groups (n = 228)	Group 1 vs 4 (n = 100)
Age at study entry, mean (SE), y	80.3 (1.0)	78.2 (1.2)	78.1 (0.7)	74.3 (1.0)	<.001	<.001
VA, mean (SE), letters	62.4 (1.8)	62.6 (2.1)	63.4 (1.3)	62.3 (1.6)	.98	.78
Lesion type, No. (%)						
Predominantly classic	11 (21.6)	7 (20.6)	21 (22.3)	10 (20.4)	.81	.62
Minimally classic	6 (11.8)	4 (11.8)	17 (18.1)	6 (12.2)	.31	.87
Occult only	33 (64.7)	22 (64.7)	52 (55.3)	31 (63.3)	.47	.86
RAP lesion, %	5 (10.0)	3 (9.4)	7 (7.8)	6 (12.5)	.49	.06
Bilateral CNV, %	21 (41.2)	13 (39.4)	21 (23.1)	13 (26.5)	.08	.17

Abbreviations: CNV, choroidal neovascularization; RAP, retinal angiomatous proliferation; VA, visual acuity.

[a] The risk allele for *CFH* is *C* and for *ARMS2* is *T*.

[b] Adjusted by age (as continuous), sex, and smoking status (3 levels).

ARMS2 and fewer RAP lesions in patients with risk alleles for *CFH*. Previously reported associations of *CFH* and *ARMS2* with classic and occult types of neovascularization on fluorescein angiography were not confirmed. Newly identified associations of *CFH*, *ARMS2,* and *C3* with retinal fluid, retinal thickness, or total thickness require confirmation in other studies. Although baseline lesion size, RAP features, retinal fluid and thickness, and total thickness have prognostic importance, anti-VEGF treatments currently are used for nearly all patients with neovascular AMD, regardless of features on angiography and OCT. In addition, previous analyses of the CATT data and analyses of the data from the Alternative Treatments to Inhibit VEGF in Patients With Age-Related Choroidal Neovascularisation trial[28,30] did not identify an association of *CFH*, *ARMS2*, or *C3* with any of several measures of response to anti-VEGF treatment. Although genotype-phenotype association may aid in understanding the effects of SNPs in the pathogenesis of AMD, results from genetic testing currently do not affect patient care for neovascular AMD.

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Drafting of the manuscript: Maguire, Grunwald.
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Invited Commentary

Genetics and the Variable Phenotype of Age-Related Macular Degeneration

Itay Chowers, MD

Age-related macular degeneration (AMD) is a multifactorial disease with a strong genetic basis. In recent years, the study of AMD genetics experienced a major advance; as a result, variants in more than 30 genes have been associated with AMD. It has been estimated that genetics contribute at least 40% of the risk associated with developing AMD.[1-3] Most of this genetic risk is attributed to common single-nucleotide polymorphisms (SNPs) in the genes that encode *CFH* (HGNC 4883), *ARMS2/HTRA1* (HGNC 32685/9476), and, to a lesser extent, *CFB* (HGNC 1037) and *C3* (HGNC 1318); interestingly, the risk variants in the *ARMS2* and *HTRA1* genes are in nearly complete linkage disequilibrium. The remaining risk variants, including common (such as ones in *VEGF* [HGNC 12680] and *CFI* [HGNC 5394]) and rare variants (such as additional variants in *CFI* and *CFH*), have only a modest or minor contribution to the overall risk of developing AMD.[1,2]

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Current challenges in the field of AMD genetics include identifying the functional consequences of these genetic variants associated with AMD and attempting to translate this genetic information into useful clinical applications. An essential first step is to characterize whether genetic factors underlie the wide range of phenotypes that comprise AMD. For example, specific genetic factors may contribute to the presence of various types, distributions, and progression of drusen. Similarly, genetics may have a role in the variable manifestations of neovascular AMD (nvAMD), including the lesion type (classic, occult, or retinal angiomatous proliferation [RAP]), lesion size, and lesion composition.

In this issue of *JAMA Ophthalmology*, Maguire et al[4] evaluated 3 major genetic risk variants for AMD in the *CFH*, *ARMS2*, and *C3* genes and examined their association with pretreatment demographic characteristics and imaging findings in patients with nvAMD. Their study was based on 835 of the 1150 participants in the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT). Their analysis revealed associations between these variants and younger age, as well as associations between *ARMS2* variants and increased lesion size, RAP, and the presence of intraretinal fluid. In contrast, presence of the *C3* variant was associated with a decreased risk of intraretinal fluid and decreased retinal thickness, whereas the *CFH* variant was associated with reduced total thickness. This study confirmed the few previously reported observations with respect to the association between the *ARMS2* variant and lesion size and RAP. Importantly, however, it also excludes many putative associations (eg, lesion location, the presence of choroidal neovascularization in the contralateral eye, and the presence of hemorrhage), including previously reported associations with lesion type, which were based on smaller, less structured data sets. Overall, the data reported by Maguire et al[4] create new avenues and uncover major challenges in the field of AMD genetics.

On the basis of these compelling findings, it is conceivable that genetic factors are associated with specific manifestations of nvAMD and not simply with the risk of developing nvAMD in general. Although seemingly intuitive, the notion that a specific genotype underlies a specific phenotype is hardly trivial in the context of AMD. Unlike monogenic retinal degenerations, in which a mutation can, but certainly does not always, underlie a particular phenotype, in AMD, most genetic variants identified to date are associated with the risk of developing the disease in general rather than being associated with a specific manifestation. Nevertheless, clues that suggest a genotype-phenotype association in AMD have been reported previously. For example, the *ARMS2/HTRA1* risk variants are more strongly associated with nvAMD than with geographic atrophy.[1,2] The study by Maguire et al[4] provides important additional information regarding the association between *ARMS2/HTRA1* risk SNPs and