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Pharmacogenetics for Genes Associated with Age-Related Macular Degeneration (AMD) in the Comparison of AMD Treatments Trials (CATT)

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Abstract

Purpose—To evaluate the pharmacogenetic relationship between genotypes of single nucleotide polymorphisms (SNPs) known to be associated with age-related macular degeneration (AMD) and response to treatment with ranibizumab (Lucentis) or bevacizumab (Avastin) for neovascular AMD.

Design—Clinical trial.

Participants—834 (73%) of 1149 patients participating in the Comparison of AMD Treatments Trials (CATT) were recruited through 43 CATT clinical centers.

Methods—Each patient was genotyped for SNPs rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs11200638 (*HTRA1*), and rs2230199 (*C3*), using TaqMan SNP genotyping assays.

Main Outcomes Measures—Genotypic frequencies were compared to clinical measures of response to therapy at one year including mean visual acuity (VA), mean change in VA, ≥15 letter increase, retinal thickness, mean change in total foveal thickness, presence of fluid on OCT,

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This article contains online-only material. The following should appear online-only: Tables 4-7.

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presence of leakage on fluorescein angiography (FA), mean change in lesion size and mean number of injections administered. Differences in response by genotype were evaluated with tests of linear trend calculated from logistic regression models for categorical outcomes and linear regression models for continuous outcomes. To adjust for multiple comparisons, $p \leq 0.01$ was considered statistically significant.

Results—No statistically significant differences in response by genotype were identified for any of the clinical measures studied. Specifically, there were no high-risk alleles that predicted final VA or change in VA, the degree of anatomical response (fluid on OCT or FA, retinal thickness, change in total foveal thickness, change in lesion size) or the number of injections. Furthermore, a stepwise analysis failed to show a significant epistatic interaction among the variants analyzed; i.e., response did not vary by the number of risk alleles present. The lack of association was similar whether patients were treated with ranibizumab or bevacizumab or whether they received monthly or *pro re nata* (PRN) dosing.

Conclusions—Although specific alleles for *CFH*, *ARMS2*, *HTRA1* and *C3* may predict the development of AMD, they did not predict response to anti-vascular endothelial growth factor (VEGF) therapy.

The treatment of neovascular age-related macular degeneration (AMD) has been dramatically improved by the development of the anti-vascular endothelial growth factor (VEGF) therapies bevacizumab (Avastin) and ranibizumab (Lucentis). The Comparison of AMD Treatments Trials (CATT) showed that bevacizumab was equivalent to ranibizumab in improving visual acuity (VA) of patients with neovascular AMD when treatment was administered either monthly or *pro re nata* (PRN).[1] At one year, participants treated monthly with bevacizumab or ranibizumab gained 8.0 and 8.5 letters, respectively, and those treated as needed gained 5.9 and 6.8 letters, respectively. The majority of CATT participants (>92%) had stable or improved VA relative to baseline. However, despite this remarkable clinical effect, there was a wide range in treatment response.

Pioneering developments in AMD genetics research have identified numerous single nucleotide polymorphisms (SNPs) in multiple genes associated with the prevalence of the early and/or late stages of AMD.[2,3] Although the risk associated with these SNPs is well-characterized, the influence of these genetic variants on response to therapy is inconclusive. To date, a limited number of studies investigating small numbers of patients have explored this topic, and their results are inconsistent. Reports investigating either bevacizumab or ranibizumab indicate that patients homozygous for the risk allele at the *CFH* Y402H polymorphism experienced worse visual outcomes or required more injections than patients with other genotypes.[4–8] However, other studies report no association with the risk genotype.[9,10] Results from studies evaluating the *ARMS2* A69S and *HTRA1* promoter polymorphisms are also conflicting with regard to treatment response and no definitive conclusions can be drawn.[9–11] Nevertheless, these studies introduce the idea that SNPs associated with the development of AMD may play a role in predicting treatment response and outcome.

The large cohort of patients treated with anti-VEGF drugs for neovascular AMD in the CATT along with the many outcome variables that were collected following standardized protocols makes this study population an ideal group to evaluate the effects of a number of genetic polymorphisms on treatment response. In this study, we investigated whether a pharmacogenetic relationship exists between response to treatment and SNPs rs1061170 (*CFH* Y402H), rs10490924 (*ARMS2* A69S), rs11200638 (*HTRA1* promoter), and rs2230199 (*C3* R80G). Although other susceptibility genes have been reported, these four SNPs have consistently been shown to have the strongest associations with the development and progression of AMD and have been postulated to influence response to therapy.[2,3,12] A

comprehensive analysis of genotypic associations with visual and anatomical outcomes evaluated by treatment group, drug and dosing regimen is described.

