CFH, VEGF, and PEDF genotypes and the response to intravitreal injection of bevacizumab for the treatment of age-related macular degeneration

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Abstract We determined whether there is an association between complement factor H (CFH), high-temperature requirement A-1 (HTRA1), vascular endothelial growth factor (VEGF), and pigment epithelium-derived factor (PEDF) genotypes and the response to treatment with a single intravitreal injection of bevacizumab for age-related macular degeneration (AMD). Eighty-three patients with exudative AMD treated by bevacizumab injection were genotyped for three single nucleotide polymorphisms (SNPs; rs800292, rs1061170, rs1410996) in the CFH gene,

a rs11200638-SNP in the HTRA1 gene, three SNPs (rs699947, rs1570360, rs2010963) in the VEGF gene, and four SNPs (rs12150053, rs12948385, rs9913583, rs1136287) in the PEDF gene using a TaqMan assay. The CT genotype (heterozygous) of CFH-rs1061170 was more frequently represented in nonresponders in vision than TT genotypes (nonrisk allele homozygous) at the time points of 1 and 3 months, while there was no CC genotype (risk allele homozygous) in our study cohort ($p=7.66\times 10^{-3}$, 7.83×10^{-3} , respectively). VEGF-rs699947 was also associated with vision changes at 1 month and PEDF-rs1136287 at 3 months ($p=5.11\times 10^{-3}$, 2.05×10^{-2} , respectively). These variants may be utilized for genetic biomarkers to estimate visual outcomes in the response to intravitreal bevacizumab treatment for AMD.

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Introduction

Angiogenesis plays a major role in many disease processes including tumor growth, atherosclerosis, arthritis, and ocular neovascular diseases. Pathological ocular angiogenesis occurs in several vision-threatening diseases such as proliferative diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration (AMD), the leading cause of blindness in developed countries. Currently, AMD is estimated to affect about 50 million people worldwide including Japan [1–4]. AMD is a clinically heterogeneous and genetically complex disease with mul-

multiple genetic and environmental risk factors [1]. Recently, the complement factor H (CFH) gene on chromosome 1q31 has been demonstrated as the major AMD susceptibility gene [5–10]. Genetic variants at another chromosomal locus, 10q26, also confer strong disease risk, including age-related maculopathy susceptibility 2 (also known as LOC387715) [11–15] and high-temperature requirement factor A1 (HTRA1) [16, 17] genes. It is now acknowledged that progress in this field has significantly increased our understanding of AMD disease susceptibility and pathogenesis.

Based on the cellular biological view, angiogenesis is a complex multistep process that involves the out-sprouting of vascular endothelial cells from existing vessels through endothelial cell proliferation, extracellular matrix remodeling, endothelial cell migration, and capillary tube formation. A number of growth factor molecules are involved in this process. Among them, the key regulator of choroidal neovascularization (CNV) in AMD is the angiogenesis stimulator, vascular endothelial growth factor (VEGF) [18]. VEGF induces both proliferation and migration of vascular endothelial cells [19]. Pigment epithelium-derived factor (PEDF), a 50-kDa protein secreted by human retinal pigment epithelial cells, has been demonstrated to be a potent antiangiogenic agent that inhibits the migration of endothelial cells *in vitro* and a more potent antiangiogenic agent than angiostatin, thrombospondin-1, or endostatin in assays [20]. Pathological angiogenesis is thought to result from an imbalance between angiogenesis stimulators and inhibitors [21], and several studies have implicated an imbalance between VEGF and PEDF as an important contributor to the development of CNV in AMD [22–24]. Currently, pharmacotherapies against VEGF-A have been introduced to treat CNVs in AMD, including pegaptanib sodium, a selective antagonist of the 165 isoform of VEGF-A; ranibizumab, a recombinant monoclonal antibody Fab fragment against all VEGF-A isoforms; and bevacizumab, a full-length monoclonal antibody against all VEGF-A isoforms [25–27]. These established therapies have met with great success in reducing the vision loss associated with neovascular AMD. The anti-VEGF therapies are now a milestone in the treatment of these disease states. Recently, several efforts are underway to identify genetic and/or pharmacological biomarkers that may predict response to therapy, thereby contributing important information to clinical decision making and care. Known and novel phenotypic biomarkers that associate with or predict variation in an individual's state of health or predict the consequences of altered genes on protein expression are strategic candidates for evaluation. Two reports demonstrated CFH genotype association with the response to bevacizumab and ranibizumab injections for neovascular AMD treatment, suggesting its role of predictive biomarker

