Variants in the *APOE* Gene Are Associated with Improved Outcome after Anti-VEGF Treatment for Neovascular AMD

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Purpose. Anti-vascular endothelial growth factor (anti-VEGF) drugs have dramatically improved the treatment of neovascular AMD. In pivotal studies, almost 90% of patients maintain vision, with approximately 30% showing significant improvement. Despite these successes, 10% to 15% of patients continue to lose vision, even with treatment. It has been reported that variants in some AMD-associated genes influence treatment outcome. This study showed an association of treatment outcome with variants in the apolipoprotein E (*APOE*) gene.

Methods. One hundred ninety-two patients receiving anti-VEGF treatment for subfoveal choroidal neovascularization secondary to AMD were enrolled. Information on demographics, lesion characteristics, delay until treatment, visual acuity (VA), and number of treatments was collected, and variants of APOE were assessed in all patients at baseline. Best corrected logarithm of the minimum angle of resolution (logMAR) VA was recorded in all patients.

Results. The presence of the *APOE* ε4 allele was associated with improved treatment outcome at 3 ($P = 0.02$) and 12 ($P = 0.06$) months, compared with the presence of the ε2 allele, after adjustment for baseline acuity, treatment delay after first symptoms, age, and sex. Patients with an APOE ε4 allele had an odds ratio (OR) of 4.04 (95% confidence interval [CI], 1.11–14.70) for a 2-line gain in vision from baseline at 3 months ($P = 0.03$) and an OR of 2.54 (95% CI, 0.61–10.52; $P = 0.20$) at 12 months after treatment, based on multivariate analysis.

Conclusions. In patients with neovascular AMD, the presence of the *APOE* ε4 allele conferred significantly better visual outcomes after anti-VEGF treatment than did the ε2 allele. These findings suggest a possible role for a personalized approach to treatment with anti-VEGF. (*Invest Ophthalmol Vis Sci.* 2011;52:4072–4079) DOI:10.1167/iovs.10-6550

Age-related macular degeneration (AMD) is the most common cause of severe, irreversible loss of vision among elderly populations in the developed world.[1–4] The neovascular, or exudative, form of the disease destroys central vision rapidly and is responsible for most severe vision loss in AMD. Ranibizumab is a recombinant, humanized, monoclonal antibody that neutralizes all isoforms of vascular endothelial growth factor (VEGF), a key mediator of the neovascular process.[5–9] The MARINA[10] and ANCHOR[11] studies demonstrated that ranibizumab was an effective treatment for neovascular disease. Vision remained stable in approximately 90% and improved in approximately one third of treated patients. Published case series of bevacizumab treatment showed similar outcomes.[12,13] Unfortunately, approximately 10% of treated patients[10,11] continued to lose vision. Trials assessing the efficacy of variable dosage regimens have generally shown a less favorable vision improvement, compared to a monthly regimen. The PIER trial[14] used a quarterly regimen of ranibizumab after three loading-dose injections. Although the treated group had better vision at 12 months, compared with the sham-treated patients, the initial gains in visual acuity observed over the loading-dose period were lost, and the vision at 12 months was similar to baseline. It was noted that not all patients treated with this regimen fared equally/ Some patients maintained vision gains that were achieved during the monthly-dose period and others either did not respond at all or initially responded well and then failed to maintain the initial gains. The SUSTAIN trial (Meyer CH, et al. *IOVS* 2008;49:ARVO E-Abstract 273) provided further evidence of this differential response to treatment. In this trial, ranibizumab treatment was examined on a pro re nata basis, after administration of three initial loading-dose injections, one in each of three consecutive months. Retreatment was based on specific visual acuity and clinical and optical coherence tomography criteria. Of these patients, approximately 50% maintained their initial sharp gains in visual acuity, the so-called gain-and-maintain group. Another third of patients enjoyed an initial gain in visual acuity, but failed to maintain the gain during follow-up: the gain-and-not-maintained group. Most of the acuity increase, in these two groups, occurred in the first 3 months of treatment. Approximately 25% of patients never experienced any gain in vision: the no-initial-gain group. In these patients, vision failed to improve from the outset, with continued losses being noted during the course of the treatment regimen. Overall, approximately 20% of the patients maintained their visual acuity without any further treatment after the initial loading doses.

