Polymorphisms in Vascular Endothelial Growth Factor Receptor 2 Are Associated with Better Response Rates to Ranibizumab Treatment in Age-related Macular Degeneration

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Purpose: Intravitreal anti-vascular endothelial growth factor (VEGF) injections are currently the standard treatment for neovascular age-related macular degeneration (AMD), but a broad range of response rates has been observed. We evaluated the association of single nucleotide polymorphisms (SNPs) in VEGF genes and their receptors (VEGFR) with the response rate to ranibizumab in 366 patients with neovascular AMD.

Design: Case series study.

Participants: A total of 366 eyes of 366 patients with neovascular AMD.

Methods: Visual acuity (VA) was determined at baseline, after 3 monthly ranibizumab injections, and after 1 year of treatment. Genotyping of 126 SNPs in the genes encoding VEGF family members VEGFA, VEGFB, VEGFC, VEGFD (Fgf), and placental growth factor (PGF); VEGF receptors VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4); and the gene encoding pigment epithelium-derived factor (PEDF) (SERPINF1) was performed.

Main Outcome Measures: The changes in VA after 3 injections and after 1 year of treatment and their association with VEGF and VEGFR genotypes.

Results: Univariate analyses of variance (ANOVAs) revealed a significant effect of SNP rs4576072 in the VEGFR2 gene on VA change after 12 months (F[1,235] = 14.05; P = 0.02). A stepwise linear regression analysis returned a model (P = 0.01) with SNPs rs4576072 and rs6828477 in the VEGFR2 gene as independent predictors for VA change after 12 months, with a mean increase in VA of 0.26 on the logarithm of the minimum angle of resolution (logMAR) scale in patients with 3 contributing minor alleles compared with a loss of 0.03 logMAR in patients with no minor allele.

Conclusions: Polymorphisms in the VEGFR2/KDR gene significantly influence visual outcome in patients receiving ranibizumab treatment for neovascular AMD. This study shows that genetic variation partially explains the wide range of response to ranibizumab treatment, which in the future might help clinicians tailoring medical interventions to individual needs. Ophthalmology 2014;121:905-910 © 2014 by the American Academy of Ophthalmology.

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In neovascular age-related macular degeneration (AMD), newly formed choroidal blood vessels (choroidal neovascularization [CNV]) invade the subretinal and intraretinal spaces, causing exudation, hemorrhage, and subsequently visual loss. Drugs such as ranibizumab targeting vascular endothelial growth factor (VEGF) are effective in preserving and improving visual acuity (VA). Although many patients respond favorably to anti-VEGF treatment, some patients lose vision despite optimal therapy. Several studies have suggested that genetic factors influence the response to anti-VEGF treatment.1-15 For example, poor response rates were demonstrated for patients carrying the complement factor H (CFH) Y402H genotype1-7,9,10,16 or polymorphisms in age-related maculopathy susceptibility 2 (ARMS2).1,12 Moreover, a cumulative effect of high-risk alleles in the CFH, ARMS2, and VEGFA genes was associated with poor response rates to ranibizumab treatment and a younger age of onset of neovascular disease.11 However, the effect of genetic variants on treatment response is still not clear.

Ranibizumab is a humanized monoclonal antibody fragment that binds all isoforms of VEGFA, which is an important pro-angiogenic factor that plays a central role in
the development of CNV. In addition to VEGFA, the VEGF family comprises VEGFB, VEGFC, VEGFD (FIGF), and placental growth factor (PGF). The VEGF members are ligands of 3 tyrosine kinase receptors: VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4). Vascular endothelial growth factor A binds to VEGFR1 and VEGFR2; VEGFB and PGF bind only to VEGFR1. Vascular endothelial growth factor R2 mediates most cellular responses to VEGF, whereas VEGFR1 might modulate VEGFR2 signaling and act as a decoy receptor competing with VEGFR2 for VEGF. Vascular endothelial growth factor R2 and VEGFR1 are both inhibited by the anti-angiogenic pigment epithelium-derived factor (PEDF). Vascular endothelial growth factor C and VEGFD are ligands for VEGFR3 involved in lymphangiogenesis.17

