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VEGFR2 Gene Polymorphisms and Response to Anti-VEGF Therapy in Age-Related Macular Degeneration

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Abstract

Purpose—A previously published study demonstrated a pharmacogenetic association between the minor alleles of two *VEGFR2* SNPs and greater improvement in visual acuity (VA) to treatment with ranibizumab, an anti-VEGF drug, in patients with neovascular age-related macular degeneration (nAMD). We evaluated whether this association was replicated among patients who participated in the Comparison of AMD Treatments Trials (CATT) or the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation (IVAN) trial.

Design—Cohort studies within randomized clinical trials.

Participants—835 patients participating in CATT and 512 patients participating in IVAN.

Methods—Each patient was genotyped for SNPs rs4576072 and rs6828477 in the *VEGFR2* gene.

Main Outcomes Measures—Mean change in VA from baseline one year after initiation of treatment with ranibizumab or bevacizumab. Differences in VA response between the patient group homozygous for the minor allele of each SNP and the other genotype groups were evaluated with analysis of variance. Differences in VA response by the number of minor alleles present for

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either SNP or both combined were evaluated with tests of linear trend. Analyses were conducted separately for CATT and IVAN participants and with both the studies combined.

Results—No statistically significant difference in mean change in VA was identified between genotypes of either SNP ($p \geq 0.05$). Furthermore, a stepwise analysis failed to show a significant interaction for either SNP based upon the number of minor alleles present. The lack of association was similar in both the CATT and IVAN cohorts and whether the analysis combined patients treated with either ranibizumab or bevacizumab or when restricted to patients treated with ranibizumab only.

Conclusions—The CATT and IVAN data do not support a pharmacogenetic association between the two *VEGFR2* SNPs, rs4576072 and rs6828477, and change in VA response to anti-VEGF therapy in patients with nAMD.

Introduction

Treatments based on inhibiting the activity of vascular endothelial growth factor (VEGF) have transformed the care of patients with neovascular age-related macular degeneration (nAMD). In nAMD, choroidal neovascularization (CNV) invades the subretinal space resulting in exudation of fluid, subretinal hemorrhage and severe visual loss. The three commonly used anti-VEGF drugs are bevacizumab, ranibizumab and aflibercept. All three drugs are highly effective and provide similar functional outcomes.[1-3] However, despite this remarkable clinical effect, there is a wide range in treatment response.[1,2] As genetic variation has been shown to strongly influence the development and progression of nAMD, attention has been focused on the influence of genetic risk alleles on treatment response to anti-VEGF therapy. Initial studies have suggested that the major risk alleles for the development of AMD do not affect response to therapy in patients with nAMD.[4,5]

VEGFA is the primary angiogenic factor involved in the development of CNV. As anti-VEGF therapeutics bind VEGFA and its isoforms, it is biologically plausible that single nucleotide polymorphisms (SNPs) that regulate VEGFA expression could also be involved in modulating the response to anti-VEGF drugs. Our recent study of eight SNPs within *VEGFA* and *VEGFR2* revealed no association between these polymorphisms and treatment response.[6] However, a recent study by Hermann et al evaluated the association of 126 SNPs in *VEGF* genes and their receptors (*VEGFR*) with response to ranibizumab in a case series of 366 patients with nAMD.[7] In an analysis that did not account for multiple comparisons, the minor alleles of two SNPs (rs4576072 and rs6828477) in *VEGFR2*, the gene encoding the receptor responsible for mediating most cellular responses to VEGF, were independently associated with a greater improvement in visual acuity (VA). At one year, the presence of the minor allele at either SNP was associated with one to two lines of VA improvement as compared to those patients without the minor allele. Furthermore, improvement was reported as additionally increased for each SNP with the presence of an additional minor allele.

The Comparison of AMD Treatments Trials (CATT) and the Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularisation (IVAN) trial are two large multicenter randomized clinical trials that compared bevacizumab and

ranibizumab in patients with nAMD. Genetic assessment of participants in these trials provides an ideal opportunity to investigate pharmacogenetic associations given that all outcomes were determined in the context of a prospective randomized clinical trial using well-defined protocols. In an effort to verify the pharmacogenetic association between *VEGFR2* SNPs and response to anti-VEGF therapy, we evaluated the two SNPs (rs4576072 and rs6828477) in participants from the CATT and IVAN trials.

