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Association analysis of genetic risk variants with response to treatment with intravitreal anti-vascular endothelial growth factor in Korean neovascular age-related macular degeneration patients

한국인 신생혈관성 나이관련황반변성 환자에서 유전학적 위험요인과 유리체강내 항혈관내피세포성장인자항체 치료반응 간의 관련성 분석

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ABSTRACT

Association analysis of genetic risk variants with response to treatment with intravitreal anti-vascular endothelial growth factor in Korean neovascular age-related macular degeneration patients

Introduction: Although intravitreal anti-vascular endothelial growth factor (VEGF) injection has revolutionized the outcome of neovascular age-related macular degeneration (AMD) treatment, there is wide spectrum of clinical response, and genetic factors seems to be one of determinants. The purpose of this study is to investigate the association between genetic risk variants for AMD and response to intravitreal anti-VEGF in Korean neovascular AMD patients.

Methods: This prospective study included 366 treatment-naïve patients (366 eyes) with subfoveal neovascular AMD. Patients were genotyped for 17 single-nucleotide polymorphisms within 13 AMD-relevant genes. Initially, patients underwent three monthly injections of intravitreal ranibizumab (0.5 mg / 0.05ml) and were followed up monthly. Additional treatments with intravitreal ranibizumab or bevacizumab (1.25 mg / 0.05ml) were administered when the retreatment criteria were met. Main outcome measures were *visual response* based on visual acuity change criteria and amount of change in best-corrected visual acuity (BCVA). *Tomographic response* and *angiographic response*, which were based on optical coherence tomography and fluorescein angiography findings respectively, and changes in central retinal thickness (CRT) and lesion size from baseline were also evaluated. Genotypic association of all categorical and continuous outcome variables at month 6, 12, and 24 was evaluated with regression analysis for each candidate polymorphisms. To evaluate the potential gene-gene interaction, multifactor dimensionality reduction (MDR) analysis was performed. In addition, to reappraise the inconsistent previous pharmacogenetic results on anti-VEGF for neovascular AMD,

meta-analysis was performed including the data from this cohort.

Results: At month 24, BCVA improved by 4.5 ± 22.5 letters and CRT decreased by 69.4 ± 112.6 μm from baseline. Regression analyses for genotypic association revealed that minor allele homozygotes of *VEGFA* gene rs3025039 had significantly higher chance of good visual response than other genotypes at month 24 (Odds ratio [OR], 5.46; 95% Confidence Interval [CI], 1.79 – 16.70; P = 0.0029). Minor allele homozygotes of *CFH* rs800292 showed significantly lower chance of good angiographic response than other genotypes (OR, 0.26; 95% CI, 0.11 - 0.62; P = 0.0021). Minor allele homozygotes for *ARMS2* rs10490924 and *HTRA1* rs11200638 showed larger amount of CRT reduction at month 12 with borderline significance when corrected for multiple testing. MDR analysis revealed the significant interaction between *CFH* rs800292 and *PEDF* rs1136287 for tomographic response at month 12. In meta-analysis, combined data from this study and 5 previous pharmacogenetic studies showed that minor allele homozygosity for *ARMS2* or *HTRA1* were associated with larger amount of visual improvement after anti-VEGF treatment for neovascular AMD.

Conclusion: In this Korean neovascular AMD cohort, *VEGFA* rs3025039, *CFH* rs800292, *ARMS2* rs10490924, *HTRA1* rs11200638, and *PEDF* rs1136287 showed possible association with response to anti-VEGF treatment. With more evidence of pharmacogenetic association with anti-VEGF agent, individualized therapeutic approaches based on genetic background may lead to optimal treatment outcome in neovascular AMD.

Keywords: Age-related macular degeneration, anti-vascular endothelial growth factor, pharmacogenetics, polymorphism

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Introduction

1. Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a late-onset neurodegenerative disease of macular region of the retina. AMD is the major cause of irreversible vision loss in elderly people in developed countries, and it has been estimated that the prevalence of AMD is 13.4% among individuals aged ≥ 60 years in the US.(1) Although much remains to be elucidated as to its etiology, AMD is thought to be influenced by both environmental and genetic factors. Epidemiological studies have shown that aging, race, and some modifiable environmental risk factors such as cigarette smoking, high body mass index, cardiovascular risk factors, and nutrition are associated with the risk for AMD.(2-4) The role of genetics in AMD has been established by population-based studies or twin studies. In Rotterdam study population, lifetime risk for advanced AMD was 4.2 times higher in first-degree relatives of AMD patients compared to relatives of controls.(5) Genetic influence in AMD was also suggested by twin studies, which reported higher AMD concordance of 37% in monozygotic twins compared to 19% in dizygotic twins.(6)

2. Genetic association of AMD

It is challenging to study AMD from genetic aspect because its development and phenotypes seem to be influenced by multiple factors including genetic variants, unlike diseases of Mendelian inheritance pattern where a disease trait is determined by a mutation in a single gene. AMD is a complex disease with heterogenous phenotypes, and its pathogenesis involves various biologic pathways such as inflammation, angiogenesis, immune regulation, lipid metabolism, extracellular matrix degradation, hence is attributable to multiple genes. In addition, potential gene-environment or gene-gene interactions might affect an individual's susceptibility.

The first identification of an association of AMD with genetic factor was in single nucleotide polymorphisms (SNP) in complement factor H (*CFH*) gene on chromosome 1q32 using 100K DNA chip.(7) The Y402H polymorphism (rs1061170) in *CFH* gene has been consistently demonstrated to have significant association with all subtypes of AMD.(8-11) Another strong genetic predictor for AMD is Age-Related Maculopathy Susceptibility 2 (*ARMS2*, formerly LOC387715) and High Temperature Requirement factor A1 (*HTRA1*) genes in 10q26 locus.(11-15) Using candidate gene approach, researchers have reported number of genes associated with AMD up to now, but they were not strongly associated with AMD as those in 1q32 and 10q26.

Apart from *CFH* gene, genetic variants in a number of complement components such as complement factor 2 (*C2*) / Complement factor B (*CFB*)(16, 17), complement factor 3 (*C3*) (17-19), complement factor I (*CFI*)(17, 20) showed AMD association. Other representative genes reported to be related to AMD, according to the biologic pathways in AMD pathogenesis, are as follows.(21-31) Genes associated with lipid metabolism: apolipoprotein E (*APOE*), scavenger receptor class B type 1 (*SCARB1*), cholesterylester transfer protein (*CETP*), lipoprotein lipase (*LPL*), ATP-binding cassette, sub-family A, member 1 (*ABCA1*); genes involved in immune regulation: chemokine receptor 1 (*CX3CR1*), toll-like receptor 3 (*TLR3*), *TLR4*, complement factor 1 inhibitor (*SERPING1*), superkiller viralicidic activity 2-like (*SKIV2L*), excision repair cross-complementation group 6 (*ERCC6*); genes associated with extracellular matrix regulation: fibulin-5 (*FBLN5*), tissue inhibitor of metalloproteinases 3 (*TIMP3*)/synapsin3 (*SYN3*), extracellular collagen matrix (*FRK*/*COL10A1*, *COL8A1*/*FILIP1L*); genes associated with angiogenesis: vascular endothelial growth factor A (*VEGFA*), pigment epithelium-derived factor (*PEDF*), transforming growth factor beta receptor-1 (*TGFBR1*). In addition to SNPs, recent studies have suggested that the structural variation such as copy number variation in AMD-relevant genes may be associated with AMD susceptibility.(32)

3. Neovascular AMD and its treatment

Advanced form of AMD presents as choroidal neovascularization (CNV) or geographic atrophic changes in the central macula. The neovascular (or exudative) form of AMD, characterized by CNV formation and proliferation of fibrous tissue, represents only 10-15% of all AMD cases but is responsible for more than 90% of severe visual loss caused by AMD.(33) Previously, neovascular AMD was treated with laser photocoagulation or photodynamic therapy (PDT) with verteporfin, but its efficacy was limited. Treatment for neovascular AMD has dramatically improved after the introduction of intravitreal treatment with anti-vascular endothelial growth factor (VEGF) monoclonal antibody. Two pivotal randomized clinical trials have shown that monthly ranibizumab injection enabled visual gain of ≥ 15 letters in 30% to 40% of neovascular AMD patients.(34, 35) The most widely used agents are on-label anti-VEGF agent ranibizumab (Lucentis; Novartis, Basel, Switzerland) and the off-label agent bevacizumab (Avastin; Roche, Basel, Switzerland). Recently, the Comparison of Age-related macular degeneration Treatments Trials (CATT) study groups reported that effects on visual acuity were equivalent for ranibizumab and bevacizumab.(36)

4. Pharmacogenetic association of neovascular AMD

Although anti-VEGF treatment is effective for most of neovascular AMD patients, some patients do not benefit the treatment and 5% to 10 % of patients lose ≥ 15 letters despite treatment. The genetic profile of a patient seems to be associated with this variability in therapeutic responsiveness. Up to date, a few studies with limited candidate genetic variants have been performed to investigate the genetic association with the response to intravitreal anti-VEGF treatment for neovascular AMD. *CFH* Y402H, *ARMS2* rs10490924, *HTRA1* rs11206038, *APOE* and some *VEGFA* polymorphisms have shown relevance to ranibizumab or bevacizumab treatment outcome, but the results were not consistent.(37-48) Recently, pharmacogenetic study for CATT study participants, which included

largest number of subjects up to now, found no association of 4 major AMD-relevant genes (*CFH*, *ARMS2*, *HTRA1*, *C3*) with the response to anti-VEGF treatment.(49) However, in East Asians, there is a chance of having a different genetic profile related to the response to AMD treatment from Caucasians because of ethnic diversity in AMD-associated polymorphisms. For example, *CFH* Y402H polymorphism, which was found to be strongly associated in Caucasians, has been reported to have no association in some reports from Chinese or Japanese.(50, 51) Furthermore, there has been no large-scale pharmacogenetic study on anti-VEGF for neovascular AMD in East Asian populations.

