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VEGF-A and *VEGFR-2* Gene Polymorphisms and Response to Anti-VEGF Therapy in the Comparison of AMD Treatments Trials (CATT)

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Abstract

Importance—Individual variation in the response and duration of anti-VEGF therapy is seen in patients with neovascular age-related macular degeneration (nAMD). Identification of genetic markers that affect clinical response may result in optimization of anti-VEGF therapy.

Objective—To evaluate the pharmacogenetic relationship between genotypes of single nucleotide polymorphisms (SNPs) in the VEGF signaling pathway and response to treatment with ranibizumab or bevacizumab for nAMD.

Design—Comparison of AMD Treatments Trials (CATT).

Setting—43 CATT clinical centers.

Participants—835 (73%) of 1149 patients participating in CATT.

Methods—Each patient was genotyped for seven SNPs in *VEGF-A* (rs699946, rs699947, rs833069, rs833070, rs1413711, rs2010963, rs2146323) and one SNP in *VEGFR-2* (rs2071559) 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, presence of leakage on fluorescein angiography, mean change in lesion size and mean number of

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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. The method of controlling the false discovery rates was used to adjust for multiple comparisons.

Results—For each of the measures of VA evaluated, there was no association with any of the genotypes or with the number of risk alleles. Four of the *VEGF-A* SNPs demonstrated an association with retinal thickness (rs699947, rs833070, rs1413711, p=0.03 to 0.04; rs2146323, p=0.006). However, adjusted p-values for these associations were all not statistically significant (p=0.24 to 0.45). Among the participants in the two PRN groups, no association was found in the number of injections among the different genotypes or for the total number of risk alleles. The effect of risk alleles on each clinical measure did not differ by treatment group, drug or dosing regimen (p >0.01).

Conclusions and Relevance—This study provides evidence that there are no pharmacogenetic associations between the studied *VEGF-A* and *VEGFR-2* SNPs and response to anti-VEGF therapy.

Trial Registration—ClinicalTrials.gov Identifier: NCT00593450.

Introduction

Vascular endothelial growth factor (VEGF) inhibition by bevacizumab, ranibizumab, and aflibercept has improved dramatically the treatment of neovascular age-related macular degeneration (nAMD). VEGF plays a key role in the regulation of angiogenesis, vascular leakage, and inflammation that is characteristic of nAMD by stimulating growth of new blood vessels.[1,2] Results from the Comparison of AMD Treatments Trials (CATT) and other multicenter clinical trials that compared bevacizumab and ranibizumab indicate that both drugs provide dramatic and lasting visual improvements in patients.[3–6] However, there is individual variation in the initial response to therapy and in the durability of the clinical effect.

One logical explanation for the variability in treatment response might be differences in genetic background. It is well established that several genetic risk variants are associated with the development and progression of AMD.[7] Recent research on outcome determinants has focused on the role of these variants on the response to anti-VEGF therapy with inconsistent results.[8] We recently reported that no statistically significant pharmacogenetic association between single nucleotide polymorphisms (SNPs) rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs11200638 (*HTRA1*), and rs2230199 (*C3*) was identified for any clinical outcome among CATT study participants.[9] Our study provides evidence that although these four SNPs clearly influence AMD risk, they are not responsible for any substantial variation in response to anti-VEGF therapy.

An obvious next strategy to identify pharmacogenetic markers that may predict anti-angiogenic therapy is to analyze polymorphisms in genes within the VEGF signaling pathway. VEGF, encoded by the *VEGF-A* gene, acts through specific tyrosine receptors, of which VEGFR-2 mediates the majority of the angiogenic effects of VEGF. Several studies suggest that genetic variations in *VEGF-A* and *VEGFR-2* may play a role in the pathogenesis

of AMD;[10–16] however, others have shown no association.[12,17–19] A recent meta-analysis designed to clarify the association between *VEGF-A* polymorphisms and AMD risk determined that there was no association but that the association was different for each polymorphism among different patient populations.[20]