Methods

Study procedures for CATT have been previously reported and are provided on ClinicalTrials.gov (NCT00593450).[1] Written informed consent was obtained from all CATT study participants involved in the genetics ancillary study. Institutional review board approval was obtained by the Cleveland Clinic and all participating CATT centers.

Patients

Between February 2008 and December 2009, 1185 patients with neovascular AMD were enrolled in CATT at 43 clinical centers in the United States. Patients were randomly assigned to one of the four treatment groups: (1) ranibizumab monthly; (2) bevacizumab monthly; (3) ranibizumab PRN; and (4) bevacizumab PRN. Between July 2010 and September 2011, 834 (73%) of the 1149 patients who were alive were enrolled in the genetics substudy.

The CATT protocol specified that eligible patients needed to be at least 50 years old, have untreated active choroidal neovascularization (CNV) due to AMD in the study eye (one eye per patient), and have visual acuity (VA) in the study eye between 20/25 and 20/320, inclusive, on electronic VA testing.[13] Active CNV was defined as the presence of leakage on fluorescein angiography (FA) and the presence of fluid on time-domain optical coherence tomography (OCT). Fluid could be located either within or below the retina or below the retinal pigment epithelium (RPE). Neovascularization or the sequela of neovascularization, i.e., pigment epithelium detachment, subretinal or sub-RPE hemorrhage, blocked fluorescence, macular edema, or subretinal sub-RPE or intraretinal fluid, needed to be present under the fovea. Patients were evaluated every month and treated according to their randomly assigned schedule of either monthly or as needed treatment.

Measures of Response to Treatment

Clinical measures of the response to treatment were based on visual acuity, anatomical features of AMD assessed by OCT and FA, and the total number of injections given in one year. Visual acuities were measured with an electronic VA testing system.[13] Mean visual acuity, mean change from baseline in visual acuity, and the proportion of patients with ≥15 letters increase from baseline were the visual measures. OCT parameters were determined by readers using a prospectively defined assessment protocol at the OCT Reading Center.[1] The proportions of patients with a thin (<120μ), normal (120–212μ), and thick (>212μ) retina; mean change from baseline in total foveal thickness, and the proportion with no fluid (“dry”) on OCT were used as the indicators of response to treatment.[14] Lesion size and leakage on fluorescein angiography was determined by readers using a prospectively defined assessment protocol at the Fundus Photograph Reading Center.[15] All examiners and readers were masked to treatment assignment.

Genotype Determination

Approximately 20 ml of peripheral blood was collected from each patient. DNA was extracted and purified from leukocytes by means of the Gentra Systems PUREGENE DNA Purification Kit (Qiagen). The following four AMD-associated single nucleotide polymorphisms (SNPs) were evaluated in each patient: (1) Complement factor H (*CFH*) Y402H (rs1061170) in exon 9 of the *CFH* gene on chromosome 1q31, resulting in a substitution of histidine for tyrosine at codon 402, (2) Age-related maculopathy susceptibility 2 (*ARMS2,* also called *LOC387715*) A69S (rs10490924) in the chromosome

10q26 region, a nonsynonymous coding SNP variant in exon 1, resulting in a substitution of the amino acid serine for alanine at codon 69, (3) High temperature requirement factor A1 (*HTRA1*) (rs11200638) in the chromosome 10q26 region, altering the promoter sequence and (4) Complement component 3 (*C3*) R80G (rs2230199), the nonsynonymous coding SNP variant in exon 3 resulting in the amino acid glycine to arginine at codon 80. Genotyping was performed using a custom made TaqMan OpenArray loaded with TaqMan SNP genotyping assays (Applied Biosystems). Typing of SNPs with OpenArray uses TaqMan nanofluidic genotyping chemistry supported on a metal-based array.[16] DNA samples were loaded and PCR-amplified on arrays as recommended by the manufacturer. Arrays were scanned on the OpenArray NT imager and genotypes were identified using the OpenArray SNP Genotyping analysis software. The allele identification of the SNP assays was verified by direct DNA sequence analysis from 10 samples for each assay yielding 100% concordance. Primer and probe sequences are available upon request. All laboratory personnel were masked to treatment assignment and patient clinical data.