The purpose of this study was to investigate whether genetic variants that have shown association with the AMD development are also related to the treatment response to intravitreal anti-VEGF treatment in Korean neovascular AMD patients. For genetic analysis, candidate genetic variants in 13 AMD-relevant genes were selected from previous reports on genetic association of AMD.(8-20, 23-30)

MATERIAL and METHODS

1. Study design and Patient eligibility

Treatment protocol and design of this study were approved by the Institutional Review Board of Seoul National University Hospital and were performed in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients before participation. For this study, we prospectively recruited outpatients who started intravitreal anti-VEGF treatment for active choroidal neovascularization (CNV) secondary to AMD at the retina center in Seoul National University Hospital between January 2009 and July 2011. Neovascular AMD was defined as the presence of any of the followings as described by the International Age-Related Maculopathy Classification (52): retinal pigment epithelial (RPE) detachments or serous detachment of the sensory retina, subretinal or sub-RPE neovascular membranes, subretinal hemorrhages, or fibrovascular disciform scarring. We treated patients with active CNV, which showed the leakage on fluorescein angiography (FA) and the fluid on optical coherence tomography (OCT) either within or below the retina or below the RPE.

Inclusion criteria were age of ≥ 50 years, subfoveal CNV secondary to AMD with an initial best corrected visual acuity (BCVA) of 5 to 70 letters using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart (Snellen equivalent of 20/40 to 20/800). Only patients of Korean descents who agreed to blood sampling for genetic analysis were included, and all angiographic lesion types were included for the study. Exclusion criteria were myopic refractive error of > 6 diopter, CNV secondary to causes other than AMD, polypoidal choroidal vasculopathy (PCV), presence of disciform macular scar or atrophy, prior treatment for neovascular AMD, previous history of vitrectomy, and failure to follow up for 24 months after the first injection. If both eyes of a patient were eligible, only one eye with shorter symptom duration before the first injection was included. If a fellow eye developed CNV

during the course of study, the second eye was treated at the discretion of treating physicians but not included in the study.

2. Clinical Examination and anti-VEGF Treatment Protocol

At initial presentation, patient demographics and peripheral blood sample for DNA extraction were collected after consent was obtained. Patients underwent a full ophthalmic examination including BCVA measurement, slit-lamp examination, intraocular pressure measurement, fundus examination, spectral domain OCT (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA), FA, and indocyanine green angiography (ICGA). Visual acuity was measured using ETDRS charts at 4-meter distance by well-trained testers after standardized refraction. Using OCT, central retinal thickness (CRT) was recorded as the 1-mm central subfield thickness obtained from the macular thickness map. If there was error in automated recognition of the inner or outer boundaries as the internal limiting membrane or the retinal pigment epithelium (RPE) respectively, CRT was manually measured with a caliper. By baseline FA, angiographic subtypes were defined as predominantly classic (> 50% classic, well-demarcated areas of hyperfluorescence appearing early in the angiogram followed by late leakage), minimally classic (< 50% classic), or occult (leakage appearing only late in the angiogram). PCV was diagnosed based on the presence of branching vascular networks and polypoidal choroidal vascular lesions on ICGA, and the patients with PCV were excluded from this study. With digitalized FA images and fundus photographs, total area of CNV lesion which includes CNV, thick blood, blocked fluorescence, and serous detachment of the RPE was measured in disc areas (DA).

Initially, all patients underwent three monthly injections of ranibizumab (month 0, 1, 2) followed by as-needed regimen until month 24. During the as-needed regimen phase, bevacizumab could be also used for injection when desired by patients whose medical insurance coverage for ranibizumab, which was up to 5 injections per eye for neovascular AMD in Korea, had been terminated. A dose of 0.5 mg

ranibizumab or 1.25 mg bevacizumab, both in 0.05 ml solution, was injected intravitreally under standard sterile condition. Patients were examined for treatment monthly and anti-VEGF re-treatments were performed when any of following OCT-guided criteria was met: evidence of persistent fluid on OCT, BCVA loss of > 5 letters or central retinal thickness (CRT) increase of > 100 μm from previous visit, new macular hemorrhage. Patients underwent BCVA assessment, dilated fundus examination, and OCT at each follow-up visit, and FA was repeated every three months.

3. Measures of response to treatment

Response to treatment was evaluated by vision and findings of OCT or FA. Primary outcome measures were visual outcome variables including *visual response* and visual acuity change in ETDRS letter. A *visual response* was defined as a gain of ≥ 15 ETDRS letters from baseline only when the BCVA at the time of assessment was ≥ 35 letters (Snellen equivalent of 20/200). For the subjects with the BCVA at the time of assessment of ≥ 70 letters (Snellen equivalent of 20/40), change in BCVA better than 5-letter loss from baseline was also regarded as visual response. Secondary outcome measures were tomographic and angiographic outcome variables including *tomographic response*, *angiographic response*, change in CRT, and change in CNV lesion size from baseline. A *tomographic response* was defined as dry status without any of following findings on OCT: subretinal fluid, intraretinal cyst, subretinal hemorrhage, and fovea-involving neurosensory retinal atrophy or subretinal fibrosis. An *angiographic response* was defined as no dye leakage or fovea-involving RPE tear on FA at the time of assessment. All categorical and continuous outcome variables were evaluated at 6, 12, and 24 months after treatment initiation, except for angiographic response and change in CNV lesion size which were evaluated only at month 24.

4. DNA preparation and Genetic analysis

A 10 mL peripheral blood sample was collected from each patient. Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (FUJIFILM, Tokyo, Japan). In this study, candidate genes for analysis were selected from the previous reports on AMD-relevant genes including *CFH*, *ARMS2*/*HTRA1*, *CFB*/*C2*, *C3*, *CFI*, *SKIV2L*, *VEGFA*, *APOE*, *PEDF*, *SCARB1*, and *SYN3*/*TIMP3*.(8-20, 23-30) Within these genes, commonly evaluated SNPs were chosen taking previous pharmacogenetic studies on anti-VEGF treatment for neovascular AMD into account.(37-48, 53, 54) In total, 17 candidate polymorphisms in 13 genes were determined. All genetic variants were genotyped using TaqMan SNP genotyping assays (Applied Biosystems Inc. [ABI], Foster City, CA, USA) or SNaPshot Multiplex kit (ABI) according to the manufacturer's recommendation. The characteristics, genotyping method, and overall genotyping results of all the candidate genetic variants are presented in Table 1.

5. Sample size calculation

Statistical power was calculated using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc).(55) To calculate the number of samples required to achieve 80% power, odds ratios (ORs) for good treatment response were obtained from the previous anti-VEGF pharmacogenetic studies which found significant association and had available OR data. Obtained ORs were 2.14 to 8.5 for *CFH* rs1061170, 3.01 for *ARMS2* rs10490924, 3.73 for *HTRA1* rs11200638, and 3.61 for *VEGFA* rs699947 respectively.(37, 38, 41, 44, 56, 57) Sample size calculation was performed with various genetic models (i.e. allelic, dominant, additive, recessive models) for each variant, using allele frequency data of this cohort. Good-to-poor responder ratio was determined according to the response rate in the study from which the OR data was obtained. Among the 4 genetic models, calculation for recessive model showed the largest number of required sample size for each polymorphism, which was 316 for rs1061170, 254 for rs10490924, 192 for rs11200638,

and 130 for rs699947 at 5% type I error rates, respectively.

6. Statistical analysis

Descriptive statistics for all demographic and clinical variables were calculated, and comparison was made using the *t*-test for means with continuous data (e.g., age, ETDRS letter, CRT) and the chi-square test for categorical data (e.g., gender). The Hardy-Weinberg equilibrium (HWE) for genotypic distribution was evaluated using the HWE exact test.

Non-genetic covariates included in the analysis were age, sex, smoking status (ever vs. never), CNV lesion type (predominantly classic vs. others), and baseline BCVA, CRT, lesion size. A linear regression model was used to determine the influence of non-genetic covariates on continuous outcome variables (i.e. mean change in BCVA, CRT, and CNV lesion size).