against anti-VEGF therapies [28, 29]. The purpose of this study is to determine whether there is an association between CFH, HTRA1, VEGF, and PEDF genotypes and response to treatment with a single intravitreal injection of bevacizumab for AMD in Japanese Asian patients.

Methods

Study subjects

Eighty-three patients with neovascular AMD who received unilateral bevacizumab injection, ranging in age from 57 to 96 years (72.2 ± 8.6 , mean $\pm$ SD), 60 male 23 female, were enrolled in this study. Baseline demographics are presented in Table 1. All patients were recruited from outpatients who visited the Department of Ophthalmology, Saitama Medical University Hospital, Saitama prefecture, Japan. The study was approved by the Ethics Committee of Saitama Medical University (approved on December 9, 2003; approval number #03-262), and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Each individual was fully informed of the purpose and the procedures involved in the study. Informed written consent was obtained from each patient. All subjects were unrelated Japanese. Patient records were reviewed retrospectively to obtain the following ophthalmic examination data.

Ophthalmic examination and definition of AMD

All patients were examined by best corrected visual acuity (BCVA), fundus photography, fluorescein, and indocyanine green angiographies. Of 83 patients recruited, 64 had an examination with optical coherence tomography (Cirrus OCT, Carl Zeiss Meditec AG, Jena, Germany). BCVA was measured at initial presentation and at each follow-up visit. The central retinal thickness (CRT) was measured at the foveola (between retinal inner surface and retinal pigment epithelium) by OCT at the baseline and each follow-up visit. Inclusion criteria were as follows: (1) age of 50 years or older, (2) diagnosis of neovascular AMD in one or both eyes, (3) no association with other retinochoroidal diseases such as angiod streaks, high myopia (greater than 6 diopter of myopic refractive error), central serous chorioretinopathy, or presumed ocular histoplasmosis. Each patient was followed up at 1, 3, and 6 months after the treatment. Clinical data at the months 1 and 3 time points were analyzed for statistical association of BCVA and CRT results with genotype. Patients were subdivided in responder and nonresponders on the basis of vision improvement. Recurrence of AMD was determined by a significant increase of the CRT (20% or more increase from baseline)

Table 1 Baseline characteristics of the study subjects

Number of patients	83
Age (mean $\pm$ SD)	72.2 $\pm$ 8.6
Age distribution; <i>n</i> (%)	50–59 8 (9.6)
	60–69 27 (32.5)
	70–79 30 (36.1)
	80–89 17 (20.5)
	90– 1 (1.2)
Subtypes	Typical neovascular AMD 60
	Polypoidal choroidal vasculopathy 23
Gender (male/female)	60/23
Mean logMAR vision (mean $\pm$ SD)	0.61 $\pm$ 0.44
Mean BCVA (approximate decimal visual acuity)	0.37
Mean CRT (mean $\pm$ SD, μ m)	445.0 $\pm$ 163.4

SD standard deviation, AMD age-related macular degeneration, logMAR logarithm of minimum angular resolution, BCVA best corrected visual acuity, CRT central retinal thickness

due to retinal edema and retinal detachment delineated by OCT or two lines or more BCVA decrease. Additional visits for retreatment were to be given when the recurrence was determined.