Data Analysis

Clinical outcomes were compared among genotypes to determine if there was an association between genotype and response to treatment. The number of risk alleles for each genotype was counted as 0, 1 or 2, and associations of genotype (in terms of number of risk alleles) with outcomes were evaluated using tests of linear trend calculated from logistic regression models for categorical outcomes and linear regression models for continuous outcomes at one year. Additionally, longitudinal analyses were performed by using all the treatment response data measured at multiple visits in one year, and the association of genotype with responses to treatment were evaluated using test of linear trend calculated from the generalized linear models with correlation from repeated measures accounted for using the generalized estimating equation.[17] To account for multiple comparisons from multiple SNPs and multiple outcomes, we considered $p \leq 0.01$ as statistically significant. Due to the genetic complexity of AMD, we performed a stepwise analysis among the SNPs studied to examine the additive effects based upon the total number of risk alleles from the four SNPs. Five groups were evaluated (0–1 risk allele, 2 risk alleles, 3 risk alleles, 4 risk alleles and ≥ 5 risk alleles).

Data from the CATT study provided high power (93% to 98%) to detect a mean difference of 2.5 letters in VA and moderate power (75% to 85%) to detect a difference of 2 letters in VA associated with one risk allele change, under the observed standard deviation of 16 to 18 letters in VA and type I error of 0.01. For categorical outcomes, the CATT study data provided high power (>85%) to detect a difference of 0.06 or more in the proportion associated with the addition of one risk allele.

Results

We evaluated 834 CATT study participants who were treated with anti-VEGF therapy across four of the most consistent and important AMD-associated genetic risk variants. Baseline demographic and ocular characteristics of all genetic study participants are shown in Table 1. The mean age (± standard deviation) of the patients at study entry was 78.5 ± 7.5 years and 61.2% of patients were female. Mean baseline VA was 61.3 ± 13.3 letters (Snellen equivalent approximately 20/63). The genetic study participants were generally comparable to those who were still alive but chose not to participate (n = 315) except that the genetic study participants were two years younger ($p<0.001$), had better baseline VA ($p=0.005$), higher percentage with hypertension ($p=0.045$), and higher percentage with occult lesion ($p=0.04$) (Table 1).

The genotypic frequencies for each SNP analyzed were balanced across treatment groups, drug and dosing regimen (data not shown). As expected, the frequency of the high-risk alleles among CATT participants was higher than in the general population since the SNPs examined are known to be associated with AMD.[18] For each measure of response to treatment, we assessed the interaction between genotypes and treatment group. The effect of risk alleles on each measure did not differ by treatment group, drug or regimen. Therefore, we collapsed all treatment groups and report our findings on the entire 834 patients as a single group (Tables 2 and 3). The genotypic associations for each treatment group are shown in Tables 4 – 7 (available at http://aaojournal.org).

Visual Outcomes by Genotype

For each of the three visual measures evaluated at one year, there was no association with any of the genotypes or with the number of risk alleles from the four SNPs (Table 2). The strongest association was for mean visual acuity with *C3* (p=0.03); however, the association was for better visual acuity among those homozygous for the risk allele (GG). Furthermore, when additional time-points (12, 24 and 36 weeks) were evaluated using longitudinal models, there was no association between genotype and mean change in VA from baseline (smallest p value = 0.30).

Anatomical Outcomes by Genotype

For each of the five anatomic outcomes evaluated at one year, there was no significant association with any of the genotypes or with the number of alleles from the four SNPs (Table 3). The strongest association was for mean change in total retinal thickness with *CFH* (p=0.03) where the association was for less improvement (decrease 142 microns) among those homozygous for the risk allele (CC) and largest improvement (decrease 188 microns) among those with heterozygous for risk allele (CT). Furthermore, when additional time-points (12 and 24 weeks) were evaluated using longitudinal models, there was no association between genotype and mean change in total foveal thickness from baseline (smallest p value = 0.27).

Number of Injections in the PRN Treatment Groups

Among the participants in the two PRN groups, no statistically significant difference was found in the number of injections among the different genotypes for any of the four SNPs, or for the total number of risk alleles from the four SNPs (Table 2). The strongest association was for *HTRA1* (p=0.25) where the highest mean number of injections (8.0) was among those homozygous for the risk allele (AA) and an equal mean number of injections (7.3) was required among those heterozygous for the risk allele (AG) or homozygous for the non-risk allele (GG).