The purpose of this study was to determine whether polymorphisms in genes encoding VEGF family members, VEGF receptors, and PEDF influence visual outcome in patients treated with ranibizumab for neovascular AMD. Although several studies have analyzed a limited number of single nucleotide polymorphisms (SNPs) in the VEGF and KDR genes,2,4,6,7,14 a comprehensive analysis of SNPs across all VEGF and VEGFR genes has not been performed. Therefore, we genotyped 126 tag-SNPs in the VEGF, VEGFB, VEGFC, VEGFD (FIGF), PGF, VEGFR1 (FLT1), VEGFR2 (KDR), VEGFR3 (FLT4), and PEDF (SERPINF1) genes in 366 patients with AMD who have been treated with ranibizumab for at least 1 year.

Methods

Study Population

This multicenter study included 366 eyes of 366 unrelated patients aged 50 years or older with active subfoveal CNV secondary to AMD. All participants were enrolled in the European Genetic Database (EUGENDA), a multicenter database for the clinical and molecular analysis of AMD, between 2008 and 2010. The study was performed in accordance with the tenets of the Declaration of Helsinki. The approval of the local ethics committee was obtained for both centers, and written informed consent was provided by all participants.

Inclusion and Exclusion Criteria

All patients had active subfoveal or juxtapfoveal CNV due to AMD confirmed by spectral-domain optical coherence tomography and fluorescein angiography (FA) with indocyanine green. Further criteria in the study eye were a best-corrected VA equivalent to ≥20/40 Early Treatment of Diabetic Retinopathy Study (ETDRS) letters and no previous treatment for exudative AMD, such as photodynamic therapy or intravitreal injections in the study eye. Exclusion criteria included any previous ophthalmic surgery, except for cataract removal, diabetic retinopathy, and progressive glaucoma.

Diagnostics and Treatment

All patients were treated for at least 12 months with ranibizumab on a pro re nata regimen. Patients initially received 3 consecutive, monthly intravitreal injections of 0.5 mg ranibizumab. After this first series of treatments, patients were monitored in monthly visits. Evaluations included spectral-domain optical coherence tomography, best-corrected VA, and fundus examination. Visual acuity was measured with Snellen charts in 288 patients and with ETDRS charts in 78 patients. A logarithm of the minimum angle of resolution (logMAR) score was recorded alongside each ETDRS measurement. Fluorescein angiography and indocyanine green were used only in unclear cases. Recurrence or persistence of CNV activity was defined as fluid seen by optical coherence tomography or leakage seen on FA, loss of ≥5 letters in ETDRS VA, or new macular intraretinal or subretinal hemorrhage. Recurrences were treated again with a series of 3 consecutive, monthly ranibizumab injections. For lesion type, all FA performed at baseline was graded by 2 independent graders.

Genotyping

The Tagger algorithm18 was used to select tag SNPs from HapMap to capture all SNPs of minor allele frequency 0.05 with an r2 of 0.8 in the VEGFA, VEGFB, VEGFC, VEGFD (FIGF), PGF, VEGFR1 (FLT1), VEGFR2 (KDR), VEGFR3 (FLT4), and PEDF (SERPINF1) genes. Tag SNPs were genotyped with 4 multiplex iPLEX Gold SNP Genotyping assays (Sequenom Inc., San Diego, CA).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (version 21; IBM Corp., New York, NY). Visual acuity assessed with Snellen charts was converted to logMAR for statistical analyses. Levene’s test for equality of variances was used to test variability of VA changes between Snellen and ETDRS measurements. Improvement in VA was calculated for each patient as the increase of VA between baseline and after 3 months (1 month after the third injection) and after 12 months’ follow-up. To identify potential confounders, we computed univariate analyses of variance (ANOVA}s) for the dependent variable “change in VA after 12 months” with the factors age (P > 0.89), gender (P > 0.97), baseline VA (P > 0.27), smoking status (P > 0.86), lesion type (P = 0.096 after Bonferroni correction), and number of injections within 12 months (P > 0.48). Only the factor of lesion type showed a trend toward statistical significance and was kept as a potential confounder. For each of the 126 tag SNPs, we computed ANOVAs with the factor “minor allele” (present, absent) and the dependent variable “change in VA after 3 months/12 months.” Lesion type (occult, predominantly classic, minimally classic, retinal angiomatosus proliferation) was used as a covariate to control for lesion type—related effects on the outcome variable. The threshold for statistical significance was set to P < 0.05. The resulting P values were corrected for multiple comparisons using Bonferroni’s approach.