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Currently, we do not fully understand what factors dictate this response. If we did, we might be able to predict patient outcomes after anti-VEGF treatment more accurately. Recent studies, including the MARINA and ANCHOR subgroup analyses, suggest that baseline visual acuity, lesion size, and age influence outcomes of anti-VEGF therapy.[15–17] The potential role of genetic variation in determining outcome has also been investigated. Although multiple studies have implicated roles for several genes, including *CFH*, *HTRA1/ARMS2*, *C3*, *CFB/C2*, and *APOE*, in the risk of developing AMD, there is no consensus in the literature regarding the influence of these genetic polymorphisms in the response to anti-VEGF treatment.[18–23] The *CFH* gene has been the most widely investigated, with conflicting reports that the CFH at risk allele CC is associated with favorable or adverse treatment responses.[18–23] No specific associations with polymorphisms of the *C3* or *ARMS2/HTRA1* genes in determining treatment outcome have been noted, although it has been suggested that polymorphisms within the C-reactive protein (*CRP*) gene are associated with worse visual outcome after anti-VEGF treatment.[20] However, these studies were limited by small patient samples that left them underpowered, and they did not account for all other potentially confounding factors that can affect visual outcome.

Nongenetic variables that have been shown to influence visual outcome and should be included in any attempt to identify genetic influences on outcomes include age, presenting visual acuity, and the size and type of lesion.[10,11,24] Traditionally, the time delay between the first symptoms of neovascular AMD and initiation of treatment was an important determinant of outcome, when the treatment was thermal laser photocoagulation, which could be applied successfully only if the choroidal neovascularization (CNV) was treated before it became subfoveal. Given that these new medical treatments are used in subfoveal lesions, it is not necessarily a given that delay to treatment would influence outcomes. It has been reported that this variable remains an important parameter in response to anti-VEGF therapy,[25,26] although it is difficult to assess accurately and thus is rarely ascertained. We have found in our present cohort of patients that delay of treatment after the first symptoms suggestive of CNV is a highly significant predictor of outcome (Lim et al., manuscript submitted). Lack of consideration of this variable may well explain the contradictory results of previously published studies in which treatment outcomes with anti-VEGF were investigated. Thus, we sought to address the possible genetic variants and their influence on treatment outcome by conducting a study that took into consideration variables that we found to have a significant effect on outcomes.

In this study we chose to look at the potential influence of polymorphisms of the *APOE* gene in determining outcome. We and others[27–31] have demonstrated a higher likelihood of development of AMD in individuals with the *APOE* ε2 polymorphism than with the ε3 and ε4 variants. As such, we were interested in determining whether there is any genetic influence on treatment outcome, within the variants of the *APOE* gene. We examined visual acuity outcome at 3, 6, and 12 months after initiation of treatment. Several pivotal RCTs,[10,11,14] have clearly demonstrated that in most cases, visual acuity increases occurred in the first 3 months of treatment. Also in those patients who responded poorly, vision failed to improve from the outset, with continued losses noted early during treatment. Investigating VA outcomes at 3 months therefore accounted for a very uniform phase in treatment strategy. At 6 and 12 months, other variables such as injection timing and number as well as missed appointments may play a role. Thus, we felt it useful to investigate an early time point as well as later ones during the course of treatment to see the extent of possible genetic influence on outcome.

Methods

Study Design and Eligibility

This study was approved by the Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital (RVEEH), as part of the Age-Related Macular Degeneration Inheritance Study. Research adhered to the tenets of the Declaration of Helsinki, and the participants provided written informed consent before participation.

Our study population consisted of patients from the Medical Retina Clinic at the RVEEH and the private rooms of the Eastern Retinal Service (Box Hill, Melbourne).

Exclusion criteria were (1) CNV secondary to other, non-AMD conditions such as angioid streaks, degenerative myopia, central serous retinopathy or hereditary retinal disorders; (2) laser photocoagulation or photodynamic therapy before anti-VEGF administration; and (3) non-Caucasian ancestry.

Data Collection and Follow-up

Data pertaining to patient demographics, medical history, visual acuity (VA), and treatment scheduling were collected after consent was obtained. A peripheral blood sample was also collected for DNA extraction.