Discussion

The CATT results confirmed that anti-VEGF therapy is highly effective in the treatment of neovascular AMD. However, there was a wide range of clinical response to therapy and variability in the number of injections required to achieve that response. Some patients had an excellent visual result with three to four injections over the course of a year while others required up to thirteen injections in a year. The explanation underlying this heterogeneity in clinical response is unknown. Given the impact of genetic factors on disease manifestation and progression, a logical assumption would be that genetic variants play a role.

The principal aim of our study was to investigate whether four strongly associated AMD-risk genotypes predict response to treatment with bevacizumab or ranibizumab for neovascular AMD. The CATT patient cohort is an ideal population to study the

pharmacogenetic relationship between genetic variants and anti-VEGF therapy. Most previous published studies involve small, retrospective reviews of a limited number of outcomes determined from routine clinical visits. CATT is a large, prospectively defined cohort of patients with neovascular AMD drawn from multiple clinical sites with all follow-up treatment and outcomes carried out under well-defined protocols. Specifically, all visual acuities were determined by masked examiners using electronic EDTRS testing, all OCT measurements were determined in a masked fashion by an independent OCT Reading Center and all photographic and fluorescein angiographic outcomes were determined by masked assessment at an independent Fundus Photographic Reading Center. The SNPs chosen for evaluation in this study represent the genes with the strongest and most consistent association with the development and progression of AMD. In addition, these SNPs have been targeted as potential markers to guide disease management.

In our study, we found no statistically significant pharmacogenetic association between these SNPs and visual acuity outcomes, anatomical outcomes or the number of injections required. There were two instances where borderline significance was present. First, better visual acuity was seen in patients who were homozygous for the risk allele at *C3* (p=0.03). This is the opposite of what would be expected if *C3* risk alleles negatively influence treatment response. Second, the lowest mean change in total foveal thickness (less clinical response) was seen in patients who were homozygous for the *CFH* risk allele (p=0.03). However, patients who were heterozygous for the risk allele had the highest mean change in total foveal thickness (best clinical response) which would not be expected if the presence of the risk allele truly influences clinical response. Further, both of these instances were isolated and, due to the adjustments for multiple comparisons, did not reach the pre-specified significance level of $p<0.01$.

The lack of any association is provocative. Although these SNPs clearly influence AMD risk, they appear to have no impact on the response or durability of anti-VEGF therapy. *CFH* and *C3* encode genes involved in the complement cascade. Dysregulation of the complement system manifest by genetic polymorphisms clearly plays an important role in the pathogenesis of AMD. The increased inflammation found in patients harboring complement-related AMD-risk alleles has been hypothesized to favor recurrence of neovascularization due to increased levels of VEGF.[5] In addition, inflammation has been postulated to reduce response to anti-VEGF treatment.[19] However, there is little biological evidence to support this idea and our data provides convincing evidence that the complement pathway, or at least these SNPs in the complement pathway, do not strongly influence response to therapy. *ARMS2* and *HTRA1* both lie in the AMD susceptibility locus identified on chromosome 10q26 and are expressed in the retina.[20] Genetic variation at this locus has been shown to confer a differential risk for CNV versus geographic atrophy.[21] The *ARMS2* gene product has been localized to the mitochondrial outer membrane, and it has been proposed that the A69S polymorphism alters *ARMS2* function and increases susceptibility of photoreceptor cells to oxidative damage and aging.[22] As such, it is understandable that it would increase the risk of developing AMD but the mechanism by which it would influence response to anti-VEGF treatment is not obvious. The SNP evaluated in *HTRA1* is located in the promoter region and is predicted to increase expression levels of the gene.[23] It has been hypothesized that overexpression of *HTRA1* may alter the integrity of Bruch's membrane and favor the development of CNV.[2] This might suggest that HTRA1 would play a role in regulating CNV and therefore affect response to anti-VEGF therapy. The precise mechanisms by which these genetic variants affect AMD susceptibility are still not fully understood and our data indicate that alteration of either *ARMS2* or *HTRA1* via these SNPs does not strongly influence anti-VEGF therapy.