As a second step to identify SNPs with an influence on visual outcome, we performed a multivariate stepwise linear regression analysis. Accordingly, we defined change in VA after 12 months as the dependent variable and the 126 analyzed SNPs (coding for the presence [1] or absence [0] of minor alleles of the respective SNP) as the independent predictor variables.

Results

The characteristics of the 366 patients included in the study are summarized in Table 1. All patients were treated for neovascular AMD with ranibizumab on a pro re nata regimen. After 3 initial injections, retreatment followed an optical coherence tomography—guided pro re nata regimen. Visual acuity was measured at baseline, at 3 months (1 month after 3 injections), and at 12 months.
**Table 1. Clinical Characteristics of the Study Population (n = 366)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>164 men (44.8%), 202 women (55.2%)</td>
</tr>
<tr>
<td><strong>Eyes (n = 366)</strong></td>
<td>51% right, 49% left</td>
</tr>
<tr>
<td><strong>Age, yrs</strong></td>
<td>76.8±7.5 (54–97)</td>
</tr>
<tr>
<td><strong>Type of CNV</strong></td>
<td>60% occult, 12% predominantly classic, 21% minimally classic, 7% retinal angiomatous proliferation</td>
</tr>
<tr>
<td><strong>No. of intravitreal injections Mean ± SD (range)</strong></td>
<td>6.0±3.3 (3–12)</td>
</tr>
<tr>
<td><strong>VA (logMAR) at baseline Mean ± SD</strong></td>
<td>0.64±0.36</td>
</tr>
<tr>
<td><strong>VA after 3 intravitreal injections (logMAR) Mean ± SD</strong></td>
<td>0.55±0.41</td>
</tr>
<tr>
<td><strong>VA after 1 yr (logMAR) Mean ± SD</strong></td>
<td>0.59±0.42</td>
</tr>
</tbody>
</table>

**Discussion**

In this cohort study, we evaluated the association of SNPs in VEGF and VEGFR genes with the response to ranibizumab treatment in patients with neovascular AMD. We identified 2 SNPs (rs4576072 and rs6828477) in the VEGFR2 gene that were independently associated with a significantly better visual outcome after 1 year. The difference in VA between patients with minor alleles and those with no minor allele was already apparent after 3 injections but increased to approximately 3 lines after 1 year. These patients gained initially more letters and did not lose vision in the course of treatment within the first year. Visual acuity increased gradually with the number of minor alleles of both SNPs. The differences in VA, which were in the range of 1 to 3 lines, were clinically relevant because a gain of 1 line on the ETDRS chart is perceived by most patients as a subjective improvement.

The 2 SNPs had a significant influence on the variability in treatment response to ranibizumab. Therefore, they represent predictive factors for the therapeutic response to anti-VEGF treatment beyond other individual factors, such as CNV characteristics, diagnostics, adherence to treatment, and other contributing minor alleles (n = 18), 0.08 (±0.35) in patients with 2 minor alleles (n = 91), and 0.02 (±0.32) in patients with 1 minor allele (n = 178) (Fig 1). Patients with no minor allele contributing had an average decrease of VA of 0.03 (±0.36).

A more detailed analysis (Table 4) revealed that the presence of at least 1 minor allele of rs4576072 or rs6828477 was associated with an improvement of VA that further increased with the presence of a second minor allele of rs6828477 but not of rs4576072. This was also confirmed when formally testing for differences in VA improvement after 12 months between different combinations of genes. Mann–Whitney U tests revealed significant differences (corrected for multiple comparisons). Differences in VA improvements after 3 months did not pass the statistical threshold after correction.