To investigate the influence of the genetic variants on long-term treatment response, the association between the genotype of each candidate variant and outcome variables at month 6, 12, and 24 were evaluated using regression models. Angiographic response and change in CNV lesion size were evaluated only at month 24. For categorical outcome variables (i.e. *visual response*, *tomographic response*, *angiographic response*), the genotype frequency was compared between responder and non-responder. Ordinary logistic regression model was used to calculate OR and 95% confidence interval (CI). For continuous outcome variables (i.e. changes in BCVA, CRT, and CNV lesion size from baseline), linear regression model was used. The genetic analyses were performed for each genetic variant independently of the other variants using allelic, dominant, recessive, and additive genetic models. The results were adjusted for age, sex, smoking status, lesion type and baseline BCVA, CRT, and CNV lesion size. For variants with significant association, the longitudinal BCVA or CRT changes throughout the 24-month follow-up were assessed according to the best fitting genetic model to verify the influence of genotype. For the *APOE* gene, allelic variants of ε2, ε3, or ε4 from the

combination of two polymorphisms (rs429358, rs7412) were used for analysis. The effect of the presence of ε2 (1 or 2 vs. no ε2 allele) and ε4 allele on treatment response was investigated respectively.

Statistical analyses were performed using PLINK, version 1.07 (available on http://pngu.mgh.harvard.edu/~purcell/plink), and SPSS for windows version 21.0 (SPSS Inc., Chicago, IL). Correction for multiple testing was performed with the Bonferroni method. For all statistical tests, corrected $P < 0.05$ was considered as statistically significant.

7. Gene-gene interaction analysis

To assess the interactions between the genetic variants in relation to anti-VEGF treatment response, the multifactor dimensionality reduction (MDR) method was used. As a non-parametric and model free method, it reduces high-dimensional genetic data into a single dimension to facilitate the detection of multi-locus effects in relatively small sample size. Using the open-source MDR software (ver. 3.0.2; available on http://www.epistasis.org), gene-gene interactions were evaluated with the flexible four-step process recommended by Moore et al (58) for each categorical outcome variable at month 6, 12, and 24 respectively. Briefly, variants unlikely to exhibit interactions were selectively filtered using a ReliefF algorithm and MDR analysis was limited to the top five variants. We tested all possible combinations of two- or three-way interaction models using the MDR constructive induction algorithm, and created new variable with two levels (good and poor response) by pooling multi-locus genotypes. A naïve Bayes classifier was used in the context of 10-fold cross-validation (CV) to estimate the balanced testing accuracy, and the single best model with the maximal testing accuracy was selected for each of the two- and three-factor level. *P*-values for the statistical significance were obtained using 1000-fold permutations testing to compare the observed testing accuracies with those expected under the null hypothesis of no association (MDR-PT software available on

http://sourceforge.net/projects/mdr/files/mdrpt). Models were considered significant at $P < 0.05$.

8. Meta-analysis for pharmacogenetic association

Because of the inconsistent results in previous pharmacogenetic studies, existing data were combined with our result and assessed by means of a meta-analysis. Relevant reports were searched in the Pubmed using keywords following terms: “neovascular AMD,” “exudative AMD,” “ranibizumab,” “bevacizumab,” “anti-VEGF,” “pharmacogenetics,” “polymorphism,” “genetic,” “variant,” “outcome,” “response,” “efficacy,” and “treatment.” A total of 26 articles that evaluated the association between genetic variants and anti-VEGF treatment outcome in neovascular AMD were identified, and each was reviewed for study design, ethnicity, treatment protocol, primary outcome measure, and candidate genetic variants. To minimize the heterogeneity resulted from the variety of criteria for treatment response, this meta-analysis was focused on amount of visual acuity change from baseline as continuous outcome variable. Studies reporting sufficient visual acuity change values for data synthesis (mean and standard error) for each genotype were included for analysis. Snellen or logMAR visual acuities were converted to ETDRS letter score using the equation described previously.(59) We identified 5 candidate studies, and genetic variants that were evaluated in two or more articles were analyzed. Change in visual acuity after anti-VEGF treatment was compared under the recessive genetic model for minor allele, and additionally between patients homozygous for minor allele and for major allele. Considering the follow-up periods of other included studies, visual acuity data at month 12 of this cohort was used for meta-analysis. The statistical significance of pooled standard difference was determined using Z-test, in which $P < 0.05$ was considered significant. Inter-study heterogeneity was checked with the Q-statistic and I^2 statistic. Heterogeneity was considered to be statistically significant when P value < 0.1.(60) If heterogeneity existed, random-effect model was used to pool the data. Otherwise, fixed-effect model was used. A leave-one-out sensitivity test was performed by

iteratively omitting one study at a time to assess the effect of excluding a specific study. All meta-analyses were performed using the software Comprehensive Meta Analysis Ver. 2.2.064.

RESULTS

1. Cohort characteristics and anti-VEGF treatment outcome

Of the 543 patients who met the inclusion/exclusion criteria at the anti-VEGF treatment initiation, 177 patients were excluded from the analysis for the following reasons: 104 patients with refusal to blood sampling for genotyping, 6 patients with failure to yield sufficient high-quality DNA, and 67 patients who failed to finish 24-month follow-up. These exclusions resulted in a final study cohort of 366 Korean patients (366 eyes).

Patient demographics and baseline characterization of the AMD phenotypes for entire study cohort are presented in Table 2. Mean age at baseline injection was 69.4 ± 7.9 years, and 55.7 % of patients were male. Mean baseline BCVA was 46.4 ± 21.1 ETDRS letters (approximate Snellen equivalent 20/118), and mean BCVA improvement from baseline were 8.9 ± 15.8 at month 6 and 7.8 ± 18.8 at month 12, and 4.5 ± 22.5 at month 24. With our criteria for visual response, 189 patients (51.6%) at month 12 and 182 patients (49.7m %) at month 24 showed good *visual response* to anti-VEGF treatment. A gain of ≥ 15 letters was seen in 29.2% and 30.9% of patients at month 12 and 24 respectively, and a loss of ≥ 15 letters was seen in 7.9% and 15.6% at month 12 and 24 respectively. *Tomographic response* was in 50.3% and 49.2% of patients at month 12 and 24, respectively. Mean baseline CRT was 322.0 ± 95.3 μm, and mean CRT reduction from baseline were 69.2 ± 97.2 μm at month 12 and 69.4 ± 112.6 μm at month 24. The changes in BCVA and CRT during 24-month follow-up are shown in Figure 1. During follow-up, mean BCVA improvement and mean CRT reduction from baseline were significant at all time points ($P < 0.001$; paired t-test). A*ngiographic response*, defined as no dye leakage or fovea-involving RPE tear on FA, was in 69.4% of patients and mean CNV lesion size did not show significant change at month 24 compared to baseline. Overall treatment outcomes are listed in Table 3.

Patients received on average 5.7 ± 1.9 injections in the first 12 months and 2.8 ± 2.3 injections in the second 12 months. During the as-needed regimen phase, the proportion of patients who received anti-VEGF treatment was lowest at month 24 (17.5%) and highest at month 4 (39.6%). Overall injection pattern is presented in Figure 2. 60 patients (16.4%) were treated with only ranibizumab, and remaining 306 patients (83.6%) received one or more injections of bevacizumab during 24-month follow-up.

Clinical features and treatment outcome according to the visual response are presented in Table 4. Patients who showed good visual response were significantly younger than poor responders both at month 12 and 24. There was no significant difference in mean baseline BCVA between the two visual response groups at month 24, but good visual responders at month 12 had better baseline BCVA compared to poor visual responders (49.4 ± 22.2 vs. 43.3 ± 19.5 letters respectively, $P = 0.006$; t-test). Patients with good visual response also showed better tomographic performance compared to the poor visual responders. At month 24, 67.6% of good visual responder showed good tomographic response at the same time, whereas only 31.3% of poor visual responder showed good tomographic response (Chi-square test, $P < 0.001$). The amount of CRT reduction was also larger in good visual responders than in poor visual responders, although both response groups showed significant reduction from baseline (84.7 ± 103.4 μm vs. 54.3 ± 119.3 μm respectively, $P = 0.01$; t-test). On FA, only good visual responders showed decrease in CNV lesion size, but angiographic response rates were not significantly different between the two visual response groups.

The regression coefficient (β) with 95% CI and *P* values for each covariate are presented in Table 5. The regression model predicted that younger patients were more likely to show a greater improvement both in BCVA and CRT and that lower baseline BCVA and thicker baseline CRT were associated with a greater increase in BCVA and a greater decrease in CRT respectively, as indicated by the regression coefficient. Baseline CNV lesion size was significantly associated with change in

BCVA and lesion size inversely, and gender, smoking and lesion type did not show significant association with continuous outcome variables.

2. Genetic analysis

Overall genotyping rate was 99.6% and missing data was less than 2% for each candidate variant. The distribution of the genotypes for each genetic variant was consistent with the HWE (*P* value > 0.05) except the variants in *ARMS2* and *HTRA1* gene as shown in Table 1. In this cohort, no polymorphism was observed at *C3* rs2230199 and all patients showed CC genotype. Pharmacogenetic analysis was performed for 16 SNPs in 12 genes except for rs2230199 and hence, uncorrected *P* value should be less than 0.003125 (= 0.05 / 16) to be considered significant when applying Bonferroni method to correct for multiple testing. Of all the regression analyses for genetic association with treatment outcome variables for visual, tomographic, and angiographic outcome variables, genetic variants that reached at least borderline significance (corrected $P < 0.10$, uncorrected $P < 0.00625$) are listed in Table 6.