All patients underwent a dilated fundus examination. Snellen VA was recorded by clinic orthoptists who were masked to the study and used a standard chart at a standard distance of 6 m to get best corrected VA. At each appointment, VA was reassessed in the same, standardized manner, albeit by different orthoptists in many cases. These values were converted to logarithm of the minimum angle of resolution (logMAR) values for all study calculations and comparisons. Fluorescein angiography was performed by the Medical Photographic Imaging Centre at the RVEEH or by an orthoptist at the private Eastern Retinal Service as part of normal patient management. Angiograms were accessed via a digital imaging system (IMAGEnet 2000; Topcon Corp., Tokyo, Japan). A retrospective review of all angiograms was undertaken, and lesion size was measured in comparison to optic disc area using the middle phase frame of the angiogram.

Treatment Decisions

All treatment decisions were based solely on the discretion of the treating retinal specialist. No specific retreatment strategies were implemented, although 75% of the participants initially received one injection during each of three consecutive months. The treatment used by the participating physicians most closely resembled an as-needed strategy, although if extension between treatments was considered, the clinical decision in general was to treat without extending treatment intervals until the retina was free of fluid. Both ranibizumab and bevacizumab were used during the treatment period, and in some cases, both drugs were used in the same eye at different time points.

Genotyping

The single-nucleotide polymorphisms (SNPs) rs429358 and rs7412 of the *APOE* gene were genotyped as previously reported.[29] In brief, the polymerase chain reaction (PCR) amplification was undertaken with the primers AF (5′GCCTCCCCACTGTGCGA3′), AR (5′GGCCGAGCATGGCCTG3′) for rs429358 and BF (5′ACCGAGGAGCTGCGGG3′), BR (5′CTCGCGGATGGCGCTGA3′) for rs7412. Digestion of each PCR product was undertaken with the restriction enzymes *Afl*III for rs429358 and *Hae*II for rs7412. Digested products were size separated on agarose gels. The combination of amino acids at these two SNPs allowed the common allelic variants of ε2, ε3, or ε4 of the *APOE* gene to be determined. Dideoxynucleotide sequencing was undertaken on a representative sample to confirm the results of each restriction digestion profile.

Statistical Analysis

The primary measure of this study was VA outcome after anti-VEGF treatment for neovascular AMD. Improvement in VA was defined as a 2-line gain in VA, as measured on a Snellen chart at 3 and 6 months after the first treatment. Stable vision was defined as VA within 2 lines of baseline after the same time period. A decrease was considered to be a reduction in VA of 2 lines or more.

In addition to patient genotype, other possible predictors of eventual outcome were investigated and defined based on the following characteristics: (1) age (years); (2) sex; (3) smoking status (nonsmoker, past or current smoker); (4) delay between first symptoms of CNV and first treatment; (5) delay between confirmed CNV diagnosis and first treatment; (6) lesion type (predominantly classic, nonpredominantly classic); (7) lesion size (≤2 disc areas [DA], >2 DA); (8) number of treatments (injections) given in the 6-month follow-up period; injection number (≤3, >3]); and (9) baseline VA.

All these treatment subgroups were selected before the study commenced.

When both eyes of a patient were recruited into the study, the eye with worse vision at the end of the 12-month follow-up period was selected for analysis. Data are reported as the median and interquartile range (IQR; p25, p75 in the tables) or the mean ± standard deviation (SD) for continuous variables, and as proportions for categorical variables. For the categorical variables, χ^2 analysis was used to determine whether there were any significant differences in the treatment outcome between each of the subgroups. For the continuous variables, ordinal logistic regression analysis was used.

To determine the extent of the effect of the specific SNPs of the *APOE* gene and their relationship with treatment outcomes, we used univariate logistic regression to generate odds ratios (ORs) for effect of genotype. To assess the effect of potentially confounding variables other than patient genotype, univariate logistic regression analysis was also used. All factors with a univariate significance level of $P < 0.10$, as well as for age and sex, were included in the multivariate ordinal regression model to see whether any significant effects of genotype in the univariate analysis remained (Stata Statistical Software Release 10.0; Stata Corp., College Station, TX).

Sample Size

Assuming an effect of the *APOE* polymorphisms on vision outcome with treatment, we calculated the sample size to allow for a 25% increased chance for vision improvement with ε4 variant compared with the ε2 variant. To detect a significant difference ($P = 0.05$) between polymorphisms of the *APOE* gene, we calculated that we would require a minimum sample size of 152 eyes for the desired statistical power of 80%.

Results

Population Characteristics

Of the 198 eyes of 192 patients that were enrolled, 30 eyes were excluded from the analysis for the following reasons: The *APOE* status of 13 patients (13 eyes) could not be determined (failed genotyping); 4 patients, with both a risk and protective allele, had a potentially confounding ε2/ε4 genotype; and 6 eyes of 6 patients received bilateral anti-VEGF treatment for CNV, and of those, only the eye with the worse outcome at 12 months was considered for analysis. Finally, seven patients contributing seven eyes failed to complete follow-up. These exclusions resulted in a final study population of 168 patients (168 eyes), of whom the majority (98; 58.3%) were women. Most lesions (122; 72.6%) were classified as nonpredominantly classic (occult and minimally classic), and most lesions (103; 66.0%) were less than or equal to 2 DA in size (103; 66.0%). The median time delay from detection of the first symptoms suggestive of CNV until treatment was 12 weeks, and at the baseline visit, the mean logMAR VA was 0.60.

Of the 168 eyes, 101 had only ranibizumab treatment, and 67 had a combination of ranibizumab and bevacizumab. No differences were observed in visual outcome between the two groups with respect to frequency of the *APOE* alleles ($P = 0.92$) and treatment outcome ($P = 0.27$). Consequently, the patients were combined and analyzed as a single cohort. Table 1 summarizes the patient and eye characteristics that we analyzed, by treatment outcome at 3, 6, and 12 months. The responses were classified as improved, stable, or decreased VA. At 3 months, in 28.3% VA had improved by 2 or more lines, in 64.5% it was stable, and in 7.2% it had worsened by 2 or more lines. Similarly, at 6 and 12 months VA in 29.2% and 24.2%, respectively, had improved, whereas in 57.7% and 57.1% it was stable; in 13.1% and 18.7% at 6 and 12 months, respectively, it had deteriorated. Of the study participants, the median age was 80 years (range, 62–94 years). There appeared to be a difference in the outcome of younger patients compared with older patients at 3, 6, and 12 months, with the median age of those improving vision at all three time periods being 78, 77, and 79 years, respectively, compared with 81 years at all three time points in those with stable vision or loss of vision. There was no significant difference in distribution across the treatment outcome categories with respect to sex or smoking status, although there was a trend for women and nonsmokers to fare better than men and previous or present smokers.

The putative AMD-risk ε2 allele for the *APOE* gene was identified in 17.4% of all patients, whereas the protective e4 allele was present in 16.1% of patients. At 3 months, the presence of at least one ε2 allele (ε2 /ε2 or ε2/ε3) was associated with a 2-line improvement in VA in only 16.7% of carriers, whereas 45.5% of ε4 (ε4 /ε4 or ε4 3/ε4) carriers showed this improvement. In patients with only the ε3 allele (ε3/ε3), 27.5% improved vision by two or more lines, $P = 0.06$. Looking specifically at the presence of the ε2 allele compared with the presence of the ε4 allele, we found a significant difference in outcome. The presence of the ε4 allele was associated with a greater likelihood of vision improvement ($P = 0.02$) at 3 months. A similar, but not significant, trend was noted at 6 and 12 months, with a greater percentage of patients with the ε4 allele compared with the ε2 allele showing vision improvement (33.3% vs. 12.3%, $P = 0.07$ and 37.5% vs. 15.4%, $P = 0.06$) at 6 and 12 months, respectively.

Contrary to the *APOE* ε4 findings, we did not detect any significant difference at 3, 6, or 12 months with regard to whether the *APOE* ε2 allele conferred a poorer outcome through loss of 2 lines or greater in vision. At 3 months 2 (6.7%) of 30 eyes with ε2 deteriorated by 2 lines or more compared with 3 (11.1%) of 27 eyes with ε4 ($P = 0.50$). Similarly at 6 months, 3 (10.0%) of 30 eyes with ε2 deteriorated compared with 4 of (14.8%) 27 eyes with ε4 ($P = 0.58$). Of note, at 12 months, although not significant, 6 (23.1%) of 26 eyes with ε2 deteriorated compared with 2 (8.3%) of 24 of those with ε4 ($P = 0.155$).

Lesion type, the number of injections, and lesion size failed to show any relationship to vision outcome, although there was borderline significance for lesion size at 3 months ($P = 0.07$), with lesions 2 DA or smaller more likely to be associated with improved vision. Also, at 12 months, predominantly classic lesions tended to have better visual outcome (35% vs. 20%, $P = 0.07$). Baseline VA was a highly significant determinant of response, with better presenting VA more likely to be associated with a deterioration in vision compared with worse vision at baseline. We hypothesize, as have others, that this association is related to a ceiling effect

Table 1. Patient and Study Eye Characteristics by Treatment Outcome at 3, 6, and 12 Months

Characteristics	3-mo Outcome (n = 166)			6-mo outcome (n = 168)			12-mo outcome (n = 149)		
	Stable/Worse VA	Improved VA	*P*	Stable/Worse VA	Improved VA	*P*	Stable/Worse VA	Improved VA	*P*
Age, median (p25, p75)	81 (76, 85)	78 (73, 82)	0.16	81 (77, 85)	77 (72, 82)	0.03	81 (77, 85)	79 (73, 81)	0.03
Sex									
Male	45 (64)	25 (36)	0.07	44 (37)	26 (53)	0.06	41 (36)	18 (50)	0.14
Female	74 (77)	22 (23)		75 (63)	23 (47)		72 (64)	18 (50)	
Smoking									
Nonsmoker	49 (79)	13 (21)	0.09	47 (43)	16 (35)	0.36	41 (38)	16 (47)	0.37
Past/present smoker	61 (66)	31 (34)		63 (57)	30 (65)		66 (62)	18 (53)	
APOE genotypes									
ε2/ε2; ε2/ε3	25 (83)	5 (17)	0.06	26 (22)	4 (8)	0.11	22 (19)	4 (11)	0.25
ε3/ε3	79 (72)	30 (28)		75 (63)	36 (74)		76 (67)	23 (64)	
ε3/ε4; ε4/ε4	15 (55)	12 (45)		18 (15)	9 (18)		15 (13)	9 (25)	
APOE alleles (ε2 vs. ε4)									
ε2	25 (83)	5 (17)	0.02	26 (87)	4 (13)	0.07	22 (85)	4 (15)	0.06
ε4	15 (55)	12 (45)		18 (67)	9 (33)		15 (62)	9 (38)	
Baseline logMAR VA median (p25, p75)*	0.60 (0.40, 0.90)	0.70 (0.48, 1.00)		0.60 (0.40, 0.78)	0.78 (0.48, 1.00)	0.02	0.60 (0.48, 0.78)	0.74 (0.50, 1.00)	0.01
Time delay: symptoms to treatment, wk									
Lowest tertile (<7)	34 (64)	19 (36)	0.22	34 (62)	20 (38)	0.32	34 (76)	11 (24)	0.72
Middle tertile (7–21)	36 (80)	9 (20)		32 (71)	13 (29)		35 (80)	9 (20)	
Highest tertile (>21)	32 (70)	14 (30)		36 (77)	11 (23)		31 (72)	12 (18)	
Time delay: diagnosis of CNV to treatment, wk									
Lowest tertile (<1)	51 (69)	23 (31)	0.21	55 (73)	20 (27)	0.33	50 (78)	14 (22)	0.02
Middle tertile (1–3)	31 (67)	15 (33)		29 (63)	17 (37)		27 (61)	17 (39)	
Highest tertile (>3)	37 (82)	8 (18)		35 (76)	11 (24)		36 (70)	5 (30)	
CNV lesion type									
Predominantly classic	29 (66)	15 (34)	0.30	32 (73)	12 (27)	0.78	26 (65)	14 (35)	0.07
Non-predominantly classic†	89 (74)	31 (16)		86 (70)	36 (30)		86 (80)	22 (20)	
Total CNV lesion size									
≤2 DA	68 (67)	33 (33)	0.07	71 (69)	32 (31)	0.27	67 (74)	24 (26)	0.47
>2 DA	43 (81)	10 (19)		41 (77)	12 (23)		38 (79)	10 (21)	
Number of treatments									
≤3	49	119	—	58 (64)	23 (36)	0.94	33 (85)	6 (15)	0.22
>3	—	—	—	59 (71)	24 (29)		78 (75)	26 (25)	

n (%) for categorical variables; median (p25, p75) for continuous variables. Percentages may not total 100 due to rounding. Apo ε2 is constituted by the ε2/ε2 and ε2/ε3 genotypes; Apo ε4 is constituted by the ε3/ε4 and ε4/ε4 genotypes.

* VA worsens as logMAR value increases.

† Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular pigment epithelial detachment.

TABLE 2. Univariate Logistic Regression Analysis of Treatment Outcomes

Characteristics	3-mo Outcome (n = 166)		6-mo Outcome (n = 168)		12-mo Outcome (n = 149)	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, y	0.95 (0.91, 1.00)	0.04	0.94 (0.89, 0.99)	0.02	0.94 (0.89, 0.99)	0.02
Sex						
Male	1	0.07	1	0.06	1	0.15
Female	0.54 (0.27, 1.06)		0.52 (0.26, 1.02)		0.57 (0.27, 1.22)	
Smoking status						
Nonsmoker	1	0.09	1	0.36	1	0.37
Past/present smoker	1.91 (0.90, 4.06)		1.39 (0.68, 2.86)		0.69 (0.32, 1.53)	
Time delay: symptoms to treatment, wk						
Lowest tertile	1		1		1	
Middle tertile	0.44 (0.18, 1.12)	0.09	0.69 (0.29, 1.62)	0.39	0.79 (0.29, 2.16)	0.65
Highest tertile	0.78 (0.34, 1.82)	0.57	0.52 (0.22, 1.25)	0.14	1.19 (0.46, 3.11)	0.71
Baseline logMAR VA*	2.67 (1.31, 5.41)	0.007	3.24 (1.56, 6.74)	0.002	4.45 (1.81, 10.98)	0.001
CNV lesion type						
Predominantly classic	1	0.30	1	0.78	1	0.07
Nonpredominantly classic†	0.67 (0.32, 1.42)		1.12 (0.52, 2.41)		0.47 (0.21, 1.06)	
Total CNV lesion size						
≤2 DA	1	0.07	1		1	0.47
>2 DA	0.48 (0.21, 1.07)		0.64 (0.30, 1.40)	0.27	0.74 (0.32, 1.70)	
Number of treatments						
≥3	—		1	0.94	1	0.23
>3	—		1.03 (0.52, 2.02)		1.83 (0.69, 4.88)	
APOE alleles						
ε2	1		1		1	
ε3	1.89 (0.66, 5.43)	0.23	3.12 (1.01, 9.64)	0.04	1.66 (0.52, 5.34)	0.39
ε4	4.00 (1.17, 13.65)	0.03	3.25 (0.86, 12.24)	0.08	3.30 (0.85, 12.77)	0.08

APOE alleles are as described in Table 1.

* VA worsens as logMAR value increases.

† Includes minimally classic, occult, retinal angiomatous proliferation, fibrovascular pigment epithelial detachment, and fibrosis, hemorrhage.

that prevents people with good presenting vision from showing improvement.

Previously, we have reported a significant association between the outcome of treatment and the length of delay between initial symptoms of CNV and treatment (Lim et al., manuscript submitted), after considering the entire range of responses to treatment (improved, stable, or worse vision). For the current analysis, we sought to define characteristics that would predict a better vision outcome, and, as such, we grouped the eyes with stable and worse vision together. In this analysis, no significance was found for time until treatment in predicting response. This result suggests that longer delays are associated with worse response, rather than shorter delays being associated with a better response.

Univariate Analysis

Table 2 shows the univariate ordinal logistic regression models for the *APOE* gene, as well as other potential contributory factors such as age, sex, size of CNV membrane, and delay from initial symptoms to initiation of treatment.

A statistically significant relationship ($P = 0.04$) was identified between the ε4 polymorphism and better posttreatment VA outcomes. The presence of the ε4 allele conferred an increased chance of better than 2 lines of vision improvement with treatment (OR, 4.00; 95% confidence interval [CI], 1.17–13.65, at 3 months, $P = 0.03$). At 6 and 12 months, a similar trend was present, although not statistically significant (OR, 3.25; 95% CI, 0.86–12.24; $P = 0.08$; and OR, 3.30; 95% CI, 0.85–12.77; $P = 0.08$, respectively). Of all other variables analyzed, baseline VA also correlated significantly with vision at 3, 6, and 12 months, with eyes that had worse initial VA more likely to have a 2-line vision improvement (OR, 2.67; 95% CI, 1.31–5.41; $P = 0.007$; OR, 3.24; 95% CI, 1.56–6.74; $P = 0.002$; and OR, 4.45; 95% CI, 1.81–10.98; $P = 0.001$, respectively).

Multivariate Analysis

Table 3 shows the multivariate regression model for the *APOE* gene. All patient or clinical characteristics with a significance level of $P < 0.10$ in the univariate analysis were incorporated into a final analysis. Younger patients tended to have an increased likelihood of vision improvement compared to older patients at 6 and 12 months (OR, 0.94; 95% CI, 0.89–0.99; $P = 0.03$ and OR, 0.94; 95% CI, 0.88–0.99; $P = 0.04$, respectively). No significant difference was seen at 3 months. Better initial acuity remained a significant predictor of a poorer response to treatment when adjusting for age, baseline VA, and genotype.

The presence of the *APOE* ε4 allele was a statistically significant predictive factor for better treatment outcome at 3 months (OR, 4.04; 95% CI, 1.11–14.70; $P = 0.03$). At 6 months (OR, 3.26; 95% CI, 0.76–13.90; $P = 0.11$) and 12 months (OR, 2.54; 95% CI, 0.61–10.52; $P = 0.20$), similar, but nonsignificant trends were observed.

DISCUSSION

Anti-VEGF drugs have revolutionized the treatment and visual outcome of patients with neovascular AMD. These treatments offer, for the first time, a real possibility of improving vision. However, vision does not improve in all treated patients; approximately 10% of patients continue to lose vision despite treatment. It is not known why some individuals do not benefit from treatment with these anti-VEGF agents, but genetic make-up may be a determinant. In this study we investigated the role of the *APOE* gene and its relation to treatment outcome in patients with neovascular

TABLE 3. Multivariate Logistic Regression Analysis of Treatment Outcomes

	3-mo Outcome (n = 166)		6-mo Outcome (n = 168)		12-mo Outcome (n = 149)	
Characteristics	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age, y	0.96 (0.91, 1.01)	0.18	0.94 (0.89, 0.99)	0.03	0.94 (0.88, 0.99)	0.04
Sex						
Male	1	0.20	1	0.16	1	0.44
Female	0.62 (0.30, 1.28)		0.60 (0.30, 1.22)		0.73 (0.33, 1.61)	
Baseline logMAR VA*	2.80 (1.31, 6.04)	0.001	4.18 (1.87, 9.33)	0.001	4.88 (1.89, 12.58)	0.001
APOE allele						
$\varepsilon 2$	1		1		1	
$\varepsilon 3$	2.27 (0.77, 6.69)	0.14	4.32 (1.24, 15.06)	0.02	1.93 (0.61, 6.11)	0.26
$\varepsilon 4$	4.04 (1.11, 14.70)	0.03	3.26 (0.76, 13.90)	0.11	2.54 (0.61, 10.52)	0.20

Each OR and *P* value is adjusted for all other characteristics listed in the table. *APOE* alleles are as described in Table 1.

* VA worsens as logMAR value increases.

AMD. In an approach that has never been undertaken, we attempted to account for all other important confounding factors by adjusting for patient and lesion characteristics as well as treatment delay.

We showed that *APOE* genotype is significantly associated with treatment outcome. The presence of an *APOE* $\varepsilon 4$ allele, significantly increased the chances of a 2-line improvement in VA at 3 months, with borderline significance at both 6 and 12 months.

We also demonstrated that presenting VA and age of the patient were other factors associated with visual outcome.

APOE may play a role in several pathologic processes thought to be occurring in AMD. *APOE* is expressed in photoreceptor outer segments, the retinal ganglion layer and in Bruch's membrane. It is also secreted by RPE cells.[32,33] It has been postulated that *APOE* $\varepsilon 4$ allele may facilitate greater lipid efflux from RPE cells and lipid transport across Bruch's membrane,[30] an effect secondary to the prevention of normal dimerization of the apolipoprotein E protein that usually occurs with the *APOE* $\varepsilon 3$ and $\varepsilon 2$ isoforms.

People with one or two copies of *APOE* $\varepsilon 4$ have reduced C-reactive protein (CRP) levels when compared to individuals lacking these copies.[34,35] CRP is a marker of systemic inflammation and is a prominent drusen-associated molecule. It is expressed in significantly higher levels among patients with advanced AMD.[36]

In addition, the effects of the *APOE* isoforms on chemokine and cytokine regulation may offer some insight into the mechanism responsible for the observed protective role of the $\varepsilon 4$ isoform. Oxidative stress has been implicated in the development of AMD,[37,38] under such conditions, increased levels of both CCL2 (macrophage chemotactic protein) and VEGF have been demonstrated.[39,40] Both VEGF and CCL2 overexpression have been associated with further degeneration, especially in advanced disease stages of AMD.[41] Bojanowski et al.[42] demonstrated an almost twofold suppression of both CCL2 and VEGF expression in RPE cells by the *APOE* $\varepsilon 4$ isoform compared to $\varepsilon 3$ and $\varepsilon 2$.

The *APOE* $\varepsilon 4$ isoform may therefore have a protective role in preventing AMD development and progression as well as allowing a better response to VEGF inhibition.

Our results suggest a significant effect of *APOE* $\varepsilon 4$ and outcome at 3 months, with borderline significance thereafter. The loss of significance with longer time of follow-up may well be because other variables, such as treatment interval or missed appointments, should be factored into these longer time period analyses. Notably, at 3 months, most patients (75%) had received three monthly loading-dose injections, whereas after this time, clinicians treated on their own discretion, with the result that a variable number of treatments were given at different intervals, depending on the treating ophthalmologist.

We did not find any significant deleterious effects of the *APOE* $\varepsilon 2$ allele on visual outcomes; however, in keeping with the suggestion that the *APOE* gene may influence treatment outcome, Lee et al.[43] found that, in the eyes of transgenic mice expressing human *APOE* $\varepsilon 2$, VEGF was overexpressed in the RPE. This works suggests that this risk allele alters or upregulates angiogenic cytokines and influences the amount of VEGF in the retinas of these patients.. Indeed, in our previous study of *APOE* and AMD, we found that individuals with the $\varepsilon 2\varepsilon 3$ genotype had a significantly younger mean age of disease diagnosis, particularly with choroidal neovascular disease, compared with patients with the $\varepsilon 3\varepsilon 3$ genotype (4.7 years, $P = 0.003$).[29]

Our finding that the presence of the *APOE* $\varepsilon 4$ allele is associated with an increased chance of better anti-VEGF treatment outcome, at least in the early stage of treatment, has important research and clinical implications. As there are possibly lower retinal VEGF levels in these patients, it may well be that less intensive dosage regimens of anti-VEGF would be sufficient to counteract the relatively lower level of VEGF present. In addition, knowing about the presence of a particular *APOE* allele will allow better prognostic information for individuals at the start of treatment, thus ushering in the start of personalized anti-VEGF treatment in neovascular AMD. Further prospective studies with larger cohorts should be conducted to validate our study results. New genetic studies are needed, to investigate the associations of anti-VEGF treatment outcomes with other genes, including the VEGF-A gene itself, as its product is the key molecule promoting abnormal blood vessel growth and is the target of therapy.

The design of our study is a major strength. We investigated and adjusted for patient and lesion characteristics, treatment delay, as well as genetic polymorphisms, all in the same cohort of patients. The observational nature of our study allowed accurate representation of normal clinic patients and normal treatment procedures, as opposed to a randomized clinical trial, best enabling retinal specialists to apply our findings to the clinical environment. Our study population was also larger than any comparable clinical study of genetic determinants of treatment outcomes. Potential limitations should also be mentioned. A larger sample size may have allowed us to find significant differences in outcomes with different genotypes when we found a trend at 6 and 12

months. Second, there was the potential for selection bias, as it was not possible to recruit every patient, such as those who required an interpreter (for non–English-speaking patients of European origin). This lowered the representation of populations groups that were not fluent in English (e.g., Mediterranean and East European persons).

In summary, we evaluated potential predictors of anti-VEGF treatment outcomes in neovascular AMD, to maximize the benefit of these therapies and to minimize harm to those who are unlikely to gain from ongoing treatment. In this study, the presence of the *APOE ε4* allele was significantly associated with a better chance of significant vision improvement in patients treated with anti-VEGF agents for neovascular AMD. Given the substantial number of people with AMD, further investigation into this potential pharmacogenetic relationship is needed to guide the use of these revolutionary treatments.

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