Polymorphisms in the *VEGF-A* gene regulate VEGF expression and therefore its angiogenic properties.[21,22] It is plausible, then, that different expression levels of VEGF may generate different responses to anti-VEGF drugs. Genetic variants in the *VEGF-A* and *VEGFR-2* genes have been investigated in small-scale studies for their influence on anti-VEGF treatment outcomes with different conclusions. One study reported a trend toward a better visual outcome after 6 months of ranibizumab treatment in those harboring the risk genotypes at *VEGF-A* SNP rs1413711, compared with those having the non-risk genotype.[23] Yet, a separate study did not find any association between SNP rs1413711 and VA outcome after treatment.[24] A Japanese study reported that SNP rs699946 in the *VEGF-A* gene is associated with a better VA response after 12 months of bevacizumab treatment.[25] Another report concluded that SNP rs3025000 was associated with better visual outcomes at 6 months of anti-VEGF treatment.[26] A recent study evaluating two *VEGF-A* SNPs and response to ranibizumab concluded that rs699947 determines early functional outcome.[27] Finally, a study evaluating seven different *VEGF-A* polymorphisms concluded that none is a major predictor of anti-VEGF treatment success with bevacizumab in patients with nAMD.[28] The collective weakness of these reports are their small sample size, the variability in treatment paradigms, and the non-standardized assessment of outcome measures.

The large cohort of patients treated with anti-VEGF drugs for nAMD in CATT along with the many outcome determinants that were collected following standardized protocols makes this study population an ideal group to evaluate the effects of genetic polymorphisms on treatment response. We investigated the pharmacogenetic relationship between the clinical outcomes of anti-VEGF treatment and eight different SNP variations in the *VEGF-A* and *VEGFR-2* genes in 835 CATT study participants. The SNPs selected were based on their potential impact on VEGF expression, their associations with nAMD in previous studies and their possible influence in anti-VEGF treatment outcome in either nAMD or other VEGF-mediated diseases. 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).[3] 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. All analyses investigating the effect of genotype on response to treatment were evaluated with outcomes data at one year to minimize confounding factors that may occur at later time points in the trial such as the second randomization at the end of year one. Furthermore, the majority of the response in morphological and visual outcomes occurred within the first six months of treatment.[3]

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. Eligibility criteria and study design for CATT has been previously defined.[3,9] Between July 2010 and September 2011, 835 (73%) of the 1149 patients who were alive were enrolled in the genetics substudy. 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 an occult lesion ($p=0.04$).[9]

Measures of Response to Treatment

Clinical measures of the response to treatment were based on visual acuity (VA), anatomical features of AMD assessed by optical coherence tomography (OCT) and fluorescein angiography (FA), and the total number of injections given in one year. Visual acuities were measured with an electronic VA testing system.[29] 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.(3) 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.[30] Lesion size and leakage on FA was determined by readers using a prospectively defined assessment protocol at the Fundus Photograph Reading Center.[31] 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 as previously described.[9] Seven SNPs in *VEGF-A* (rs699946, rs699947, rs833069, rs833070, rs1413711, rs2010963, rs2146323) and one SNP in *VEGFR-2* (rs2071559) were evaluated in each patient. Genotyping was performed using a custom made TaqMan OpenArray loaded with TaqMan SNP genotyping assays (Applied Biosystems) as previously described.[9] The call rate was 100% for all patients. 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. To account for multiple comparisons from multiple SNPs and multiple outcomes, we calculated adjusted p-values using the approach of false discovery rate.[32] Because we

compared 3 types of outcomes (VA, anatomy, and number of injections) among genotypes of each SNP, and outcomes in the same type are likely to be highly correlated, we counted the number of tests performed within each type of outcome for the adjustment for multiple comparisons. Specifically, we considered 24 statistical tests performed for VA outcomes (i.e., 8 SNPs for 3 VA outcomes), 40 tests for anatomic outcomes, and 8 tests for number of injections, and calculated their adjusted p-value separately. We considered adjusted p-values <0.05 to be 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 eight SNPs. Five groups were evaluated (0–6 risk allele, 7 risk alleles, 8 risk alleles, 9 risk alleles and ≥ 10 risk alleles).

Data from the CATT study provided good power (83% to 93%) to detect a mean difference of 2.5 letters in VA and moderate power (56% to 71%) 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 a false discovery rate of 0.05. For anatomic outcomes, the CATT study data provided good power (>80%) to detect a difference of 0.07 or more in the proportion associated with the addition of one risk allele.