Genotyping and statistical analysis

Genomic DNA was extracted from the peripheral blood of each individual using a DNA extraction and purification Kit (Wizard Genomic DNA Purification Kit; Promega, Madison, WI, USA), according to the manufacturer's instructions. The samples were genotyped (TaqMan genotyping assay with the ABI Prism 7000 Sequence Detection System; Applied Biosystems, Inc., Foster City, CA, USA), and the data were analyzed (Allelic Discrimination Program; ABI). Assessed were three single nucleotide polymorphisms (SNPs; rs800292, rs1061170, rs1410996) in the CFH gene, a rs11200638-SNP in the HTRA1 gene, three SNPs (rs699947, rs1570360, rs2010963) in the VEGF gene, and four SNPs (rs12150053, rs12948385, rs9913583, rs1136287) in the PEDF gene. All analyses were performed using commercially available software (SNPAlyze ver. 6.0.1, Dynacom, Chiba, Japan; SSRI ver. 1.20, SSRI, Tokyo, Japan).

Results

Eighty-three eyes of 83 patients with neovascular AMD were treated with single intravitreal bevacizumab injection, and the genotype association with short-term treatment outcomes was analyzed. Patients were subdivided in responder and nonresponders on the basis of vision improvement. Table 2 displays the genotype association with the BCVA changes from baseline at each time point. Although there was no CC genotype (risk allele homozygous) among our study cohort, the CT genotype (heterozygous) was more frequently represented in nonresponders

than TT genotypes (nonrisk allele homozygous) at the time points of 1 and 3 months ($\chi^2=7.194$, $p=7.66\times 10^{-3}$; $\chi^2=7.193$, $p=7.83\times 10^{-3}$; respectively). VEGF-rs699947 was also associated with BCVA changes at 1 month time point, and PEDF-rs1136287 was at 3 months significantly ($\chi^2=7.986$, $p=5.11\times 10^{-3}$; $\chi^2=5.590$, $p=2.05\times 10^{-2}$, respectively).

We also examined the genotype association with CRT changes from baseline measured by OCT. Of the 64 AMD patients examined by OCT, 54 patients (84%) had CRT reduction at 1 month, and 45 (70%) patients had at 3 month. Among the tested SNPs, none had a significant association with CRT change from baseline ($p>0.05$; Table 3). However, mean CRT reduction of nonrisk allele homozygous of VEGF-rs699947 and PEDF-rs1136287 tended to be higher than those of heterozygous and risk allele homozygous, which genotypes were associated with visual responses to intravitreal bevacizumab as shown in Table 2. We also examined how often CNV recurrence occurred in part determined by an increase of the retinal thickness examined by OCT. Table 4 shows the p value and odds ratio (OR) for the incidence of recurrence after initial bevacizumab treatment. Among the tested 11 polymorphisms, no significant difference was demonstrated in the recurrence of CNV at the time points of 3 and 6 months ($p>0.05$).

Discussion

In this study, we have described a significant association between CFH-rs1061170, VEGF-rs699947, and PEDF-rs1136287 variants and visual outcomes after intravitreal bevacizumab treatment. Regarding CRT changes and the CNV recurrence, we did not identify significance in genetic association with the response to bevacizumab therapy, possibly due to, at least in part, smaller sample size. However, mean CRT reduction of nonrisk allele homozygous of VEGF-rs699947 and PEDF-rs1136287 tended to be

Table 2 Responders and non-responders in BCVA after bevacizumab injection and the studied CFH, HTRA1, VEGF, and PEDF genotypes

dbSNP ID	1 month after the injection		3 months after the injection	
	χ^2	p^a	χ^2	p^a
CFH				
rs800292	0.726	0.398	0.930	0.339
rs1061170	7.194	7.66×10^{-3}	7.193	7.83×10^{-3}
rs1410996	0.215	0.646	0.057	0.813
HTRA1				
rs11200638	0.884	0.353	0.410	0.527
VEGF				
rs699947	7.986	5.11×10^{-3}	1.031	0.314
rs1570360	0.114	0.739	0.213	0.650
rs2010963	1.323	0.254	0.461	0.502
PEDF				
rs12150053	0.161	0.690	3.590	0.060
rs12948385	0.037	0.849	3.218	0.075
rs9913583	0.557	0.460	0.010	0.922
rs1136287	0.014	0.906	5.590	2.05×10^{-2}

BCVA best corrected visual acuity, CFH complement factor H, HTRA1 high-temperature requirement A-1, VEGF vascular endothelial growth factor, PEDF pigment epithelium-derived factor, SNP single nucleotide polymorphism (dbSNP ID; <http://www.ncbi.nlm.nih.gov/SNP/>), OR odds ratio, CI confidence intervals, NA not available

^a Chi-square test

higher than those of heterozygous and risk allele homozygous, which were consistent to visual outcomes. Our data may indicate that these variants may be utilized for genetic biomarkers to estimate visual outcomes in the response to intravitreal bevacizumab treatment for neovascular AMD.

A group of us has previously reported a significant association between diabetic retinopathy and three VEGF variants (rs699947, rs1570360, rs2010963) tested in this current study as well as diabetic macular edema [30, 31]. These VEGF SNPs are located in the promoter region or 5'-

untranslated region and are associated with VEGF production [30–32]. Haplotypes of these SNPs are reported to be associated with plasma VEGF levels and VEGF gene transcription [32]. Other studies have recently reported an association between VEGF SNPs and AMD development, including VEGF-rs2010963 studied here [33, 34]. However, in our recent reports, we failed to provide an evidence of the association of these three VEGF SNPs with disease susceptibility [35] and the response to photodynamic therapy treatment [36]. The population-based Rotterdam

Table 3 Mean CRT changes from baseline and the studied CFH, HTRA1, VEGF, and PEDF genotypes

dbSNP ID	1 month after the injection		3 months after the injection	
	Mean CRT change (μm)	p^a	Mean CRT change (μm)	p^a
CFH				
rs800292	−119.00/−124.92/−64.23	0.253	−33.40/−94.29/−74.70	0.794
rs1061170	−90.96/−110.40/NA	0.693	−85.6/−47.11/NA	0.581
rs1410996	−199.40/−107.00/−61.04	0.110	−223.00/−92.06/−43.92	0.151
HTRA1				
rs11200638	−91.69/−111.82/−84.38	0.812	−106.00/−71.92/−81.17	0.886
VEGF				
rs699947	−275.00/−109.69/−79.83	0.0667	−266.00/−127.65/−48.37	0.0780
rs1570360	NA/−60.86/−96.50	0.636	NA/−148.25/−45.59	0.337
rs2010963	−128.79/−87.30/−78.44	0.575	−104.40/−108.07/−18.32	0.245
PEDF				
rs12150053	+102.00/−113.33/−96.08	0.131	+163.50/−92.31/−84.40	0.194
rs12948385	−96.70/−106.64/+102.00	0.134	−82.36/−100.27/+163.50	0.179
rs9913583	−118.72/−116.94/−65.21	0.349	−99.00/−66.29/−76.39	0.876
rs1136287	−139.11/−108.87/+17.00	0.0650	−95.21/−125.64/−17.20	0.482

Data are mean CRT change from baseline (nonrisk allele homozygous/heterozygous/risk allele homozygous) from baseline and p values

CRT central retinal thickness

^a One-way ANOVA

Table 4 *p* values and odds ratios for CNV recurrence during the 6 months following intravitreal injection of bevacizumab

dbSNP ID	3 months after the injection		6 months after the injection	
	χ^2	<i>p</i> ^a	χ^2	<i>p</i> ^a
CFH				
rs800292	3.265	0.0750	0.147	0.704
rs1061170	0.185	0.670	0.010	0.923
rs1410996	0.258	0.615	0.315	0.578
HTRA1				
rs11200638	0.671	0.418	0.425	0.519
VEGF				
rs699947	2.431	0.133	0.004	0.949
rs1570360	0.597	0.447	1.482	0.229
rs2010963	0.031	0.861	2.890	0.0926
PEDF				
rs12150053	0.008	0.929	0.158	0.693
rs12948385	0.031	0.860	0.544	0.464
rs9913583	1.751	0.190	0.166	0.687
rs1136287	0.121	0.734	0.028	0.869

^a Chi-square test

study, which examined 4,228 participants, also demonstrated no significant association with AMD susceptibility [37], which is consistent to our report [35]. This study is the first to demonstrate that the VEGF-rs699947 polymorphism is significantly associated with visual outcomes after anti-VEGF therapy, intravitreal bevacizumab. The risk allele (−2578C) carriers of VEGF-rs699947 SNP were more frequent within the nonresponders. VEGF SNPs tested here have also been reported to associate with overall survival of patients with advanced breast cancer treated with additional use of bevacizumab, indicating that patients with VEGF genotypes that predict low VEGF production and/or expression gain the most substantial benefit with anti-VEGF therapy [38, 39]. Although disease pathogenesis is different between AMD and breast cancer, VEGF genotypes correlating with VEGF production may have a potential as genetic biomarkers to predict the efficacy of bevacizumab for the treatment of neovascular AMD.

We have also demonstrated a significant association between the PEDF-rs1136287 variant and visual outcomes after intravitreal bevacizumab treatment. As well as VEGF genotypes tested, we failed to provide an evidence of the association of PEDF SNPs with disease susceptibility and the response to photodynamic therapy treatment in our recent reports [35, 36]. Several lines of evidence indicate a role of PEDF in the pathogenesis of exudative AMD: decreased immunoreactivity for PEDF in both RPE cells and in Bruch's membrane of AMD eyes in immunohistochemical study [40], significantly reduced vitreous PEDF concentrations in eyes with exudative AMD [41], and inhibition and regression of CNV with the administration of

viral vector-delivered PEDF [42, 43]. Considering the antiangiogenic effects and an important role in AMD pathogenesis of PEDF, it is reasonable to determine whether PEDF gene polymorphisms, as well as VEGF variants, may modulate the efficacy of anti-VEGF treatment. For further investigations, functional analysis of these PEDF polymorphisms is necessary to clarify the exact role of these SNPs and their possible interaction with VEGF SNPs in the response to anti-VEGF therapy.

Two reports have already been published describing CFH Y402H genotype association with the response to bevacizumab and ranibizumab treatments for neovascular AMD, suggesting its role of predictive biomarker against anti-VEGF therapies [28, 29]. Although ethnic genotypic variation, especially in Japanese population, has been reported with an AMD-associated CFH Y402H polymorphism, our results replicated and were consistent with these reports in Caucasian population [28, 29]. The association between CFH Y402H genotypes and response to anti-VEGF therapies may indirectly indicate potential molecular interaction between CFH and VEGF in AMD pathology.

In conclusion, we demonstrated that CFH-rs1061170, VEGF-rs699947, and PEDF-rs1136287 variants were associated with response to intravitreal bevacizumab in this study population. These variants may be utilized for genetic biomarkers to estimate visual outcomes in the response to intravitreal bevacizumab treatment for neovascular AMD. In contrast, our previous study provided an evidence of the association of HTRA1-rs11200638 but not of these VEGF, PEDF, and CFH SNPs with the response to photodynamic therapy treatment [36]. The incidence of polypoidal

choroidal vasculopathy in the Asian populations with neovascular AMD has been reported to be much higher than in Caucasians [35, 44, 45]. Photodynamic therapy is thought to be good treatment modality for polypoidal choroidal vasculopathy, and therefore, photodynamic therapy is still important in the treatment of Asian AMD as well as anti-VEGF therapies [45]. While our previous [36] and present works support a hypothesis that known genetic polymorphisms may be utilized as genetic biomarkers to predict responses to photodynamic therapy and anti-VEGF therapy in AMD, the implications are still certainly broader. The full utility of such an approach is not yet known, but ethnic groups with relative genetic homogeneity such as the Japanese population studied here present unique and distinct opportunities to begin to understand the potential of genotype-driven treatment decision making in choosing photodynamic therapy or anti-VEGF therapy. Although we have tested 11 SNPs in total using chi-square test and ANOVA in this study, we should utilize multiple comparison post hoc tests with larger sample size of study cohort for further analysis to obtain sufficiently strong statistic results. Warranted are also further investigations of subtype analysis for both typical neovascular AMD and polypoidal choroidal vasculopathy and of genome-wide association study searching novel variants associating with the treatment response.

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