This study provides convincing evidence that the major risk alleles that influence the development of AMD do not strongly affect clinical response to therapy. This lack of association is supported by the high power provided by our large sample size and rigorously assessed outcome variables. We cannot exclude the possibility that other SNPs that are less predictive of AMD risk may be associated with response to therapy. Additional studies are underway including investigations targeting biological pathways that directly modulate cytokine behavior in neovascular AMD, such as VEGF and other growth factor pathways. Identification of markers that do affect clinical response may result in optimization of anti-VEGF therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Comparison of baseline demographic and ocular characteristics between participants and non-participants in the genetic study (N=1149)

Baseline Characteristics	Subjects in genetic study (N=834)	Alive subjects not in genetic study (N=315)	P Value
Age (years): Mean (SD)	78.5 (7.5)	80.9 (7.2)	<0.0001
Female (%)	510 (61.2)	204 (64.8)	0.28
Former or current cigarette smoker (%)	483 (57.9)	169 (53.7)	0.36
Presence of hypertension (%)	563 (67.5)	232 (73.7)	0.045
Taking AREDS supplement (%)	536 (64.3)	189 (60.0)	0.21
Baseline VA (letters): Mean (SD)	61.3 (13.3)	58.8 (13.7)	0.005
Baseline area of CNV (DA): Mean (SD)	1.70 (1.69)	1.91 (1.90)	0.096
Baseline total area of CNV lesion (DA): Mean (SD)	2.47 (2.55)	2.49 (2.54)	0.87
Presence of occult lesion (%)	505 (60.6)	169 (53.7)	0.04
Presence of RAP lesion (%)	80 (9.6)	41 (13.0)	0.12
Total foveal thickness (microns): Mean (SD)	462 (190)	456 (180)	0.60

SD = standard deviation; AREDS = age-related eye disease study; VA = visual acuity; CNV = choroidal neovascularization; DA = disc area; RAP = retinal angiomatous proliferation.

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Table 2

Genotypic associations with visual outcome measures and number of injections at one year (N=834)

SNP	Genotype*	n	Mean VA in letters (SE)	Mean VA change from baseline in letters (SE)	≥ 15 letters increase from baseline (%)	Mean number of injections in Year 1 in PRN Groups (SE) †
***CFH* rs1061170**	***CC***	270	70.8 (0.9)	7.9 (0.8)	76 (28.4)	7.4 (0.3)
	TC	391	68.4 (0.9)	8.2 (0.7)	116 (29.8)	7.4 (0.3)
	TT	173	69.5 (1.3)	8.6 (1.1)	59 (34.1)	7.6 (0.4)
	Linear trend P§		0.30	0.61	0.22	0.72
***ARMS2* rs10490924**	***TT***	170	69.1 (1.1)	8.2 (1.0)	48 (28.4)	8.0 (0.4)
	GT	398	69.1 (0.9)	8.3 (0.7)	126 (31.9)	7.2 (0.3)
	GG	266	70.0 (1.0)	7.9 (0.9)	77 (28.9)	7.4 (0.3)
	Linear trend P§		0.51	0.77	0.97	0.35
***HTRA1* rs11200638**	***AA***	162	69.2 (1.1)	8.4 (1.0)	47 (29.2)	8.0 (0.4)
	AG	398	69.2 (0.9)	8.2 (0.7)	123 (31.1)	7.3 (0.3)
	GG	274	69.8 (1.0)	7.9 (0.9)	81 (29.6)	7.3 (0.3)
	Linear trend P§		0.68	0.69	0.99	0.25
***C3* rs2230199**	***GG***	56	71.0 (2.1)	8.1 (2.0)	16 (28.6)	7.0 (0.6)
	CG	318	70.9 (0.9)	8.9 (0.8)	101 (31.8)	7.9 (0.3)
	CC	460	68.1 (0.8)	7.6 (0.7)	134 (29.4)	7.2 (0.2)
	Linear trend P§		0.03	0.34	0.72	0.30
# of Risk Alleles*	0–1	123	68.2 (1.6)	7.0 (1.4)	36 (29.3)	7.6 (0.5)
	2	141	69.7 (1.4)	9.1 (1.2)	47 (33.3)	7.0 (0.4)
	3	175	69.2 (1.3)	7.9 (1.0)	48 (27.6)	7.2 (0.4)
	4	170	69.2 (1.4)	8.1 (1.2)	53 (31.5)	7.3 (0.4)
	≥5	225	70.2 (0.9)	8.5 (0.8)	67 (29.9)	7.9 (0.3)
	Linear trend P§		0.42	0.66	0.71	0.27

SNP = single nucleotide polymorphism; SE = standard error; VA = visual acuity.

*The risk alleles are highlighted in bold and italics and are C for *CFH*, T for *ARMS2*, A for *HTRA1* and G for *C3*.

†Patients (n=100) were excluded if they missed 3 or more missed visits, had no treatment due to contraindication, or had reached treatment futility.