Results

We evaluated 835 CATT study participants who were treated with anti-VEGF therapy across eight SNPs within the VEGF signaling pathway. Seven polymorphisms are located in the VEGF gene, *VEGF-A*, and one in its primary signaling receptor, *VEGFR-2*. Patient demographics and baseline characteristics of all CATT participants have been previously described.[9] In brief, 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 ETDRS letters (Snellen equivalent approximately 20/63).

The genotypic frequencies for each SNP analyzed were balanced across treatment groups. For each measure of response to treatment, we assessed the interaction between genotypes and treatment group. The effect of risk alleles on each clinical measure did not differ by treatment group, drug or dosing regimen (p >0.01). Therefore, we collapsed all treatment groups and report our findings on the entire 835 patients as a single group (Tables 1 and 2).

For each of the three vision measures evaluated at one year, there was no association with any of the genotypes or with the number of risk alleles from the eight SNPs (Table 1). For each of the five anatomical measures, there were few noteworthy associations (Table 2). Four of the *VEGF-A* SNPs demonstrated an association with retinal thickness (rs699947, rs833070, rs1413711, p=0.03 to 0.04; rs2146323, p=0.006). However, the adjusted p-values for these associations were all not statistically significant (adjusted p-value range from 0.24 to 0.45). Furthermore, none of these modest associations were supported by any other anatomical measure. Finally, among the participants in the two PRN groups, no association was found in the number of injections among the different genotypes for any of the eight SNPs, or for the total number of risk alleles from the eight SNPs (Table 1).

Discussion

Choroidal neovascularization, the hallmark of nAMD, is an angiogenic process that is finely regulated between inhibitory and stimulating factors such as VEGF. Anti-VEGF drugs are highly effective for the treatment of nAMD[3–6,33–35] and induce their therapeutic action by blocking the binding of VEGF to its receptors and subsequent initiation and progression of CNV. It is logical, then, to assume that any factor that alters this pathway, such as a genetic polymorphism, might influence the therapeutic effect of these drugs.

The polymorphisms selected for this study are genetic variants in *VEGF-A* and *VEGFR-2* that are best known to be associated with clinical outcomes in VEGF-mediated diseases such as nAMD, diabetic retinopathy, and several malignancies.[21] Some of these SNPs are located in the promoter region and are known to influence the expression and plasma concentration of VEGF.[22] Others are located within the introns where there are putative regulatory elements influencing binding of VEGF to its receptor.[11] One SNP is located in the promoter region of the gene encoding for VEGFR-2, the primary receptor responsible for the majority of the angiogenic effects of VEGF.

The three SNPs that we evaluated in the promoter region of *VEGF-A* are rs699946, rs699947, and rs2010963. These variants affect gene splicing, resulting in changes in VEGF expression levels. In a pharmacogenetic study analyzing rs699946, mean VA was significantly better in patients with the GG genotype compared to patients with the AG or AA genotypes after 12 months of treatment with bevacizumab for nAMD.[25] Studies of rs699947 in nAMD patients have also suggested pharmacogenetic associations, but with inconsistent results. Two studies suggest that patients carrying the C allele were less likely to respond to treatment, either with bevacizumab[36] or with photodynamic therapy (PDT).[37] Another study, however, suggests that patients homozygous for the C allele show significantly improved VA following ranibizumab injections.[27] The third promoter SNP, rs2010963, has been shown to increase VEGF expression in the retina.[38] Although one report has shown an association between the development of AMD and this SNP,[10] a separate study did not confirm this observation.[18] In another study, no association was detected between rs2010963 and response to bevacizumab.[36]

The four SNPs that we evaluated in the intron regions of *VEGF-A* are rs1413711, rs2146323, rs833069, and rs833070. The first of these, rs1413711, is located in intron 1. It has been proposed that the proximity of this SNP to a putative stress response element binding site may influence VEGF receptor binding and increase protein production.[11] Several studies have reported an increased risk of developing AMD in patients homozygous for the CC genotype.[11,13] In contrast, several other reports do not detect an association.[16,19,24] One pharmacogenetic analysis suggested that patients with high risk genotypes (TC or CC) at this SNP trend towards a better response to ranibizumab.[23] However, a separate study did not confirm this result.[24]