Other individual factors, such as age, sex, smoking, baseline VA, and number of injections, did not show significant effects on VA improvements after 3 or 12 months (P > 0.1 for each comparison).

**Table 3. Improvement of Visual Acuity on the Logarithm of the Minimum Angle of Resolution Scale Depending on the Genotype of Vascular Endothelial Growth Factor Receptor 2 Single Nucleotide Polymorphisms rs4576072 and rs6828477**

<table>
<thead>
<tr>
<th>SNP Genotype</th>
<th>No. (%)</th>
<th>Age, yrs (Mean ± SD)</th>
<th>Baseline VA (Mean logMAR ± SD)</th>
<th>VA Improvement after 3 Mos (Mean logMAR ± SD)</th>
<th>VA Improvement after 12 Mos (Mean logMAR ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4576072</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>259 (71)</td>
<td>77.1±7.5</td>
<td>0.63±0.37</td>
<td>0.07±0.27 (P = 0.007)</td>
<td>0.00±0.33 (P = 0.007)</td>
</tr>
<tr>
<td>CT</td>
<td>96 (26)</td>
<td>76.4±7.9</td>
<td>0.66±0.33</td>
<td>0.12±0.31 (P = 0.019)</td>
<td>0.13±0.35 (P = 0.004)</td>
</tr>
<tr>
<td>CC</td>
<td>11 (3)</td>
<td>74.3±5.6</td>
<td>0.65±0.33</td>
<td>0.18±0.27 (P = 0.058)</td>
<td>0.14±0.28 (P = 0.182)</td>
</tr>
<tr>
<td>CT or CC</td>
<td>107 (29)</td>
<td>76.2±7.7</td>
<td>0.66±0.33</td>
<td>0.13±0.30 (P = 0.007)</td>
<td>0.13±0.34 (P = 0.002)</td>
</tr>
<tr>
<td>rs6828477</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>122 (33)</td>
<td>77.3±8.6</td>
<td>0.58±0.33</td>
<td>0.09±0.27 (P = 0.002)</td>
<td>0.00±0.35 (P = 0.002)</td>
</tr>
<tr>
<td>CT</td>
<td>193 (53)</td>
<td>76.4±7.5</td>
<td>0.65±0.33</td>
<td>0.07±0.29 (P = 0.779)</td>
<td>0.06±0.33 (P = 0.147)</td>
</tr>
<tr>
<td>CC</td>
<td>51 (14)</td>
<td>77.0±8.6</td>
<td>0.75±0.39</td>
<td>0.10±0.30 (P = 0.991)</td>
<td>0.06±0.36 (P = 0.035)</td>
</tr>
<tr>
<td>CT or CC</td>
<td>244 (67)</td>
<td>76.5±7.8</td>
<td>0.67±0.36</td>
<td>0.08±0.29 (P = 0.815)</td>
<td>0.06±0.35 (P = 0.137)</td>
</tr>
</tbody>
</table>

logMAR = logarithm of the minimum angle of resolution; SD = standard deviation; SNP = single nucleotide polymorphism.

P values given (Mann–Whitney U) when compared with TT genotype.
underdosing, or delay of treatment, which all interfere with the therapeutic outcome.²⁰,²¹ Therefore, this is the first study of AMD to show a correlation between polymorphisms in the VEGFR2 gene and visual outcome in ranibizumab therapy.

Considered separately, the presence of at least 1 C-allele of SNP rs4576072 led to an improvement of 0.13 logMAR compared with wild-type. This trend toward improved visual outcome was already observed after 3 injections and not influenced by age or VA at baseline. A similar but weaker effect was seen for SNP rs6828477. In line with previous findings, the CNV type showed no association with response to ranibizumab treatment.