§The genotype is coded as 2 for two copies of risk alleles, 1 for one copy of risk allele, 0 for no risk allele.

For continuous outcomes, the linear trend P value is from linear regression with the genotype as continuous variable.

For categorical outcomes, the linear trend P value is from logistic regression with the genotype as continuous variable.

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Table 3

Genotypic associations with anatomical outcome measures at one year (N=834)

SNP	Genotype*	n	Retinal thickness in microns (%)			Mean change of total foveal thickness from baseline in microns (SE)	Dry on OCT (%)	Leakage on FA (%)	Mean change in lesion size from baseline in disc area (SE)
			<120	120–212	>212				
***CFH* rs1061170**	***CC***	270	46 (17.2)	187 (70.0)	34 (12.7)	−142 (9.9)	72 (27.3)	120 (46.2)	0.2 (0.2)
	TC	391	85 (22.1)	262 (68.2)	37 (9.6)	−188 (9.4)	112 (29.6)	173 (47.1)	0.2 (0.1)
	TT	173	37 (21.9)	112 (66.3)	20 (11.8)	−174 (16.3)	55 (33.5)	71 (43.8)	0.4 (0.1)
	Linear trend P§			0.62		0.03	0.18	0.71	0.48
***ARMS2* rs10490924**	***TT***	170	34 (20.5)	114 (68.7)	18 (10.8)	−184 (16.3)	46 (28.0)	79 (48.8)	0.5 (0.2)
	GT	398	79 (20.2)	275 (70.2)	38 (9.7)	−176 (9.0)	129 (33.4)	164 (43.5)	0.0 (0.1)
	GG	266	55 (21.0)	172 (65.6)	35 (13.4)	−152 (10.8)	64 (24.9)	121 (48.4)	0.4 (0.2)
	Linear trend P§			0.33		0.06	0.30	0.89	0.80
***HTRA1* rs11200638**	***AA***	162	34 (21.5)	107 (67.7)	17 (10.8)	−178 (15.8)	45 (28.8)	76 (49.4)	0.5 (0.2)
	AG	398	79 (20.2)	273 (69.6)	40 (10.2)	−179 (9.2)	128 (33.1)	162 (43.0)	0.1 (0.1)
	GG	274	55 (20.4)	181 (67.0)	34 (12.6)	−152 (10.8)	66 (25.0)	126 (48.8)	0.3 (0.1)
	Linear trend P§			0.64		0.10	0.23	0.84	0.42
***C3* rs2230199**	***GG***	56	11 (20.4)	37 (68.5)	6 (11.1)	−182 (24.8)	19 (35.8)	27 (50.0)	0.0 (0.4)
	CG	318	69 (22.0)	217 (69.1)	28 (8.9)	−161 (10.4)	99 (32.1)	135 (45.3)	0.1 (0.1)
	CC	460	88 (19.5)	307 (67.9)	57 (12.6)	−174 (8.7)	121 (27.1)	202 (46.2)	0.3 (0.1)
	Linear trend P§			0.51		0.67	0.07	0.85	0.28
# of Risk Alleles*	0–1	123	27 (22.5)	75 (62.5)	18 (15.0)	−153 (15.3)	30 (25.9)	63 (54.8)	0.2 (0.2)
	2	141	26 (18.6)	101 (72.1)	13 (9.3)	−157 (15.2)	33 (24.1)	60 (45.1)	0.5 (0.2)
	3	175	36 (20.9)	115 (66.9)	21 (12.2)	−200 (15.6)	51 (29.8)	63 (38.4)	0.1 (0.1)
	4	170	41 (24.6)	113 (67.7)	13 (7.8)	−174 (14.8)	61 (37.0)	71 (43.8)	−0.0 (0.2)
	≥5	225	38 (17.2)	157 (71.0)	26 (11.8)	−160 (11.7)	64 (29.4)	107 (49.8)	0.3 (0.2)
	Linear trend P§			0.29		0.68	0.30	0.93	0.80

SNP = single nucleotide polymorphism; SE = standard error; OCT = optical coherence tomography; FA = fluorescein angiography.

*The risk alleles are highlighted in bold and italics and are C for *CFH*, T for *ARMS2*, A for *HTRA1* and G for *C3*.

§The genotype is coded as 2 for two copies of risk alleles, 1 for one copy of risk allele, 0 for no risk allele.

For continuous outcomes, the linear trend P value is from linear regression with the genotype as continuous variable.

For categorical outcomes, the linear trend P value is from logistic regression with the genotype as continuous variable.