The remaining three intronic SNPs are located with intron 2. SNP rs2146323 has been reported to be associated with the development of AMD;[10] although a separate study did not confirm these results.[17] This SNP has also been associated with anatomic outcome

following PDT.[37] Polymorphisms rs833069 and rs833070 have also been associated with the development and progression of AMD;[10,14] although no pharmacogenetic analysis has been performed to date. However, we believed these to be potential targets for modification of response to therapy on the basis of *in silico* analysis. Results from the Ensembl genome browser indicate that both rs833069 and rs833070 are located within a putative regulatory element that is enriched with CTCF and DNaseI sites that could affect VEGF expression levels.[39] A second bioinformatics program (FastSNP) confirmed this possibility.[40]

Finally, rs2071559 is located in the gene encoding the primary receptor responsible for the majority of the angiogenic effects of VEGF (*VEGFR-2*). Studies have shown that the T allele increases transcription activity of the gene and therefore increases receptor function.[15] Individuals homozygous for the T allele have been shown to have a higher risk for the development of AMD.[14]

The lack of any significant associations between SNPs in the VEGF pathway and response to anti-VEGF treatment differs from several previous studies, many of which were limited by small sample size and non-standardized assessment of outcomes. The strengths of our study include the large prospectively defined cohort of patients with nAMD in CATT drawn from multiple clinical sites, and the well-defined protocols that were employed to guide follow-up treatment and determine outcomes. 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. Our findings are supported by a recent report from the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation (IVAN) Study Group. This comparable pharmacogenetic study evaluated two of the same SNPs in our study (rs833069 and rs833070) and also demonstrated no association between an anatomical outcome (total retinal thickness) and genotype.[41]

This study provides evidence that there are no substantial pharmacogenetic associations between the studied *VEGF-A* and *VEGFR-2* SNPs and response to anti-VEGF therapy in patients participating in CATT. We cannot exclude the possibility that other SNPs in *VEGF-A, VEGFR-2* or in other genes that regulate angiogenesis may be associated with response to therapy. Although identification of markers that do affect clinical response may result in optimization of anti-VEGF therapy, there is currently no rationale for modifying therapy for individuals based on their genetic profiles. Additional studies, including a genome-wide analysis, are underway to identify novel polymorphisms that may be associated with response to anti-VEGF therapy in patients with nAMD.

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Table 1

Genotypic associations with visual outcome measures and number of injections at one year (N=835).

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)[†]	N in PRN Groups
VEGF-A rs699946	AA	535	69.2 (0.7)	8.2 (0.6)	167 (31.3)	7.3 (0.2)	224
	AG	269	69.7 (1.0)	8.5 (0.8)	78 (29.2)	7.5 (0.3)	116
	GG	31	69.2 (3.5)	4.7 (3.0)	6 (19.4)	8.0 (0.9)	17
Linear Trend P[§]			0.77	0.61	0.21	0.39	
VEGF-A rs699947	AA	201	69.6 (1.2)	8.4 (1.0)	60 (30.0)	7.3 (0.4)	80
	AC	409	69.2 (0.8)	8.1 (0.7)	126 (30.8)	7.5 (0.3)	171
	CC	225	69.5 (1.2)	8.1 (1.0)	65 (29.3)	7.5 (0.3)	106
Linear Trend P[§]			0.97	0.82	0.86	0.67	
VEGF-A rs833069	CC	103	69.6 (1.9)	8.1 (1.6)	29 (28.4)	8.1 (0.4)	53
	TC	359	69.3 (0.8)	8.2 (0.7)	111 (31.1)	7.3 (0.3)	153
	TT	373	69.4 (0.9)	8.1 (0.8)	111 (29.8)	7.3 (0.3)	151
Linear Trend P[§]			0.93	0.97	0.96	0.17	
VEGF-A rs833070	CC	228	69.4 (1.2)	8.0 (1.0)	66 (29.3)	7.5 (0.3)	109
	CT	402	69.2 (0.8)	8.3 (0.7)	124 (30.8)	7.4 (0.3)	167
	TT	205	69.8 (1.2)	8.1 (1.0)	61 (29.9)	7.4 (0.3)	81
Linear Trend P[§]			0.82	0.91	0.89	0.79	
VEGF-A rs1413711	CC	230	69.5 (1.2)	8.0 (1.0)	67 (29.5)	7.5 (0.3)	109
	CT	398	69.1 (0.8)	8.2 (0.7)	122 (30.7)	7.4 (0.3)	167
	TT	207	69.8 (1.2)	8.2 (1.0)	62 (30.1)	7.4 (0.3)	81
Linear Trend P[§]			0.85	0.91	0.89	0.79	
VEGF-A rs2010963	GG	375	69.3 (0.9)	8.1 (0.8)	111 (29.7)	7.3 (0.3)	151
	GC	361	69.4 (0.8)	8.3 (0.7)	112 (31.2)	7.4 (0.3)	156
	CC	99	69.4 (1.9)	7.9 (1.6)	28 (28.6)	8.1 (0.4)	50
Linear Trend P[§]			0.97	0.98	0.97	0.18	0.18
VEGFR-2 rs2071559	AA	193	68.5 (1.2)	8.1 (1.0)	59 (30.7)	7.7 (0.4)	71
	AG	414	69.5 (0.9)	8.0 (0.8)	128 (31.0)	7.2 (0.2)	182
	GG	228	69.8 (0.9)	8.5 (0.8)	64 (28.3)	7.6 (0.3)	104

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)†	N in PRN Groups
Linear Trend P§			0.42	0.77	0.57	1.00	
VEGF-A rs2146323	AA	97	71.0 (1.5)	8.7 (1.2)	27 (27.8)	7.2 (0.5)	36
	AC	377	68.7 (0.9)	7.9 (0.8)	120 (31.8)	7.7 (0.3)	156
	CC	361	69.6 (0.9)	8.2 (0.8)	104 (29.1)	7.3 (0.3)	165
Linear Trend P§			0.81	0.93	0.86	0.61	
# of Risk Alleles	0–6	31	71.6 (2.5)	6.9 (1.8)	5 (16.7)	7.6 (0.8)	14
	7	143	70.4 (1.1)	9.2 (1.1)	41 (28.7)	7.5 (0.4)	71
	8	310	68.6 (1.0)	7.1 (0.9)	95 (30.8)	7.4 (0.3)	132
	9	266	67.5 (1.8)	7.2 (1.5)	25 (29.4)	7.6 (0.5)	105
	≥10	85	70.1 (1.0)	9.3 (0.8)	85 (32.1)	7.3 (0.3)	35
Linear Trend P§			0.42	0.88	0.28	0.96	

†Patients (n=100) are excluded due to 3 or more missed visits or ever had no treatment due to contraindication or futility in Year 1.

§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 outcome, the linear trend P value is from linear regression with the genotype as continuous variable.

For categorical outcome, the linear trend P value is from logistic regression with the genotype as continuous variable, (cumulative logit model for three level outcome).

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Table 2

Genotypic associations with anatomical outcome measures at one year (N=835).

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				
VEGF-A rs699946	AA	535	106 (20.3)	356 (68.1)	61 (11.7)	−59.1 (4.8)	150 (29.0)	227 (44.7)	0.2 (0.1)
	AG	269	56 (21.0)	183 (68.5)	28 (10.5)	−61.2 (7.4)	78 (29.9)	125 (49.6)	0.3 (0.2)
	GG	31	6 (19.4)	23 (74.2)	2 (6.5)	−49.1 (15.3)	11 (37.9)	13 (43.3)	0.3 (0.3)
Linear Trend P§				0.59		0.92	0.44	0.38	0.38
VEGF-A rs699947	AA	201	33 (17.0)	129 (66.5)	32 (16.5)	−55.8 (7.8)	44 (22.8)	101 (52.1)	0.1 (0.1)
	AC	409	93 (22.9)	267 (65.8)	46 (11.3)	−58.1 (5.8)	137 (34.4)	169 (44.1)	0.4 (0.1)
	CC	225	42 (19.0)	166 (75.1)	13 (5.9)	−65.0 (7.3)	58 (26.7)	95 (44.6)	0.0 (0.1)
Linear Trend P§				0.03		0.40	0.45	0.14	0.58
VEGF-A rs833069	CC	103	21 (20.8)	76 (75.2)	4 (4.0)	−59.2 (10.4)	23 (23.5)	45 (46.4)	0.4 (0.2)
	TC	359	71 (19.9)	244 (68.5)	41 (11.5)	−61.2 (6.3)	114 (32.9)	146 (43.3)	0.2 (0.1)
	TT	373	76 (20.9)	242 (66.5)	46 (12.6)	−57.7 (5.6)	102 (28.1)	174 (48.9)	0.2 (0.1)
Linear Trend P§				0.33		0.79	0.94	0.33	0.71
VEGF-A rs833070	CC	228	42 (18.8)	168 (75.0)	14 (6.3)	−63.3 (7.2)	60 (27.4)	94 (43.9)	0.0 (0.1)
	CT	402	92 (23.1)	263 (65.9)	44 (11.0)	−60.1 (5.9)	133 (33.9)	169 (44.7)	0.4 (0.1)
	TT	205	34 (17.2)	131 (66.2)	33 (16.7)	−53.6 (7.6)	46 (23.4)	102 (51.5)	0.1 (0.1)
Linear Trend P§				0.043		0.38	0.42	0.13	0.63
VEGF-A rs1413711	CC	230	42 (18.6)	170 (75.2)	14 (6.2)	−64.5 (7.2)	61 (27.6)	95 (44.0)	0.0 (0.1)
	CT	398	92 (23.3)	259 (65.6)	44 (11.1)	−58.7 (5.9)	130 (33.5)	168 (44.9)	0.4 (0.1)
	TT	207	34 (17.0)	133 (66.5)	33 (16.5)	−55.1 (7.6)	48 (24.1)	102 (51.0)	0.1 (0.1)
Linear Trend P§				0.045		0.38	0.49	0.16	0.65
VEGF-A rs2010963	GG	375	76 (20.8)	246 (67.2)	44 (12.0)	−58.1 (5.6)	104 (28.5)	173 (48.5)	0.2 (0.1)
	GC	361	72 (20.1)	242 (67.6)	44 (12.3)	−60.4 (6.3)	112 (32.1)	149 (44.0)	0.2 (0.1)
	CC	99	20 (20.6)	74 (76.3)	3 (3.1)	−60.7 (10.7)	23 (24.5)	43 (45.7)	0.4 (0.2)
Linear Trend P§				0.39		0.78	0.92	0.37	0.72
VEGFR-2 rs2071559	AA	193	38 (20.0)	120 (63.2)	32 (16.8)	−60.5 (8.9)	58 (31.0)	87 (47.0)	0.1 (0.1)
	AG	414	80 (19.6)	294 (71.9)	35 (8.6)	−51.6 (4.9)	108 (26.7)	195 (49.5)	0.3 (0.1)

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				
VEGF-A rs2146323	GG	228	50 (22.5)	148 (66.7)	24 (10.8)	−72.8 (8.2)	73 (33.6)	83 (39.3)	0.3 (0.1)
	Linear Trend P[§]			0.13		0.22	0.50	0.11	0.37
	AA	97	16 (17.4)	58 (63.0)	18(19.6)	−45.9 (9.2)	23 (25.3)	41 (44.6)	0.1 (0.1)
	AC	377	76 (20.4)	247 (66.2)	50(13.4)	−55.7 (6.0)	113 (30.6)	179 (50.3)	0.4 (0.1)
	CC	361	76 (21.3)	257 (72.2)	23(6.5)	−66.7 (6.0)	103 (29.6)	145 (42.4)	0.1 (0.1)
	Linear Trend P[§]			0.006		0.07	0.64	0.22	0.34
# of Risk Alleles	0–6	31	9 (30.0)	17 (56.7)	4 (13.3)	−64.8 (22.7)	10 (34.5)	9 (30.0)	0.0 (0.3)
	7	143	32 (22.4)	101 (70.6)	10 (7.0)	−79.7 (10.9)	42 (30.2)	67 (49.6)	0.2 (0.1)
	8	310	59 (19.5)	210 (69.3)	34 (11.2)	−52.7 (5.7)	90 (30.1)	134 (45.7)	0.4 (0.2)
	9	266	18 (21.7)	48 (57.8)	17 (20.5)	−54.9 (7.0)	25 (30.5)	120 (47.6)	0.0 (0.2)
	≥10	85	50 (19.1)	186 (71.0)	26 (9.9)	−60.9 (12.4)	72 (27.8)	35 (43.8)	0.1 (0.1)
	Linear Trend P[§]			0.08		0.18	0.51	0.73	0.45

[§]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 outcome, the linear trend P value is from linear regression with the genotype as continuous variable.

For categorical outcome, the linear trend P value is from logistic regression with the genotype as continuous variable, (cumulative logit model for three level outcome).