Methods

CATT Participants

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. We recruited 835 CATT participants for the genetics study and details about this cohort are well documented elsewhere.[4,6] All analyses investigating the effect of genotype on response to treatment for this study were evaluated with outcomes data at one year to minimize confounding factors that may occur at later time points in the trial. Furthermore, the majority of the response in morphological and visual outcomes occurred within the first six months of treatment.[1] Finally, we chose to look at one year outcomes so that we could directly compare our results to those of Hermann et al.[7]

IVAN Participants

Study procedures for IVAN have been previously reported and are provided on ControlledTrials.com (ISRCTN92166560).[2] Informed consent for participating in this additional genetics study was obtained from all IVAN genetic study participants. A UK National Health Service Research Ethics Committee gave approval (reference 07/NIR03/37). The IVAN Study Investigators recruited 512 IVAN patients for the genetics study and details about this cohort are well documented elsewhere.[5] Similar to the CATT analyses, this analysis of the IVAN data focused on one year outcomes.

Genotype Determination

In CATT, approximately 10-20 ml of peripheral blood were collected from each patient. DNA was extracted and purified from leukocytes as previously described.[4] Two SNPs in *VEGFR2* (rs4576072 and rs6828477) were evaluated in each patient. Genotyping was performed using TaqMan SNP genotyping assays (Applied Biosystems) as previously described.[4] All laboratory personnel were masked to treatment assignment and patient clinical data. For the genetic analysis of IVAN samples, DNA was extracted and normalized from 10 ml of peripheral blood using an established method.[8] The SNP assays were performed using KASPar biochemistry as previously described.[5]

Measures of Response to Treatment

For the purposes of this study the main outcome measure of responsiveness to treatment was defined as the mean change in best corrected visual acuity from baseline at 1 year in study eyes. In both CATT and IVAN, visual acuity examiners were masked to treatment status and

best corrected acuity obtained at every visit in study eyes. Acuity was measured using either electronic VA charts (CATT) or backlit early treatment diabetic retinopathy charts (IVAN). Regardless of the method of acuity testing, the measure of acuity was ETDRS letters read in both of the clinical trials thereby allowing easy pooling of data for analysis.

Statistical Analysis

The mean VA change from baseline at one year was compared among all three genotype groups (TT, CT, CC) for each SNP using linear trend test. Following the same analysis approach of Hermann et al,[7] three genotype groups (CC, CT, and CC or CT) having a minor C allele were compared to the genotype TT using analysis of variance. This analysis was performed among patients treated with either ranibizumab or bevacizumab and among patients treated with ranibizumab only. Data from the CATT and IVAN studies were considered separately and in a combined analysis controlling for study. An uncorrected p-value less than 0.025 was considered statistically significant after applying the Bonferroni adjustment to account for the evaluation of 2 SNPS; no further adjustments were made for multiple statistical tests for each SNP or for subgroup analyses.

Results

We evaluated a total of 1347 patients with nAMD across two SNPs within the *VEGFR2* gene previously reported to have a significant influence on the treatment response to ranibizumab. The minor allele frequencies for both SNPs were nearly identical in the 835 CATT participants, 512 IVAN participants, and in the Hermann cohort (0.43, 0.42, 0.40, respectively for rs6828477; 0.16, 0.16, 0.16 respectively for rs4576072) (Table 1).[7]

Among CATT participants, there was no significant difference in mean change in VA at one year between patients homozygous for the C allele (minor allele) for either of the two *VEGFR2* SNPs of interest (rs4576072 and rs6828477) versus those who were homozygous for the T allele (p=0.46 and p=0.26, respectively) (Table 1). When the analysis was restricted to patients who were treated with ranibizumab only (n=432), no significant difference in mean change in VA was detected for patients homozygous for the C allele for rs4576072 (p=0.63) as compared to those homozygous for the T allele. However, for rs6828477, there was a significant difference in mean change in VA for patients homozygous for the T allele (10.6 letters TT vs 5.5 letters CC, p=0.007) (Table 1). This difference was in the opposite direction of that reported by Hermann et al.[7]

When we analyzed the possibility of an additive effect on mean change in VA from the number of minor alleles present (0 – 4 alleles) from the two SNPs, we found no association (linear trend p=0.24) (Table 2). Similarly, no correlation was observed between mean change in VA and the number of minor alleles present when evaluating patients treated solely with ranibizumab (linear trend p=0.07) (Table 2). When analyzing all possible combinations of alleles between the two SNPs, there was no significant association noted between the combinations and mean change in VA (p=0.64) (Table 3). Similarly, no correlation was observed when the combination of allele analysis was restricted to patients treated with ranibizumab only (p=0.21) (Table 3).

Among IVAN participants, there was no significant difference in mean change in VA between patients homozygous for the C allele (minor allele) for either of the two *VEGFR2* SNPs versus those who were homozygous for the T allele (p=0.62 and p=0.81, respectively) (Table 1). In addition, when the analysis was restricted to patients who were treated with ranibizumab only (n=271), no significant difference was detected for patients homozygous for the C allele for either of the two *VEGFR2* SNPs versus those who were homozygous for the T allele (p=0.19 and p=0.14, respectively) (Table 1).

We found no association among IVAN participants between the number of minor alleles present (0 – 4 alleles) from the two SNPs (linear trend p=0.72) (Table 2). Furthermore, no correlation was observed between mean change in VA and the number of minor alleles present when evaluating patients treated solely with ranibizumab (linear trend p=0.13) (Table 2). When analyzing all possible combinations of alleles between the two SNPs, there was no significant association noted between the combinations and mean change in VA (p=0.73) (Table 3). Similarly, no correlation was observed when the combination of allele analysis was restricted to patients treated with ranibizumab only (p=0.87) (Table 3).

When the data from both CATT and IVAN cohorts were combined for the analyses, there was no significant difference in mean change in VA between patients homozygous for the C allele (minor allele) versus those homozygous for the T allele for rs4576072 (p=0.81) or for rs6828477 (p=0.29) (data not shown). When the analysis was restricted to patients who were treated with ranibizumab only, no significant difference was detected for patients homozygous for the C allele versus those homozygous for the T allele for rs4576072 (p=0.51) or for rs6828477 (p=0.25) (data not shown).

Finally, for both CATT and IVAN, no statistically significant association was observed between mean change in VA and either of the two *VEGFR2* SNPs tested when the data from 3 months was analyzed (data not shown).

Discussion

We evaluated the association of two *VEGFR2* SNPs with response to anti-VEGF therapy in two independent, large patient cohorts because a strong association with VA had been reported previously by Hermann et al. We found no statistically significant associations that would support the findings of the Hermann study in our analysis that involved a total of 1347 patients with nAMD. In their study of 366 patients evaluating the pharmacogenetic effects of 126 SNPs from 9 genes, only two SNPs (rs4576072 and rs6828477) were found to be associated with greater VA improvement in patients treated with ranibizumab. However, most of their analyses did not account for their evaluation of many (n=126) different SNPs. Although there was a statistically significant association (p<0.025) within the CATT data for rs6828477 in patients treated with ranibizumab, the allele associated with lower visual acuity in the Hermann study was associated with better visual acuity in the CATT patients and no association was identified among the IVAN patients or the combined group of patients in each study treated with ranibizumab or bevacizumab. In addition to the possibility that the associations were attributable to chance variation, it is possible that the Hermann cohort was different given the fact that there were no baseline variables that were

associated with one-year visual outcomes in the Hermann study. In CATT and most other nAMD studies, age and baseline visual acuity, for example, were strongly associated with one year visual outcomes.[9-12]

The rationale as to why these two SNPs located in the *VEGFR2* gene would influence the visual outcome of anti-VEGF treatment is not clear. Hermann et al suggest that these SNPs lead to altered expression of VEGFR2, leading to a benefit on visual acuity of VEGF neutralization by ranibizumab. However, both rs4576072 and rs6828477 are located in the intronic sequences and to our knowledge, there are no reports that have tested this hypothesis and confirmed that either of these polymorphisms influence the expression or functional activity of VEGFR2.

In conclusion, the combined analysis of data from the CATT and IVAN do not support a pharmacogenetic association between the two *VEGFR2* SNPs, rs4576072 and rs6828477, and the visual acuity response to anti-VEGF therapy in patients with nAMD.

Acknowledgments

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Two *VEGFR2* SNPs, rs4576072 and rs6828477, previously associated with greater visual acuity improvement in patients treated with ranibizumab did not strongly affect visual acuity response to anti-VEGF therapy in CATT and IVAN study participants.

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Table 1

Visual acuity change from baseline at one year among genotype groups of *VEGFR2* SNPs rs4576072 and rs6828477 in CATT and IVAN

	Ranibizumab and Bevacizumab Combined			Ranibizumab only		
SNP Genotype	**n (%)**	**Change in visual acuity (letters) Mean (SD)**	**P for comparison with TT[§]**	**n (%)**	**Change in visual acuity (letters) Mean (SD)**	**P for comparison with TT[§]**
		CATT (N=835)			**CATT (N=432)**	
rs4576072		Linear Trend P=0.77			Linear trend P=0.66	
TT	595 (71)	8.2 (14.5)		312 (72)	8.7 (12.8)	
CT	219 (26)	8.3 (13.6)	0.90	111 (26)	9.9 (12.5)	0.41
CC	21 (3)	5.9 (11.3)	0.46	9 (2)	6.7 (13.0)	0.63
CT or CC	240 (29)	8.1 (13.4)	0.94	120 (28)	9.6 (12.6)	0.50
rs6828477		Linear Trend P=0.20			Linear trend P=0.01	
TT	250 (30)	9.2 (13.7)		132 (31)	10.6 (11.2)	
CT	454 (54)	7.8 (14.2)	0.20	230 (53)	9.1 (12.6)	0.29
CC	131 (16)	7.5 (14.8)	0.26	70 (16)	5.5 (15.0)	0.007
CT or CC	585 (70)	7.7 (14.4)	0.16	300 (69)	8.3 (13.3)	0.09
		IVAN (N=512)[*]			IVAN (N=271)	
rs4576072		Linear trend P=0.53			Linear trend P= 0.56	
TT	364 (71)	6.0 (11.9)		196 (72)	6.4 (11.9)	
CT	124 (24)	4.2 (11.4)	0.19	60 (22)	5.6 (12.3)	0.67
CC	20 (4)	7.4 (18.8)	0.62	12 (4)	11.5 (17.4)	0.19
CT or CC	144 (28)	4.7 (12.5)	0.30	72 (27)	6.5 (13.0)	0.93
rs6828477		Linear trend P=0.97			Linear trend P= 0.13	
TT	185 (36)	5.0 (12.0)		95 (35)	5.1 (12.8)	
CT	221 (43)	6.6 (11.8)	0.22	121 (45)	6.6 (11.2)	0.39
CC	105 (21)	4.7 (12.8)	0.81	55 (20)	8.3 (13.2)	0.14
CT or CC	326 (64)	5.9 (12.1)	0.43	176 (65)	7.2 (11.8)	0.21

SD = standard deviation, SNP=single nucleotide polymorphism, VA=visual acuity.

CATT = Comparison of AMD Treatments Trials.

IVAN = Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularization.

[§] P values were calculated using analysis of variance.

[*] Invalid genotype data occurred in 4 patients for rs4576072 and 1 patient for rs6828477 and were excluded from the statistical analysis.

Table 2

Visual acuity change from baseline at one year by the number of minor alleles in *VEGFR2* SNPs rs4576072 and rs6828477 in CATT and IVAN

	Ranibizumab and Bevacizumab Combined		Ranibizumab Only	
Number of minor (C) alleles	**n (%)**	**Letters Mean (SD)**	**n (%)**	**Letters Mean (SD)**
	CATT			
0	183 (22)	9.3 (14.2)	97 (22)	10.9 (10.9)
1	387 (46)	8.0 (14.2)	193 (45)	8.7 (12.8)
2	207 (25)	7.9 (14.2)	120 (28)	8.5 (13.4)
3	56 (7)	7.2 (14.2)	22 (5)	5.6 (15.2)
4	2 (0)	0.0 (0.0)	0 (0)	--
Linear Trend P		0.24		0.07
	IVAN			
0	133 (26)	5.3 (11.2)	69 (26)	5.0 (10.9)
1	196 (39)	6.3 (12.6)	106 (40)	6.2 (12.5)
2	145 (29)	5.3 (12.1)	75 (28)	7.8 (13.6)
3	26 (5)	3.7 (10.8)	15 (6)	5.1 (4.4)
4	7 (1)	5.7 (22.5)	3 (1)	19.7 (17.8)
Linear Trend P		0.72		0.13

SD = Standard deviation.
CATT = Comparison of AMD Treatments Trials.
IVAN = Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularization.

Table 3

Visual acuity change from baseline at one year by the combination of *VEGFR2* SNPs rs4576072 and rs6828477 in CATT and IVAN

	Ranibizumab and Bevacizumab Combined			Ranibizumab only		
		rs4576072			**rs4576072**	
	Mean (SD) in CATT					
rs6828477	TT	CT	CC	TT	CT	CC
TT	9.3 (14.2)	9.1 (12.6)	--	10.9 (10.9)	9.5 (12.2)	--
CT	7.8 (14.5)	8.1 (13.8)	5.8 (12.0)	8.5 (12.9)	11.1 (11.6)	5.3 (13.4)
CC	7.5 (14.9)	7.7 (15.0)	--	5.4 (14.8)	5.7 (16.5)	--
	P=0.64			P=0.21		
	Mean (SD) in IVAN					
rs6828477	TT	CT	CC	TT	CT	CC
TT	5.3 (11.2)	3.7 (13.2)	10.4 (22.4)	5.0 (10.9)	4.8 (15.8)	--
CT	7.0 (12.3)	5.2 (10.6)	6.9 (14.4)	6.6 (11.6)	6.6 (11.4)	6.5 (5.2)
CC	5.1 (12.5)	2.3 (9.0)	5.7 (22.5)	8.3 (13.9)	4.4 (4.1)	--
		P=0.73			P=0.87	

SD = Standard deviation.
CATT = Comparison of AMD Treatments Trials.
IVAN = Alternative Treatments to Inhibit VEGF in Patients with Age-Related Choroidal Neovascularization.
--: Mean (SD) was not calculated due to because the number of patients is less than 5.