Table 4. Improvement of Visual Acuity after 3 and 12 Months on the Logarithm of the Minimum Angle of Resolution Scale for All Allele Combinations of the Vascular Endothelial Growth Factor Receptor 2 Single Nucleotide Polymorphisms rs4576072 (Minor Allele T) and rs6828477 (Minor Allele T)

<table>
<thead>
<tr>
<th>SNP</th>
<th>TT</th>
<th>CT</th>
<th>CC</th>
<th>VA improvement after 3 mos (mean logMAR ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4576072</td>
<td>0.07±0.26</td>
<td>0.11±0.28</td>
<td>0.26±0.24</td>
<td></td>
</tr>
<tr>
<td>rs6828477</td>
<td>0.06±0.27</td>
<td>0.11±0.33</td>
<td>0.13±0.31</td>
<td></td>
</tr>
</tbody>
</table>

Other pharmacogenetic studies of anti-VEGF therapy in AMD have demonstrated that risk alleles in CFH and ARMS2 are associated with vision loss or a higher number of injections,³,⁷,¹⁰,¹¹,¹³,¹⁶ although other studies could not confirm these findings.⁹,¹²,¹⁵ Although the VEGFA gene confers only a minor genetic risk for the development of AMD,²² genetic variants in VEGFA have been demonstrated to significantly influence the outcome of ranibizumab treatment in AMD.²,⁴,⁶ The latter association was not confirmed in this study because none of the tag-SNPs across the VEGFA gene were significantly associated with change in VA after treatment.

The effect of ranibizumab treatment is mediated primarily through VEGFA and its main receptor VEGFR2. Ranibizumab binds and inactivates VEGFA, which constitutes a key component of neovascularization. In this study, we identified 2 polymorphisms located in different haplotype blocks of the VEGFR2 gene that are independently associated with the outcome of ranibizumab treatment. Several studies have evaluated SNPs in VEGFR2 but so far failed to find significant associations with treatment response. This may be due to the limited sample size in those studies or the limited number of SNPs that have been analyzed.⁴,⁷,¹¹,¹⁴ In contrast, the present study tested for a broad range of SNPs in a relatively large cohort of subjects (n = 366 eyes), which facilitated the identification of significant associations between genetic factors and treatment response. We did not detect a significant association with genetic variants in other VEGF or VEGFR genes. Although this finding is in agreement with other studies,⁷,¹⁴ there is evidence that variants in the VEGFA gene affect treatment outcome.²,⁸

This is the first study to demonstrate a highly significant effect of polymorphisms in VEGFR2 on the therapeutic outcome of ranibizumab treatment by systematically analyzing tag SNPs across the VEGF and VEGFR genes. Further studies are now needed that clarify the pathophysiology underlying these gene-treatment associations with respect to the 2 candidate SNPs and to other genetic variants that are in high linkage disequilibrium with these SNPs. Hints for a putative pathophysiologic mechanism stem from data obtained in patients with metastatic pancreatic adenocarcinoma, in whom response to anti-VEGF therapy was related to VEGFR gene polymorphisms. Here, a synonymous SNP in VEGFR1 caused increased VEGFR1 expression and increased downstream signaling by a shift in codon use.²³ It has been suggested that increased VEGFR1 concentrations can sequester VEGF, thereby decreasing its proangiogenic effects transduced via VEGFR2 and subsequently limiting the benefits of additional VEGF neutralization by bevacizumab. In line with these findings, we hypothesize that the VEGFR2 SNPs identified in this study may lead to altered expression or functional activity of VEGFR2, leading to an increased benefit of VEGF neutralization by ranibizumab on visual outcome. Whether such effects are responsible for treatment effects in AMD needs to be elucidated in future studies.

The findings need to be replicated in other cohorts and in a larger sample of subjects to confirm a putative diagnostic
predictor for treatment response. In this study, VA at baseline and the initial gain of VA after 3 injections were comparable to other trials. However, we observed some loss of VA after this initial gain after 12 months.

In conclusion, this study is the first to systematically analyze tag SNPs across the VEGF and VEGFR genes. This approach identified 2 SNPs in VEGFR2 that are independently associated with improved treatment response to ranibizumab in neovascular AMD. In the future, such data may help to identify high-risk patients and to individualize therapy.

References


Footnotes and Financial Disclosures

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