In analysis for visual outcome measures, *VEGFA* rs3025039 was the only polymorphism that showed significant association with the good visual response at month 24 under recessive genetic model. Patients with TT genotype at *VEGFA* rs3025039 were 5.5-times (OR, 5.46; 95% CI, 1.79 - 16.68; uncorrected $P = 0.0029$) more likely to show good visual response after 24-month anti-VEGF treatment compared to those with CC or CT genotypes. Visual outcome variables during 24-month follow-up are presented in Figure 3. Patients with TT genotype showed higher chance of good visual response with uncorrected *P* value < 0.05 at all time points except month 3, 12, and 18 (Figure 3A), and showed a tendency of larger extent of vision improvement from month 6 to 24 (Figure 3B). Compared to other genotypes, TT genotype showed better tomographic performance both at month 12 and 24. They also had higher chance of good angiographic response, which was significant only when

uncorrected for multiple testing (OR, 4.65; 95% CI, 1.04 - 20.8; uncorrected $P = 0.0444$). No genetic variant showed significant association with BCVA change from baseline at month 6, 12, and 24 in linear regression model. Table 7 shows the association of all candidate genetic variants with visual outcome variables under recessive model at month 6, 12, and 24.

In analysis for tomographic outcome measures, good tomographic response was not associated with any candidate genetic variants at month 6, 12, and 24. Significant association with CRT change from baseline was found at *SKIV2L* gene rs429608 under recessive model at month 24. In patients with AA genotype at rs429608, who were only four, mean CRT increased 78.3 ± 155.9 μm from baseline, whereas patients with GG and GA genotype experienced mean reduction of 69.4 ± 108.7 μm (uncorrected $P = 0.0008$). Under dominant genetic model, there was no significant genetic association with tomographic outcome variables at month 6, 12, and 24. CRT changes during 24-month follow-up according to rs429608 genotypes are presented in Figure 4. Apart from rs429608, genetic variants at 10q26 locus (*AMRS2* rs10490924, *HTRA1* rs11200638) showed possible association with CRT change at month 12, which reached borderline significance under recessive model (corrected $P < 0.10$). At month 12, mean CRT reduction from baseline in patients homozygous for the minor allele (GG genotype for both SNP) were greater than those in patients with other genotypes (uncorrected $P = 0.0041$ for rs10490927, 0.0035 for rs11200638). Changes in mean CRT during 24-month follow-up are shown in Figure 5. Compared to other genotypes, larger amount of CRT reduction in minor allele homozygotes persisted through month 24, and the statistical significance with uncorrected $P < 0.05$ was achieved at all time points except month 3 for both variants. Homozygotes of 10q26 locus also showed better visual performance than other genotypes consistently at month 6, 12, and 24, but were not significant. Table 8 shows the association of all candidate genetic variants with tomographic outcome variables under recessive model at 6, 12, and 24.

Angiographic response was analyzed only at month 24 because considerable patients did not

undergo FA timely at month 6 and 12. *CFH* rs800292 was the only polymorphism that showed significant association with the angiographic outcome. Under recessive model, patients with AA genotype at rs800292 were less likely to show good angiographic response (OR, 0.26; 95% CI, 0.11 - 0.62; uncorrected P = 0.00213) at month 24 compared to those with GG and GA genotypes. Patients with rs800292 AA genotype showed larger extent of BCVA improvement and CRT reduction than other genotypes at month 6, 12, and 24, but all the associations were not significant. In linear regression model, no genetic variant showed significant association with mean CNV lesion size change from baseline. Table 9 shows the association of all candidate genetic variants with angiographic outcome variables under recessive model at month 24.

3. Results of gene-gene interaction analysis

Table 10 summarizes the results of an exhaustive MDR analysis that evaluated all possible combinations of two- and three-order models using the variants selected from the ReliefF algorithm for each categorical outcome variables. The best model for each order is shown along with its accuracy, cross-validation consistency (CVC), and significance level. Of the overall best model in analyses for each outcome variables at various time points, only two-locus model including *CFH* gene rs800292 and *PEDF* gene rs1136287 for good tomographic response at month 12 was statistically significant as determined by permutation testing. This best model had a significant testing accuracy of 0.602 and CVC of 10/10. The carriers of genotype combinations conferring increased probability for good response were 2.4-fold more likely to show good tomographic response at month 12 (OR, 2.36; 95% CI, 1.54 - 3.62; P = 0.018). Figure 6 shows the distribution of responders and non-responders for each of the genotype combinations in the *CFH-PEDF* model for good tomographic response at month 12.

4. Meta-analysis results

Previous pharmacogenetic studies on anti-VEGF treatment in neovascular AMD are summarized in Table 11. We identified 5 articles with available visual acuity data for meta-analysis, and all studies were performed in Caucasians except one study.(39-41, 49, 53, 61) The combined sample size for this meta-analysis was 1279 when including 366 patients from this cohort. *CFH* rs1061170, *ARMS2* 10490924, and *HTRA1* 11200638 was evaluated in two or more candidate studies, and visual acuity change data according to the genotypes were pooled including this cohort.

In Q-statistic, analysis between major and minor allele homozygotes for rs1061170 and analysis under recessive model for rs11200638 showed significant heterogeneity ($P < 0.1$), and random-effect model was used for the analyses. When visual acuity data were pooled together, GG genotype of *ARMS2* rs10490924 showed 3.6 letters better visual improvement compared to TT and TG genotypes under recessive model (95% CI, 1.3 to 5.9; $P = 0.002$) and 4.3 letters better visual improvement compared to TT genotype alone (95% CI, 2.1 to 6.5; $P < 0.001$). GG genotype of *HTRA1* rs11200638 showed 4.7 letters better visual improvement compared to AA genotype (95% CI, 0.9 to 8.4; $P = 0.014$). In the leave-one-out sensitivity analysis, no study changed the significant result of meta-analyses. Results of meta-analyses are listed in Table 12, and forest plots for difference in mean visual acuity changes are presented in Figure 7.

Table 1. Characteristics of candidate genetic markers

Chr	Gene	Location	dbSNP ID	Major/ Minor Allele	MAF	Genotyping Method	HWE *P* - value	Genotyping rate	References
1	***CFH***	I62V	rs800292	G/A	0.291	SNaPshot	0.505	100%	Tian et al(15); Liu et al(30)
1	***CFH***	Y402H	rs1061170	T/C	0.098	SNaPshot	1.0	98.9%	Hageman et al(8); Souied et al(9); Sepp et al(10); Tian et al(15); Liu et al(30); Yu et al(17)
1	***CFH***	IVS14-543A/G	rs1410996	G/A	0.324	Taqman	0.211	100%	Yu et al(17); Tian et al(15)
6	***CFI***	64060C/T	rs10033900	T/C	0.304	Taqman	0.643	99.7%	Fagerness et al(20); Yu et al(17)
6	***C2***	E318D	rs9332739	G/C	0.012	Taqman	0.097	100%	Gold et al(16);Yu et al(17)
6	***CFB***	R32Q	rs641153	G/A	0.065	SNaPshot	1.0	99.5%	Gold et al(16);Yu et al(17)
6	***SKIV2L***	3493G/A	rs429608	G/A	0.069	Taqman	0.154	99.5%	Kopplin et al(28); Liu et al(29)
6	***VEGFA***	C-2578A	rs699947	C/A	0.295	SNaPshot	0.086	99.5%	Immonen et al(62); Imai et al(42)
6	***VEGFA***	C936T	rs3025039	C/T	0.232	Taqman	0.400	100%	Lin et al(24)
10	***ARMS2***	A69S	rs10490924	T/G	0.31	Taqman	0.041	100%	Seddon et al(11); Shuler et al(12); Andreoli et al(13); Hadley et al(14); Yu et al(17); Tian et al(15)
10	***HTRA1***	-625A/G	rs11200638	A/G	0.305	Taqman	0.009	100%	Hadley et al(14); Tian et al(15); Andreoli et al(13)
12	***SCARB1***	A350A	rs5888	C/T	0.265	Taqman	0.435	100%	Zerbib et al(26)
17	***PEDF***	Met72Thr	rs1136287	C/T	0.499	Taqman	1.0	99.7%	Lin et al(27); Imai et al(42)
19	***C3***	R80G	rs2230199*	C/-	0	Taqman	1.0	99.7%	Yates et al(18); Maller et al(19); Yu et al(17)
19	***APOE***	Cys112Arg	rs429358	T/C	0.093	Taqman	0.326	98.9%	Baird et al(23); Wickremasinghe et al(46)
19	***APOE***	Arg158Cys	rs7412	C/T	0.061	Taqman	1.0	98.6%	Baird et al(23); Wickremasinghe et al(46)
22	***SYN3/TIMP3***	IVS5-91789G/T	rs9621532	T/G	0.029	SNaPshot	0.511	100%	Chen et al(25); Yu et al(17)

SNP = Single nucleotide polymorphism (dbSNP ID; available at: http://www.ncbi.nlm.nih.gov/SNP/); MAF = Minor allele frequency; HWE = Hardy-Weinberg Equilibrium; IVS = Intervening sequence; UTR = Untranslated region

*: Excluded from analysis because all patients showed CC genotype

Table 2. Demographic and baseline clinical features of the patients

	Total
Number of patients	366
Mean Age (years)	69.4 ± 7.9 (51-91)
Male	204 (55.7 %) / 162 (44.3 %)
Smoking status	
Never	218 (59.6 %)
Ever (current / ex-smoker)	148 (40.4 %)
Angiographic lesion type	
Predominantly classic lesion	75 (20.5 %)
Minimally classic lesion	104 (28.4 %)
Occult lesion	187 (51.1 %)
Baseline Visual Acuity, ETDRS letter	46.4 ± 21.1
Baseline Central Retinal Thickness, µm	322.0 ± 95.3
Baseline CNV lesion area, DA	3.6 ± 4.1

ETDRS = Early Treatment Diabetic Retinopathy Study; CNV = Choroidal neovascularization; DA = Disc area

Table 3. Outcome of anti-vascular endothelial growth factor treatment for neovascular age-related macular degeneration

	Month 6	Month 12	Month 24
Visual Outcome			
Mean visual acuity score, ETDRS letters (SD)	55.3 (21.8)	54.2 (21.5)	51.0 (24.5)
Visual response, no. (%)	200 (54.6 %)	189 (51.6 %)	182 (49.7 %)
Change in visual acuity score from baseline			
Mean (SD)	+ 8.9 (15.8)	+ 7.8 (18.8)	+ 4.5 (22.5)
≥ 15 letter increase, no. (%)	115 (31.4 %)	107 (29.2 %)	113 (30.9 %)
≥ 15 letter decrease, no. (%)	20 (5.5 %)	29 (7.9 %)	57 (15.6 %)
Tomographic Outcome			
Tomographic response, no. (%)	225 (61.5 %)	184 (50.3 %)	180 (49.2 %)
Mean change in central retinal thickness from baseline, µm (SD)	- 70.7 (100.1)	- 69.2 (97.2)	- 69.4 (112.6)
Angiographic Outcome			
Angiographic response, no. (%)	-	-	254 (69.4 %)
Mean change in CNV lesion size from baseline, DA (SD)	-	-	- 0.1 (4.1)
Mean number of injections (SD)	4.3 (1.0)	5.7 (1.9)	8.5 (3.5)

ETDRS = Early Treatment Diabetic Retinopathy Study; CNV = Choroidal neovascularization; DA = Disc area

Table 4. Comparison between patients with and without good visual response at month 6, 12, and 24

	Month 6			Month 12			Month 24		
	Good visual response	**Poor visual response**	***P*-value**	**Good visual response**	**Poor visual response**	***P*-value**	**Good visual response**	**Poor visual response**	***P*-value**
Number of patients (%)	200 (54.6 %)	166 (45. 4 %)		189 (51.6 %)	177 (48.4 %)		182 (49.7%)	184 (50.3 %)	
Mean Age, years (SD)	68.9 (8.4)	70.1 (7.4)	0.136	68.4 (8.2)	70.6 (7.5)	**0.007**	68.4 (7.9)	70.5 (7.9)	**0.012**
Male	112 (56.0 %)	92 (55.4 %)	0.916	99 (52.4%)	105 (59.3%)	0.207	101 (55.5 %)	103 (56.0 %)	1.000
Smoking (ever, %)	80 (40.0 %)	68 (41.0 %)	0.915	73 (38.6%)	75 (42.4%)	0.523	73 (40.1 %)	75 (40.8 %)	0.916
Baseline visual acuity, ETDRS letter (SD)	50.0 (21.4)	42.2 (20.1)	**< 0.001**	49.4 (22.2)	43.3 (19.5)	**0.006**	47.6 (22.4)	45.3 (19.8)	0.306
Mean change in visual acuity from baseline (SD)	17.5 (13.8)	-1.5 (11.0)	**< 0.001**	18.3 (16.3)	- 3.5 (14.3)	**< 0.001**	19.4 (14.4)	- 10.2 (19.0)	**< 0.001**
Tomographic Outcome									
Tomographic response, no. (%)	145 (72.5 %)	80 (48.2 %)	**< 0.001**	118 (62.4 %)	66 (37.3 %)	**< 0.001**	123 (67.6 %)	57 (31.3 %)	**< 0.001**
Mean change in central retinal thickness from baseline, µm (SD)	-80.6 (99.6)	-58.8 (99.6)	**0.038**	- 79.0(100.0)	- 58.8 (93.6)	**0.047**	- 84.7 (103.4)	- 54.3 (119.3)	**0.01**
Angiographic Outcome									
Angiographic response, no. (%)	-	-	-	-	-	-	133 (73.1 %)	121 (65.8 %)	0.141
Mean change in CNV lesion size from baseline, DA (SD)	-	-	-	-	-	-	- 0.6 (4.5)	0.3 (3.6)	**0.046**
Number of injection	4.2 (1.0)	4.3 (1.0)	0.426	5.6 (1.9)	5.8 (1.9)	0.25	7.9 (3.5)	9.1 (3.5)	**0.001**

ETDRS = Early Treatment Diabetic Retinopathy Study; CNV = Choroidal neovascularization; DA = Disc area

Table 5. Regression coefficients (β) and *P* values for association of non-genetic covariates with continuous outcome variables at month 6, 12, and 24

		Age	Sex	Smoking	Baseline BCVA	Baseline CRT	Baseline CNV lesion size	Lesion type
BCVA change from baseline	**at month 6**	-0.248 ± 0.099 (***P* = 0.013**)	-1.018 ± 1.931 (*P* = 0.598)	1.771 ± 1.956 (*P* = 0.366)	-0.257 ± 0.039 (***P* < 0.001**)	0.008 ± 0.008 (*P* = 0.353)	-0.423 ± 0.192 (***P* = 0.028**)	0.250 ± 2.009 (*P* = 0.901)
	at month 12	- 0.242 ± 0.114 (***P* = 0.034**)	0.164 ± 2.214 (*P* = 0.941)	3.836 ± 2.242 (*P* = 0.088)	-0.394 ± 0.045 (***P* < 0.001**)	-0.003 ± 0.009 (*P* = 0.729)	-0.443 ± 0.220 (***P* = 0.045**)	0.467 ± 2.303 (*P* = 0.840)
	at month 24	-0.461 ± 0.138 (***P* = 0.001**)	0.661 ± 2.683 (*P* = 0.805)	2.796 ± 2.717 (*P* = 0.304)	-0.434 ± 0.054 (***P* < 0.001**)	0.002 ± 0.011 (*P* = 0.836)	-0.726 ± 0.267 (***P* = 0.007**)	-0.561 ± 2.791 (*P* = 0.841)
CRT change from baseline	**at month 6**	1.422 ± 0.481 (***P* = 0.003**)	-3.818 ± 9.376 (*P* = 0.684)	-3.323 ± 9.495 (*P* = 0.727)	-0.198 ± 0.189 (*P* = 0.297)	-0.735 ± 0.040 (***P* < 0.001**)	0.326 ± 0.933 (*P* = 0.727)	3.987 ± 9.752 (*P* = 0.683)
	at month 12	1.340 ± 0.462 (***P* = 0.004**)	-3.937 ± 9.016 (*P* = 0.663)	-4.691 ± 9.130 (*P* = 0.608)	0.162 ± 0.182 (*P* = 0.375)	-0.718 ± 0.039 (***P* < 0.001**)	1.314 ± 0.897 (*P* = 0.144)	16.823 ± 9.377 (*P* = 0.074)
	at month 24	1.452 ± 0.545 (***P* = 0.008**)	-34.408 ± 10.623 (*P* = 0.101)	-30.274 ± 10.758 (*P* = 0.105)	-0.312 ± 0.214 (*P* = 0.146)	-0.796 ± 0.045 (***P* < 0.001**)	2.176 ± 1.057 (*P* = 0.140)	-7.058 ± 11.049 (*P* = 0.523)
Lesion size change from baseline	**at month 24**	0.024 ± 0.025 (*P* = 0.344)	-0.591 ± 0.477 (*P* = 0.216)	-0.001 ± 0.484 (*P* = 0.999)	-0.006 ± 0.010 (*P* = 0.546)	0.004 ± 0.002 (*P* = 0.079)	-0.603 ± 0.050 (***P* < 0.001**)	0.105 ± 0.508 (*P* = 0.837)

BCVA = Best-corrected visual Acuity; CRT = Central retinal thickness; CNV = Choroidal neovascularization

Table 6. Pharmacogenetic association with anti-vascular endothelial growth factor treatment outcome in neovascular age-related macular degeneration patients

Outcome variable		Gene	dbSNP ID	Best fitting genetic model	Uncorrected *P* - value	Corrected *P* - value*
Visual Response	**at month 6**	-	-	-		
	at month 12	-	-	-		
	at month 24	*VEGFA*	rs3025039	Recessive (CC+CT versus TT)	0.0029	0.04641
BCVA change from baseline	**at month 6**	-	-	-		
	at month 12	-	-	-		
	at month 24	-	-	-		
Tomographic Response	**at month 6**	-	-	-		
	at month 12	-	-	-		
	at month 24	-	-	-		
CRT change from baseline	**at month 6**	-	-	-		
	at month 12	*ARMS2*	rs10490924	Recessive (TT+TG versus GG)	0.00410	0.06563
		HTRA1	rs11200638	Recessive (AA+AG versus GG)	0.00346	0.05532
	at month 24	*SKIV2L*	rs429608	Recessive (GG+GA versus AA)	0.0008	0.01272
Angiographic Response	**at month 24**	*CFH*	rs800292	Recessive (GG+GA versus AA)	0.00213	0.03402
CNV lesion size change from baseline	**at month 24**	-	-	-		

SNP = Single nucleotide polymorphism (dbSNP ID; available at: http://www.ncbi.nlm.nih.gov/SNP/); BCVA = Best-corrected visual Acuity; CRT = Central retinal thickness; CNV = Choroidal neovascularization

*: *P* - value corrected for multiple testing with Bonferroni method; Polymorphisms with borderline significance of corrected $P < 0.1$ are listed

Table 7. Association of candidate genetic variants with visual outcome variables under recessive genetic model

Gene	Variant		Genotype (N)	Visual Responder	OR (95% CI)	*P* - value*	Mean BCVA change (letter)	*P* - value†
CFH	rs800292	6 mo	GG (187) + GA (145)	180 (54.2%)	1.00		+8.8	
			AA (34)	20 (58.8%)	1.00 (0.48-2.12)	0.9947	+12.6	0.6292
		12 mo	GG (187) + GA (145)	169 (50.9%)	1.00		+7.3	
			AA (34)	20 (58.8%)	1.13 (0.53-2.41)	0.7426	+15.3	0.1216
		24 mo	GG (187) + GA (145)	162 (48.8%)	1.00		+4.5	
			AA (34)	20 (58.8%)	1.27 (0.60-2.68)	0.5285	+7.8	0.8741
CFH	rs1061170††	6 mo	TT (294)	159 (54.1%)	1.00		+8.7	
			TC (65) + CC (3)	39 (57.4%)	1.24 (0.72-2.15)	0.438	+11.2	0.1341
		12 mo	TT (294)	150 (51.0%)	1.00		+7.8	
			TC (65) + CC (3)	37 (54.4%)	1.28 (0.74-2.21)	0.3779	+9.2	0.4847
		24 mo	TT (294)	146 (49.7%)	1.00		+4.4	
			TC (65) + CC (3)	34 (50.0%)	1.10 (0.64-1.89)	0.7317	+6.6	0.2948
CFH	rs1410996	6 mo	GG (173) + GA (149)	175 (54.3%)	1.00		+9.0	
			AA (44)	25 (56.8%)	0.87 (0.45-1.72)	0.6976	+10.9	0.8986
		12 mo	GG (173) + GA (149)	165 (51.2%)	1.00		+7.6	
			AA (44)	24 (54.5%)	0.89 (0.45-1.76)	0.7484	+11.8	0.4819
		24 mo	GG (173) + GA (149)	161 (50.0%)	1.00		+5.2	
			AA (44)	21 (47.7%)	0.70 (0.36-1.37)	0.2996	+2.4	0.09
CFI	rs10033900	6 mo	TT (179) + TC (150)	182 (55.3%)	1.00		+9.3	
			CC (36)	18 (50.0%)	0.84 (0.42-1.70)	0.6328	+9.1	0.8235
		12 mo	TT (179) + TC (150)	169 (51.4%)	1.00		+8.2	
			CC (36)	20 (55.6%)	1.25 (0.61-2.56)	0.5342	+8.1	0.7973
		24 mo	TT (179) + TC (150)	161 (48.9%)	1.00		+4.9	
			CC (36)	20 (55.6%)	1.38 (0.68-2.80)	0.3692	+4.7	0.87
C2	rs9332739††	6 mo	GG (358)	195 (54.5%)	1.00		+9.2	
			GC (7) + CC (1)	5 (62.5%)	1.49 (0.33-6.74)	0.6034	+9.4	0.4731
		12 mo	GG (358)	185 (51.7%)	1.00		+8.1	
			GC (7) + CC (1)	4 (50.0%)	0.94 (0.21-4.14)	0.9352	+7.5	0.398
		24 mo	GG (358)	178 (49.7%)	1.00		+4.7	
			GC (7) + CC (1)	4 (50.0%)	0.87 (0.20-3.73)	0.8541	+11.4	0.9144
CFB	rs641153††	6 mo	GG (318)	176 (55.3%)	1.00		+9.7	
			GA (45) + AA (1)	23 (50.0%)	0.85 (0.45-1.62)	0.6259	+6.0	0.146
		12 mo	GG (318)	166 (52.2%)	1.00		+8.4	
			GA (45) + AA (1)	21 (45.7%)	0.76 (0.40-1.45)	0.4109	+6.0	0.3232
		24 mo	GG (318)	157 (49.4%)	1.00		+4.7	
			GA (45) + AA (1)	25 (54.3%)	1.27 (0.67-2.42)	0.466	+7.5	0.3907
SKIV2L	rs429608††	6 mo	GG (318)	174 (54.7%)	1.00		+9.6	
			GA (42) + AA (4)	24 (52.2%)	0.33 (0.03-3.38)	0.3475	+6.6	0.1521
		12 mo	GG (318)	164 (51.6%)	1.00		+8.2	
			GA (42) + AA (4)	23 (50.0%)	0.94 (0.49-1.81)	0.8632	+7.2	0.5003
		24 mo	GG (318)	155 (48.7%)	1.00		+4.2	
			GA (42) + AA (4)	25 (54.3%)	1.28 (0.67-2.45)	0.4576	+9.4	0.1768
VEGFA	rs699947	6 mo	CC (188) + CA (137)	175 (53.8%)	1.00		+8.7	
			AA (39)	23 (59.0%)	1.23 (0.62-2.46)	0.5588	+12.6	0.1822
		12 mo	CC (188) + CA (137)	170 (52.3%)	1.00		+7.6	
			AA (39)	17 (43.6%)	0.69 (0.34-1.37)	0.2891	+11.5	0.252
		24 mo	CC (188) + CA (137)	163 (50.2%)	1.00		+4.8	
			AA (39)	17 (43.6%)	0.73 (0.37-1.45)	0.3714	+4.4	0.7739
VEGFA	rs3025039	6 mo	CC (219) + CT (124)	181 (52.8%)	1.00		+8.9	
			TT (23)	19 (82.6%)	4.56 (1.49-14.0)	**0.0079**	+13.0	0.2156

		12 mo	CC (219) + CT (124)	173 (50.4%)	1.00		+7.8	
			TT (23)	16 (69.6%)	2.48 (0.63-6.36)	0.0599	+12.0	0.2652
		24 mo	CC (219) + CT (124)	163 (47.5%)	1.00		+4.4	
			TT (23)	19 (82.6%)	5.46 (1.79-16.7)	**0.0029**	+11.4	0.129
ARMS2	rs10490924	6 mo	TT (183) + TG (139)	172 (53.4%)	1.00		+8.9	
			GG (44)	28 (63.6%)	1.20 (0.60-2.42)	0.6057	+11.3	0.8201
		12 mo	TT (183) + TG (139)	164 (50.9%)	1.00		+7.8	
			GG (44)	25 (56.8%)	0.93 (0.47-1.86)	0.8388	+10.1	0.7868
		24 mo	TT (183) + TG (139)	157 (48.8%)	1.00		+4.4	
			GG (44)	25 (56.8%)	1.06 (0.54-2.10)	0.8666	+8.4	0.9389
HTRA1	rs11200638	6 mo	AA (188) + AG (133)	172 (53.6%)	1.00		+9.0	
			GG (45)	28 (62.2%)	1.14 (0.57-2.27)	0.7085	+10.8	0.9592
		12 mo	AA (188) + AG (133)	164 (51.1%)	1.00		+7.8	
			GG (45)	25 (55.6%)	0.89 (0.45-1.76)	0.7413	+9.9	0.6985
		24 mo	AA (188) + AG (133)	157 (48.9%)	1.00		+4.3	
			GG (45)	25 (55.6%)	1.02 (0.52-2.00)	0.9597	+8.4	0.9413
SCARB1	rs5888	6 mo	CC (201) + CT (136)	186 (55.2%)	1.00		+9.4	
			TT (29)	14 (48.3%)	0.75 (0.34-1.64)	0.4659	+7.2	0.7704
		12 mo	CC (201) + CT (136)	173 (51.3%)	1.00		+8.2	
			TT (29)	16 (55.2%)	1.19 (0.53-2.63)	0.6754	+7.0	0.8179
		24 mo	CC (201) + CT (136)	169 (50.1%)	1.00		+4.6	
			TT (29)	13 (44.8%)	0.78 (0.35-1.72)	0.5397	+7.8	0.2408
PEDF	rs1136287	6 mo	CC (92) + CT (182)	145 (52.9%)	1.00		+9.2	
			TT (91)	55 (60.4%)	1.37 (0.83-2.24)	0.2157	+9.3	0.9317
		12 mo	CC (92) + CT (182)	136 (49.6%)	1.00		+7.6	
			TT (91)	53 (58.2%)	1.43 (0.87-2.35)	0.1541	+9.8	0.3863
		24 mo	CC (92) + CT (182)	134 (48.9%)	1.00		+5.2	
			TT (91)	48 (52.7%)	1.17 (0.72-1.90)	0.5344	+4.2	0.6267
APOE	apoE	6 mo	ε2 carriers(40)+ε3/ε3(253)	158 (53.9%)	1.00		+9.0	
			ε4 carriers(66)	41(62.1%)	1.43 (0.82-2.51)	0.2081	+8.9	0.8864
		12 mo	ε2 carriers(40)+ε3/ε3(253)	151 (51.5%)	1.00		+7.8	
			ε4 carriers(66)	37 (56.1%)	1.19 (0.68-2.07)	0.5432	+8.1	0.9851
		24 mo	ε2 carriers(40)+ε3/ε3(253)	143 (48.8%)	1.00		+4.5	
			ε4 carriers(66)	38 (57.6%)	1.44 (0.83-2.50)	0.1963	+5.9	0.7327
SYN3/ TIMP3	rs9621532††	6 mo	TT (346)	188 (54.3%)	1.00		+9.4	
			TG (19) + GG (1)	12 (60.0%)	1.16 (0.45-3.01)	0.7547	+5.6	0.3966
		12 mo	TT (346)	175 (50.6%)	1.00		+8.4	
			TG (19) + GG (1)	14 (70.0%)	2.15 (0.77-5.96)	0.1428	+2.8	0.2663
		24 mo	TT (346)	169 (48.8%)	1.00		+4.9	
			TG (19) + GG (1)	13 (65.0%)	1.82 (0.69-4.79)	0.2279	+3.3	0.8497

OR = Odds ratio; CI = Confidence interval; CNV = Choroidal neovascularization; DA = Disc area
*: Uncorrected *P* - value from logistic regression model
†: Uncorrected *P* - value from linear regression model
††: Analyzed under dominant model because of extremely rare minor allele homozygote

Table 8. Association of candidate genetic variants with tomographic outcome variables under recessive genetic model

Gene	Variant		Genotype	Tomo-graphic Responder	OR (95% CI)	*P* - value*	Mean CRT change (µm)	*P* - value†
CFH	rs800292	6 mo	GG (187) + GA (145)	207 (62.3%)	1.00		-70.4	
			AA (34)	18 (52.9%)	0.48 (0.22-1.05)	0.0673	-73.9	0.7902
		12 mo	GG (187) + GA (145)	161 (48.5%)	1.00		-67.3	
			AA (34)	23 (67.6%)	1.99 (0.90-4.44)	0.0913	-89.1	0.2311
		24 mo	GG (187) + GA (145)	162 (48.8%)	1.00		-69.5	
			AA (34)	18 (52.9%)	1.01 (0.47-2.16)	0.9767	-74.2	0.5663
CFH	rs1061170††	6 mo	TT (294)	178 (60.5%)	1.00		-75.0	
			TC (65) + CC (3)	46 (67.6%)	1.73 (0.94-3.19)	0.0793	-56.6	0.5179
		12 mo	TT (294)	146 (49.7%)	1.00		-71.9	
			TC (65) + CC (3)	36 (52.9%)	1.27 (0.73-2.22)	0.403	-62.4	0.9622
		24 mo	TT (294)	143 (48.6%)	1.00		-77.1	
			TC (65) + CC (3)	34 (50.0%)	1.12 (0.64-1.96)	0.6963	-42.6	**0.0428**
CFH	rs1410996	6 mo	GG (173) + GA (149)	197 (61.2%)	1.00		-71.7	
			AA (44)	28 (63.6%)	0.91 (0.43-1.89)	0.7937	-63.7	0.7929
		12 mo	GG (173) + GA (149)	154 (47.8%)	1.00		-67.8	
			AA (44)	30 (68.2%)	1.87 (0.91-3.88)	0.0901	-81.1	0.1561
		24 mo	GG (173) + GA (149)	154 (47.8%)	1.00		-70.5	
			AA (44)	26 (59.1%)	1.30 (0.65-2.63)	0.4588	-65.8	0.6077
CFI	rs10033900	6 mo	TT (179) + TC (150)	201 (61.1%)	1.00		-72.1	
			CC (36)	24 (66.7%)	1.27 (0.59-2.78)	0.5414	-61.1	0.7755
		12 mo	TT (179) + TC (150)	164 (49.8%)	1.00		-70.8	
			CC (36)	20 (55.6%)	1.27 (0.61-2.61)	0.526	-59.8	0.6488
		24 mo	TT (179) + TC (150)	160 (48.6%)	1.00		-71.2	
			CC (36)	19 (52.8%)	1.17 (0.57-2.40)	0.6764	-59.6	0.5769
C2	rs9332739††	6 mo	GG (358)	220 (61.5%)	1.00		-69.8	
			GC (7) + CC (1)	5 (62.5%)	1.36 (0.28-6.58)	0.7016	-110.0	0.7056
		12 mo	GG (358)	178 (49.7%)	1.00		-68.3	
			GC (7) + CC (1)	6 (75.0%)	4.76 (0.87-26.1)	0.0726	-119.3	0.331
		24 mo	GG (358)	175 (48.9%)	1.00		-70.4	
			GC (7) + CC (1)	5 (62.5%)	3.04 (0.63-14.7)	0.1674	-47.8	0.0956
CFB	rs641153††	6 mo	GG (318)	193 (60.7%)	1.00		-69.1	
			GA (45) + AA (1)	31 (67.4%)	1.13 (0.56-2.30)	0.7288	-81.3	0.5178
		12 mo	GG (318)	162 (50.9%)	1.00		-67.7	
			GA (45) + AA (1)	22 (47.8%)	0.88 (0.45-1.71)	0.6988	-80.3	0.2582
		24 mo	GG (318)	156 (49.1%)	1.00		-69.3	
			GA (45) + AA (1)	24 (52.2%)	1.23 (0.63-2.39)	0.5393	-74.0	0.9508
SKIV2L	rs429608††	6 mo	GG (318)	194 (61.0%)	1.00		-67.5	
			GA (42) + AA (4)	30 (65.2%)	0.19 (0.02-2.22)	0.1834	-84.8	0.6978
		12 mo	GG (318)	159 (50.0%)	1.00		-64.9	
			GA (42) + AA (4)	24 (52.2%)	1.26 (0.64-2.45)	0.5045	-87.0	0.1993
		24 mo	GG (318)	154 (48.4%)	1.00		-66.7	
			GA (42) + AA (4)	24 (52.2%)	1.42 (0.72-2.78)	0.3108	-78.7	0.9412
VEGFA	rs699947	6 mo	CC (188) + CA (137)	203 (62.5%)	1.00		-69.6	
			AA (39)	20 (51.3%)	0.72 (0.35-1.48)	0.3763	-78.2	0.7553
		12 mo	CC (188) + CA (137)	161 (49.5%)	1.00		-66.5	
			AA (39)	21 (53.8%)	1.19 (0.59-2.41)	0.6207	-91.9	0.4309
		24 mo	CC (188) + CA (137)	159 (48.9%)	1.00		-67.2	
			AA (39)	19 (48.7%)	0.95 (0.47-1.91)	0.8881	-90.6	0.5505
VEGFA	rs3025039	6 mo	CC (219) + CT (124)	212 (61.8%)	1.00		-69.9	

			TT (23)	13 (56.5%)	0.89 (0.35-2.28)	0.8138	-82.4	0.8923
		12 mo	CC (219) + CT (124)	168 (49.0%)	1.00		-66.9	
			TT (23)	16 (69.6%)	2.61 (0.99-6.92)	0.0534	-106.0	0.0615
		24 mo	CC (219) + CT (124)	165 (48.1%)	1.00		-67.5	
			TT (23)	15 (65.2%)	2.35 (0.92-5.98)	0.0731	-105.4	0.0862
ARMS2	rs10490924	6 mo	TT (183) + TG (139)	189 (58.7%)	1.00		-64.6	
			GG (44)	36 (81.8%)	3.12 (1.30-7.48)	**0.0106**	-115.0	**0.0438**
		12 mo	TT (183) + TG (139)	156 (48.4%)	1.00		-62.6	
			GG (44)	28 (63.6%)	1.49 (0.72-3.07)	0.2771	-118.4	**0.0041**
		24 mo	TT (183) + TG (139)	152 (47.2%)	1.00		-62.2	
			GG (44)	28 (63.6%)	1.80 (0.88-3.70)	0.1086	-126.2	**0.0269**
HTRA1	rs11200638	6 mo	AA (188) + AG (133)	188 (58.6%)	1.00		-64.6	
			GG (45)	37 (82.2%)	3.21 (1.35-7.66)	**0.0084**	-114.2	**0.0416**
		12 mo	AA (188) + AG (133)	155 (48.3%)	1.00		-62.7	
			GG (45)	29 (64.4%)	1.63 (0.79-3.34)	0.1824	-116.7	**0.0035**
		24 mo	AA (188) + AG (133)	151 (47.0%)	1.00		-62.3	
			GG (45)	29 (64.4%)	1.93 (0.95-3.94)	0.0711	-123.7	**0.0291**
SCARB1	rs5888	6 mo	CC (201) + CT (136)	206 (61.1%)	1.00		-71.9	
			TT (29)	19 (65.5%)	1.30 (0.54-3.11)	0.5606	-56.2	0.9843
		12 mo	CC (201) + CT (136)	167 (49.6%)	1.00		-70.8	
			TT (29)	17 (58.6%)	1.43 (0.63-3.27)	0.3926	-52.5	0.7034
		24 mo	CC (201) + CT (136)	163 (48.4%)	1.00		-71.1	
			TT (29)	17 (58.6%)	1.52 (0.67-3.46)	0.3146	-55.7	0.883
PEDF	rs1136287	6 mo	CC (92) + CT (182)	169 (61.7%)	1.00		-70.9	
			TT (91)	56 (61.5%)	1.07 (0.63-1.80)	0.809	-69.0	0.9624
		12 mo	CC (92) + CT (182)	129 (47.1%)	1.00		-69.0	
			TT (91)	55 (60.4%)	1.82 (1.09-3.04)	**0.0220**	-69.2	0.9465
		24 mo	CC (92) + CT (182)	130 (47.4%)	1.00		-69.8	
			TT (91)	50 (54.9%)	1.32 (0.80-2.17)	0.2821	-69.9	0.9473
APOE	apoE	6 mo	ε2 carriers(40)+ε3/ε3(253)	178 (60.8%)	1.00		-76.0	
			ε4 carriers(66)	46 (69.7%)	1.41 (0.77-2.59)	0.2633	-47.7	0.0589
		12 mo	ε2 carriers(40)+ε3/ε3(253)	147 (50.2%)	1.00		-74.4	
			ε4 carriers(66)	36 (54.5%)	1.19 (0.68-2.10)	0.5467	-47.2	0.0705
		24 mo	ε2 carriers(40)+ε3/ε3(253)	143 (48.8%)	1.00		-71.0	
			ε4 carriers(66)	36 (54.5%)	1.25 (0.71-2.19)	0.4448	-62.5	0.783
SYN3/	rs9621532††	6 mo	TT (346)	214 (61.8%)	1.00		-71.2	
TIMP3			TG (19) + GG (1)	11 (55.0%)	0.61 (0.23-1.62)	0.3185	-62.9	0.3039
		12 mo	TT (346)	173 (50.0%)	1.00		-69.2	
			TG (19) + GG (1)	11 (55.0%)	1.03 (0.39-2.71)	0.9553	-72.3	0.9317
		24 mo	TT (346)	169 (48.8%)	1.00		-69.3	
			TG (19) + GG (1)	11 (55.0%)	1.19 (0.45-3.14)	0.7273	-80.2	0.8501

OR = Odds ratio; CI = Confidence interval; CNV = Choroidal neovascularization; DA = Disc area
*: Uncorrected *P* - value from logistic regression model
†: Uncorrected *P* - value from linear regression model
††: Analyzed under dominant model because of extremely rare minor allele homozygote

Table 9. Association of candidate genetic variants with angiographic outcome variables under recessive genetic model

Gene	Variant	Genotype	Angio-graphic Responder	OR (95% CI)	*P* - value*	Mean CNV lesion size change (DA)	*P* - value†
CFH	rs800292	GG (169) + GA (126)	208 (70.5%)	1.00		-0.2	
		AA (28)	21 (42.9%)	0.26 (0.11-0.62)	**0.0021**	+0.8	0.4201
CFH	rs1061170††	TT (257)	171 (66.5%)	1.00		0.0	
		TC (59) + CC (3)	45 (72.6%)	1.39 (0.73-2.65)	0.3177	-0.5	0.3519
CFH	rs1410996	GG (157) + GA (130)	199 (69.3%)	1.00		-0.3	
		AA (36)	21 (58.3)	0.46 (0.21-1.01)	0.0529	+0.7	0.52
CFI	rs10033900	TT (163) + TC (127)	200 (69.0%)	1.00		-0.3	
		CC (32)	19 (59.4%)	0.56 (0.26-1.23)	0.1506	+1.0	0.0454
C2	rs9332739††	GG (316)	214 (67.7%)	1.00		-0.1	
		GC (6) + CC (1)	6 (85.7%)	5.75 (0.62-53.2)	0.1235	-0.7	0.8166
CFB	rs641153††	GG (282)	194 (68.8%)	1.00		-0.2	
		GA (38) + AA (1)	26 (66.7%)	0.94 (0.44-2.00)	0.8642	+0.4	0.1862
SKIV2L	rs429608††	GG (283)	193 (68.2%)	1.00		-0.2	
		GA (34) + AA (4)	25 (65.8%)	1.11 (0.51-2.42)	0.7964	+0.1	0.3347
VEGFA	rs699947	CC (167) + CA (119)	191 (66.8%)	1.00		-0.2	
		AA (35)	27 (77.1%)	1.61 (0.69-3.78)	0.2726	+0.2	0.615
VEGFA	rs3025039	CC (193) + CT (110)	202 (66.7%)	1.00		-0.1	
		TT (20)	18 (90.0%)	4.65 (1.04-20.8)	**0.0444**	-0.7	0.5093
ARMS2	rs10490924	TT (165) + TG (125)	195 (67.2%)	1.00		-0.2	
		GG (33)	25 (75.8%)	1.74 (0.69-4.37)	0.2366	0.0	0.4972
HTRA1	rs11200638	AA (169) + AG (120)	194 (67.1%)	1.00		-0.2	
		GG (34)	26 (76.5%)	1.85 (0.74-4.62)	0.1851	0.0	0.5048
SCARB1	rs5888	CC (180) + CT (119)	201 (67.2%)	1.00		-0.2	
		TT (24)	19 (79.2%)	1.70 (0.59-4.90)	0.3269	+0.2	0.6436
PEDF	rs1136287	CC (78) + CT (157)	155 (66.0%)	1.00		0.0	
		TT (87)	65 (74.7%)	1.60 (0.90-2.86)	0.1119	-0.8	0.0543
APOE	apoE	ε2 carriers(33)+ε3/ε3(231)	181 (68.6%)	1.00		-0.1	
		ε4 carriers(54)	38 (70.4%)	0.98 (0.54-1.79)	0.9531	-0.5	0.4278
SYN3/ TIMP3	rs9621532††	TT (304)	209 (68.8%)	1.00		-0.2	
		TG (18) + GG (1)	11 (57.9%)	0.57 (0.21-1.51)	0.2566	+0.4	0.5903

OR = Odds ratio; CI = Confidence interval; CNV = Choroidal neovascularization; DA = Disc area
*: Uncorrected *P* - value from logistic regression model
†: Uncorrected *P* - value from linear regression model
††: Analyzed under dominant model because of extremely rare minor allele homozygote

Table 10. Summary of multifactor dimensionality reduction analysis for anti-vascular endothelial growth factor treatment outcome variables with the candidate genetic variants

Model		Training accuracy	Testing accuracy	CVC	*P* value
Good Visual Response					
at month 6	*CFI* rs10033900 + *VEGFA* rs3025039	0.5811	0.5595	9/10	0.1668
	CFI rs10033900 + *VEGFA* rs3025039 + *VEGFA* rs699947	0.6104	0.5205	10/10	0.5155
at month 12	*ARMS2* rs10490924 + *VEGFA* rs699947	0.5651	0.5078	9/10	0.5904
	ARMS2 rs10490924 + *VEGFA* rs699947 + *SCARB1* rs5888	0.6072	0.5359	8/10	0.3227
at month 24	*CFI* rs10033900 + *VEGFA* rs699947	0.5793	0.4839	7/10	0.8072
	CFI rs10033900 + *VEGFA* rs699947 + *SCARB1* rs5888	0.6238	0.5243	6/10	0.4525
Good Tomographic Response					
at month 6	*HTRA1* rs11200638 + *APOE*	0.5891	0.5132	4/10	0.5205
	ARMS2 rs10490924 + *PEDF* rs1136287 + *APOE*	0.6393	0.5512	7/10	0.2098
at month 12	*CFH* rs800292 + *PEDF* rs1136287	0.6016	0.6016	10/10	**0.011**
	CFH rs800292 + *PEDF* rs1136287 + *VEGFA* rs699947	0.6329	0.5392	4/10	0.2977
at month 24	*HTRA1* rs11200638 + *PEDF* rs1136287	0.584	0.5294	6/10	0.4226
	CFH rs800292 + *CFI* rs10033900 + *ARMS2* rs10490924	0.6199	0.5142	5/10	0.5724
Good Angiographic response					
at month 24	*CFH* rs800292 + *CFH* rs1410996	0.5629	0.4901	6/10	0.7263
	CFH rs800292 + *CFH* rs1410996 + *SCARB1* rs5888	0.5799	0.4659	4/10	0.8771

CVC = Cross-validation consistency

Table 11. Summary of previous studies on pharmacogenetics of anti-vascular endothelial growth factor agent for neovascular age-related macular degeneration treatment

Study	Year	Ethnicity	N	Design	Previous neovascular AMD Treatment	Anti-VEGF agent	Protocol	Assess time point	Primary outcome measure	VA measure	Candidate genes						Significant association
											No of variants	CFH	ARMS2	HTRA1	VEGFA	Others	
Brantley et al (37)	2007	Caucasian	86	Retrospective	Excluded	BVC	1+prn	Final FU (≥ 6 mo)	Δ BCVA	Snellen	2	+	+			-	*CFH* Y402H
Teper et al (40)	2010	Caucasian	90	Prospective	Included	RNB	3+prn	12 mo	ΔBCVA, ