

A pharmacogenetics study to predict outcome in patients receiving anti-VEGF therapy in age related macular degeneration

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Purpose: To ascertain whether single nucleotide polymorphisms (SNPs) in the *Vascular Endothelial Growth factor (VEGFA)*, *Complement Factor H (CFH)*, and *LOC387715* genes could predict outcome to anti-VEGF therapy for patients with age related macular degeneration (AMD).

Methods: Patients with “wet” AMD were identified by chart review. Baseline optical coherence tomography (OCT) and visual acuity (VA) data, and at least 6 months of clinical follow up after 3 initial monthly injections of bevacizumab or ranibizumab were required for inclusion. Based on OCT and VA, patients were categorized into two possible clinical outcomes: (a) responders and (b) non-responders. DNA was extracted from saliva and genotyped for candidate SNPs in the *VEGFA*, *LOC387715*, and *CFH* genes. Clinical outcomes were statistically compared to patient genotypes.

Results: 101 patients were recruited, and one eye from each patient was included in the analysis. 97% of samples were successfully genotyped for all SNPs. We found a statistically significant association between the *LOC387715 A69S TT* genotype and outcome based on OCT.

Conclusion: Genetic variation may be associated with outcome in patients receiving anti-VEGF therapy.

Keywords: age related macular degeneration, ARMS2, bevacizumab, complement factor H (CFH), LOC387715, ranibizumab, single nucleotide polymorphisms, vascular endothelial growth factor

Introduction

Exudative (“wet”) age related macular degeneration (AMD) is a leading cause of vision loss in people over 65. An estimated 1.75 million Americans suffer from this severe form of AMD and the number is predicted to reach 2.95 million by 2020.¹ Standard therapeutic approach includes the inhibition of vascular endothelial growth factor (VEGFA) with intraocular injections of bevacizumab or ranibizumab.²⁻⁴ Although these agents are efficacious, substantial heterogeneity is seen with the implementation of anti-VEGF therapeutics in terms of duration and degree of response.^{5,6} This heterogeneity and ambiguous duration of treatment demonstrates the need for predictive biomarkers.

Previous work has reported that candidate single nucleotide polymorphisms (SNPs) might serve as prognostic or predictive markers for AMD. A SNP in the *LOC387715* gene has previously been correlated with progression from intermediate forms of non-exudative (dry) AMD to wet AMD.⁷⁻¹¹ Prior work also demonstrated that SNPs in *CFH*, *LOC387715*, and *VEGF* have been associated with response to

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anti-VEGF therapy in patients with AMD.⁹⁻¹¹ In addition, our group has previously identified SNPs in the *VEGFA* gene that predicted efficacy and toxicity for bevacizumab in women with metastatic breast cancer.⁶ In this current study, we sought to determine whether genetic variations could predict outcome as measured by optical coherence tomography (OCT) and visual acuity (VA) in AMD patients receiving anti-VEGF therapy, and to identify a subgroup of patients that may not benefit optimally from this modality of treatment. To our knowledge, studies in which both OCT and VA were investigated in the same study cohort are scarce.

Methods

Patient selection

Patients with wet AMD between 2001-2010 were identified by a retrospective chart review. Inclusion criteria were 1) history of documented exudative AMD with baseline OCT and VA, 2) at least three previous consecutive injections of intravitreal bevacizumab or ranibizumab as initial therapy, and 3) at least 6 monthly follow-ups after the third injection of bevacizumab/ranibizumab. The majority of patients received ranibizumab (supplementary material Table S1). One of four retina specialists determined disease activity for each patient; however, practice patterns were uniform among the four treating physicians. These patients returned monthly for follow up after 3 monthly injections and retreatment was based on the presence or absence of subretinal and intraretinal fluid on OCT. Change in VA did not influence retreatment. Exclusion criteria included: 1) prior vitrectomy surgery or prior therapy (laser, photodynamic therapy, steroid injections), 2) primary pigment epithelial detachment, 3) significant fibrosis, or 4) vitreous hemorrhage.

Phenotype definition and sample acquisition

Patients that met the prespecified inclusion and exclusion criteria were initially categorized into one of two clinical response phenotypes by OCT and VA data from chart review: responder and non-responder (Table 1A and B). Zeiss Stratus Oct™ and Zeiss Cirrus OCT machines (Carl Zeiss Meditech AG, Jena, Germany) performing macular

Table 1A Phenotype based on OCT

Phenotype	OCT
Group 1 (responders)	No fluid after third injection for at least 1 month (no fluid at month 4)
Group 2 (non-responders)	Fluid present 1 month after third injection (fluid present at month 4)

Abbreviation: OCT, optical coherence tomography.

Table 1B Phenotype based on VA

Phenotype	VA
Group 1 (responders)	Gained ≥ 3 lines at month 9
Group 2 (non-responders)	Did not gain ≥ 3 lines at month 9

Abbreviation: VA, visual acuity.

thickness map and line scans were utilized to look for the presence of change. VA was measured using the Snellen eye chart consisting of Sloan letter optotypes at a standard 20 feet. These patients were then invited to provide a saliva specimen for DNA extraction and genotyping. Informed written consent was obtained from all participants. Analysis was not performed on fluorescein angiograms. The study was approved by the Sterling Institutional Review Board and the Indiana University School of Medicine Institutional Review Board, and all patients were consented in person.

DNA extraction and genotyping

Candidate SNPs included those in the *VEGFA*, *CFH*, and *LOC387715* genes (Table 2). The *VEGFA* SNPs included those that tagged for the common haplotypes or those that were previously shown to have predictive capacity for anti-VEGF therapy in the literature. The *CFH* and *LOC387715* SNP selections were based on those with literature to support an association with AMD at the time this trial was initiated. DNA was extracted from saliva using the Oragene® DNA sample collection kit by DNA Genotek (Ottawa, ON, Canada). Samples were genotyped for candidate SNPs by TaqMan®-based real time-PCR (Applied Biosystems, Foster City, CA, USA). Pre-designed TaqMan SNP genotyping assays were used for *LOC387715 A69S*, *VEGFA -3818G/T, -1498C/T, -2578C/A, -634G/C, -7C/T, and -1154G/A*. Custom TaqMan SNP genotyping assays were designed for *CFHY402H*, *VEGFA -2305G/T*, and *-1210C/A*

Table 2 Candidate SNPs

GENE	Variant	dbSNP ID
<i>VEGFA</i>	-2578C/A	rs699947
	-1154G/A	rs1570360
	-3818G/T	rs833060
	-2305G/T	rs36208049
	-1498C/T	rs833061
	-7C/T	rs25648
	-1210C/A	rs59260042
	-634G/C	rs2010963
<i>LOC387715</i>	S69AG/T	rs10490924
<i>CFH</i>	Y402HC/T	rs1061170

Abbreviations: SNPs, single nucleotide polymorphisms; *VEGFA*, vascular endothelial growth factor A; *LOC*, otherwise known as age-related maculopathy susceptibility gene 2 (ARMS2); *CFH*, complement factor H; dbSNP, single nucleotide polymorphism database.

(supplementary material Tables S2 and S3). Samples that were previously sequenced by Sanger sequencing were used as positive controls.

Endpoints

Clinical responses (Tables 1A and B) were assessed monthly for 6 months after 3 monthly injections of bevacizumab or ranibizumab. Patient demographics, VA, prior OCT data, as well as prior injection history were collected and utilized to classify each of the enrolled patients into one of the two possible clinical outcomes. For VA, patients with a gain of three lines or greater on the Snellen eye chart after conclusion of the study (9 month time period) were considered “responders” and all others were considered “non-responders”. For OCT, patients that had no sub- or intraretinal fluid present at least one month after the third monthly injection (no fluid at month 4) were considered “responders” and all others (fluid present at month 4) were considered “non-responders”.

Statistical design

There were 101 patients recruited for this study. Analyses for recessive, dominant and allele dose effect were performed for all candidate SNPs. Association between demographics and phenotype was assessed by Fisher’s exact test. Association between genotype and phenotype (responder versus non-responder) was assessed for both VA and OCT. Dominant, recessive, and allele dose effects were assessed using a logistic regression test. *P*-values < 0.006 were considered statistically significant after correcting for multiple comparisons, which was done using Bonferroni methodology.

Results

Study population, phenotype and demographics

101 patients were recruited based on inclusion and exclusion criteria. Patients were grouped into responders and non-responders based on distinct OCT and VA criteria. Therefore, responders based on OCT were not necessarily responders based on VA and vice versa. Based on OCT data, 79% of the patients were classified as responders and 21% were classified as non-responders. Patient demographics are outlined in Table 3 and demographics by clinical outcome based on OCT and VA are outlined in Table 4 and supplementary Table S4, respectively. VA data was available on only 100 patients. Based on VA, 30% of the patients were classified as responders and 70% were classified as non-responders as outlined in Table 5. There were no significant associations between demographic data points (ie, age, sex, tobacco use, and race) and clinical response.

Table 3 Patient demographics

Characteristics	Number of patients
Sex	
Male	33
Female	68
Mean age	80
Race	
White	101
Other	0
Tobacco use	
None	84
Past/present use	17

Association by genotype

Ninety nine of 101 patients were successfully genotyped for the *VEGFA* -2578C/A, -1154G/A, -3818G/T, -1498C/T, -7C/T, and -634G/C SNPs. One hundred of 101 were successfully genotyped for the *LOC387715 A69S* and *VEGFA* -2305G/T SNPs. Ninety-seven of 101 were successfully genotyped for *CFHY402H*. The *VEGFA*-1210C/A and *VEGFA*-2305G/T SNPs were excluded, as there were no variants in our population. Genetic analyses for dose, dominant and recessive effects were performed for all other SNPs on all available samples and were evaluated for correlation with outcome (Tables 6, 7, and supplementary Table S5).

Patients who carried the *VEGFA*-2578CC, *VEGFA* -1498TT or the *VEGFA*-1154GG genotypes were more likely to be non-responders based on VA (Figure 1 and Table 6). However, this association was not statistically significant after correction for multiple comparisons. There were no associations with *CFH* and *LOC387715*. Based on OCT, patients who carried the *LOC387715 A69STT* genotype were significantly more likely to be classified as a non-responder (9/16) compared to those with the GG and GT genotypes

Table 4 Patient demographics by clinical outcome (OCT)

Characteristics	Non-responder	Favorable responder phenotype	(<i>P</i> -value)
Total number of patients	21	80	–
Sex			
Male	4	29	0.19
Female	17	51	
Mean age	80	80	1.00
Race			
White	21	80	–
Other	0	0	
Tobacco use			
None	20	65	0.18
Past/present use	1	15	

Table 5 Snellen visual acuity (VA) outcomes

Patient group	Number of patients	Mean initial VA	Mean final VA (at 9 months)
All patients	100	20/80	20/60
Responders (gained \geq 3 lines)	30	20/100	20/40
Non-responders (gained $<$ 3 Lines)	70	20/70	20/70

(12/84); ($P = 0.00071$; odds ratio: 7.69; 95% confidence interval: 2.38–25). There were no significant associations between *VEGFA* and *CFH* genotypes with OCT (Figure 2 and Table 7).

Discussion

There is substantial heterogeneity in the degree and duration of response among AMD patients treated with anti-VEGF therapy. In this retrospective study we examined germline genetic variation in *VEGFA*, *LOC387715* and *CFH* genes as possible predictive biomarkers for outcome in patients receiving bevacizumab or ranibizumab for the treatment of wet AMD.

Our prior work in metastatic breast cancer had identified several *VEGFA* SNPs that predicted outcome for systemic bevacizumab.⁶ While there are clearly some similarities between cancer-mediated angiogenesis and AMD-mediated angiogenesis making cross disease comparisons rational, there are also likely fundamental differences to consider.

In this study we report a strong trend between *VEGFA* SNPs (*-2578CC*, *-1498TT* and *-1154*) and VA. However, these SNPs lost their significance after correction for multiple comparisons. One of the limitations of this study was sample size and the resultant statistical power. Therefore, it is possible that this study was underpowered to detect associations between genotype and phenotype of smaller effect size. An additional potential limitation of this study was relying upon Snellen VA as opposed to ETDRS vision charts. It is possible

that using ETDRS vision charts would have led to different statistical outcomes due to the variable letter size and letters per line on the Snellen chart. However, we used “three lines or greater” on the Snellen eye chart as the best metric available given the retrospective nature of this study.

Other groups have also evaluated these SNPs in the AMD setting. A study by Imai et al¹⁰ found a correlation between *VEGFA -2578C/A* and VA changes in response to anti-VEGF therapy. Similar to our study, *VEGFA-2578 C* carriers were more likely to be non-responders based on VA. Although our results would support this finding, our data would suggest that the effect is modest. Additionally, this effect was not seen when response was measured by OCT (measuring retinal thickness), which is similar to what we observed. Another study by Boltz et al¹² reported significant findings with *VEGFA-634* and *VEGFA IVS -99* (rs3024997). However, these results were not significant after correction for multivariate analysis.

We also evaluated OCT as a second phenotype. At the time of this trial, OCT was commonly used as a surrogate for the frequency and duration of therapy independent of changes in VA. The goal of using OCT as the endpoint was to predict the duration and frequency of therapy necessary to achieve good vision. This is very different from a biomarker for VA which predicts for vision outcome regardless of the intensity/frequency of therapy and which is impacted by other variables. We identified a statistically significant association between *LOC387715 A69S* carriers and the non-responder phenotype based on OCT. Those patients with two variant alleles had a markedly higher likelihood of not responding to therapy (recessive effect) and each variant allele added to this likelihood (allele-dose effect).

As the exact function of *LOC387715* is yet to be elucidated, the role of the non-synonymous *LOC387715 A69S* SNP in the pathogenesis of AMD remains unknown. Previous studies, however, have clearly demonstrated an association with *LOC387715 A69S* and poor outcome independent

Table 6 Genotype compared to visual acuity (VA)

Gene/SNP	Dominant analysis			Recessive analysis			Additive analysis		
	P-value	OR	CI	P-value	OR	CI	P-value	OR	CI
<i>VEGFA-3818G/T</i>	0.46	1.39	0.58, 3.31	0.11	0.32	0.08, 1.30	0.87	0.95	0.50, 1.82
<i>VEGFA-2578C/A</i>	0.80	1.14	0.42, 3.04	0.013	0.27	0.10, 0.76	0.17	0.63	0.33, 1.22
<i>VEGFA-1498C/T</i>	0.80	1.14	0.42, 3.04	0.013	0.27	0.10, 0.76	0.17	0.63	0.33, 1.22
<i>VEGFA-1154A/G</i>	0.42	0.69	0.29, 1.68	–	–	–	0.10	0.59	0.31, 1.12
<i>VEGFA-634C/G</i>	0.80	1.12	0.47, 2.66	0.04	0.27	0.08, 0.93	0.51	0.81	0.43, 1.53
<i>VEGFA-7C/T</i>	0.93	0.95	0.36, 2.54	0.09	0.33	0.09, 1.18	0.93	0.95	0.36, 2.54
<i>CFH Y402H</i>	0.21	0.53	0.20, 1.42	0.12	3.43	0.72, 16.30	1.00	1.00	0.53, 1.90
<i>LOC387715 A69S</i>	0.58	1.28	0.54, 3.03	0.56	1.44	0.42, 4.91	0.50	1.23	0.67, 2.24

Abbreviations: OR, odds ratio; CI, 95% confidence interval; SNP, single nucleotide polymorphism.

Table 7 Genotype compared to optical coherence tomography (OCT)

Gene/SNP	Dominant analysis			Recessive analysis			Additive analysis		
	P-value	OR	CI	P-value	OR	CI	P-value	OR	CI
VEGFA-3818G/T	1.00	1.08	0.40, 2.94	0.20	0.19	0.04, 1.96	0.71	1.06	0.78, 1.45
VEGFA-2578C/A	0.58	0.74	0.24, 2.27	0.76	0.65	0.16, 2.56	0.38	0.87	0.65, 1.19
VEGFA-1498C/T	0.58	1.56	0.40, 6.25	0.76	1.35	0.45, 4.17	0.38	1.14	0.85, 1.56
VEGFA-1154A/G	0.46	3.03	0.36, 33.3	0.45	1.47	0.54, 4.17	0.70	1.06	0.79, 1.45
VEGFA-634C/G	1.00	2.78	0.32, 25.00	0.45	0.98	0.36, 2.70	0.17	1.23	0.92, 1.67
VEGFA-7C/T	0.58	1.35	0.45, 4.17	1.00	–	–	0.59	0.88	0.56, 1.41
CFH Y402H	1.00	1.12	0.38, 3.45	1.00	0.95	0.24, 4.00	0.71	1.05	0.78, 1.45
LOC387715 A69S	0.32	1.89	0.66, 5.26	0.0007	7.65	2.38, 25	0.016	1.39	1.06, 1.85

Abbreviations: OR, odds ratio; CI, 95% confidence interval; SNP, single nucleotide polymorphism.

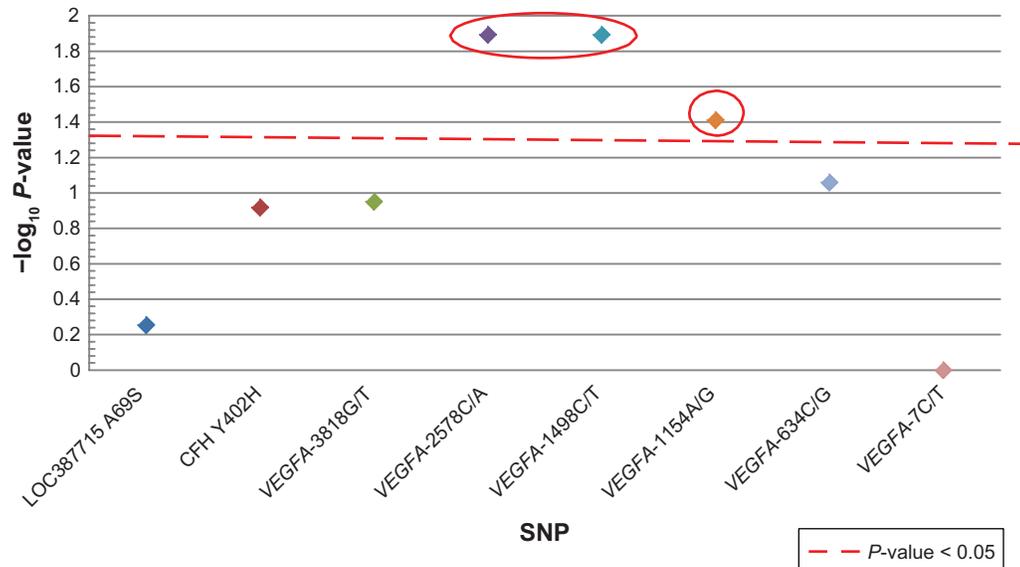


Figure 1 Genotype compared to visual acuity (VA).

Notes: A strong trend between VEGFA -2578CC, VEGFA -1498TT (odds ratio: 3.7, 95% confidence interval: 1.3, 10.2) and VEGFA -1154 GG (odds ratio: 3.7, 95% confidence interval: 1.1, 12.9) and VA. The x-axis indicates the SNP analyzed and the y-axis denotes magnitude of the evidence for association, shown as $-\log_{10}(P\text{-value})$. Each colored diamond represents the $-\log_{10}(P\text{-value})$ for that specific SNP.

Abbreviations: VEGFA, vascular endothelial growth factor; SNP, single nucleotide polymorphism.

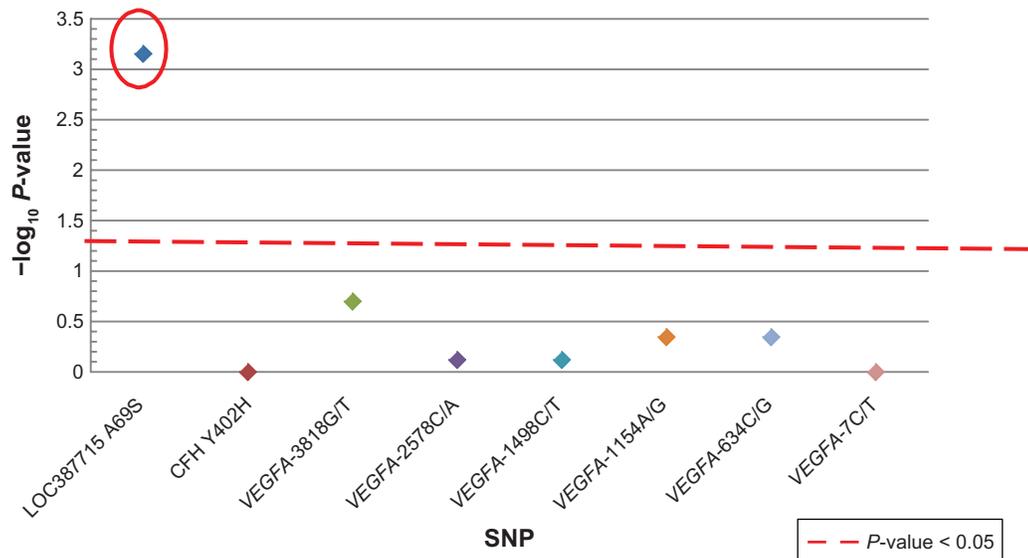


Figure 2 Genotype compared to OCT.

Notes: A significant association between LOC387715 A69S (odds ratio: 7.69, 95% confidence intervals: 2.38, 25) and OCT response. The colored diamonds represent the correlation between each of the SNPs and OCT.

Abbreviations: SNP, single nucleotide polymorphism; OCT, optical coherence tomography.

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of therapy (ie, a prognostic marker)^{7,8,13}. The results here would further suggest that this genotype does poorly even with the use of standard anti-VEGF therapy; thus making it a powerful predictive biomarker as well. Brantley et al⁹ demonstrated a correlation between the *CFH CC* genotype and worse outcome after anti-VEGF injections compared with the alternate genotypes. They did not, however, find a significant association between *LOC387715 A69S* and treatment outcome. In congruence with our results, a more recent study showed a correlation between the *LOC387715 A69S TT* genotype and a poor response to ranibizumab injections with no correlation between *CFH* genotypes and response.¹⁴ There are several variables that might explain the differing results among these studies. First, the treatment duration and dosing were not uniform. In our study, response was assessed after 3 monthly injections; whereas Brantley et al used 6-week intervals.⁹ More importantly, the phenotype was markedly different between the three studies. The prior studies used changes in VA as the endpoint whereas the associations in this study were between genotype and OCT in addition to genotype and VA.

Although we used both ranibizumab and bevacizumab, both CATT (Comparison of AMD Treatment Trial) and IVAN (Inhibit VEGF in Age-related choroidal Neovascularization) suggest that there is no statistically significant difference in visual acuity improvement between bevacizumab and ranibizumab in the treatment of neovascular AMD.^{15,16}

In conclusion, we have demonstrated a genetic association between the *LOC387715 A69S TT* genotype and a group of patients that may not respond well to bevacizumab or ranibizumab injections. This study would suggest that patients carrying this genotype should be candidates for studies evaluating alternative or novel therapeutic approaches to wet AMD. Prior studies have also suggested that other (non-VEGF directed) interventions such as photodynamic therapy (PDT) may also be influenced by genotype.^{17–19} Thus, personalized therapy based on genotype may provide a rational direction for selection of therapeutic approach. Alternatively, this patient cohort may simply represent ideal candidates for trials of higher dose anti-VEGF therapy. Further studies to confirm this genotype-phenotype association are underway by our collaborative group.

Disclosure

John W Kitchens has been a speaker/consultant for Genentech and Regeneron and a consultant for Thrombogenics, Allergan, Alcon, and Synergetics. He also holds stock in Synergetics. Bryan P Schneider is on the Genentech speaker's bureau and advisory board (compensated). The authors report no other conflicts of interest in this work.

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Supplementary materials

Table S1 Number of patients receiving bevacizumab, ranibizumab, or both

Anti-VEGF therapy used	Number of patients
Bevacizumab	17
Ranibizumab	81
Both	3

Table S2 Predesigned TaqMan™ assays

dbSNP ID	Assay ID
rs1570360	C_1647379_10
rs25648	C_791476_10
rs833060	C_1647392_20
rs699947	C_8311602_10
rs833061	C_1647381_10
rs2010963	C_8311614_10
rs10490924	C_29934973_20

Abbreviation: dbSNP ID, single nucleotide polymorphism database identification.

Table S3 Custom made TaqMan™ assays

dbSNP ID	Primers (forward/reverse)	Reporter sequences
rs36208049	GGAGAAGTAGC CAAGGGATCCT/ GCCAGACTCA TAGCTCATCTTC	TCGTCTCAGCTCCCCCA/ TCGTCTCAGATCCCCCA
rs59260042	TCGAGCTTCCC CTTCATTGC/ GGACAGGCG AGCCTCAG	CCGCAGCCCGCC/ CCGCATCCCGCC
rs1061170	TGTTATGTCCTTAGG AAAATGTTATTTTCCTT/ GGCAGGCAACGTCTAT AGATTTACC	CTTCTCCATGATTTTG/ TTTCTCCATAATTTTG

Abbreviation: dbSNP ID, single nucleotide polymorphism database identification.

Table S4 Patient demographics by clinical outcome (visual acuity)

Characteristics	Non-responder	Favorable responder phenotype	(P-value)
Total number of patients	70	30	–
Sex			
Male	26	8	0.36
Female	44	22	
Mean age	79	81	0.44
Race			
White	70	30	–
Other	0	0	
Tobacco use			
None	57	27	0.38
Past/present use	13	3	

Table S5 Non-responder/responder ratio by genotype

Gene/SNP	Genotype (never responder/ favorable responder)		
	1*	2*	3*
VEGF-3818G/T	10/51	10/39	0/9
VEGF-2578C/A	6/25	11/54	3/20
VEGF-1498C/T	3/20	11/54	6/25
VEGF-1154A/G	1/12	9/45	10/42
VEGF-634C/G	9/45	10/43	1/11
VEGF-7C/T	14/74	6/25	0/0
CFH Y402H	6/31	11/51	3/15
LOC387715 A69S	7/45	5/39	9/16

Notes: *1, Homozygous wild type; 2, Heterozygous variant; 3, Homozygous variant.

Abbreviation: SNP, single nucleotide polymorphism.

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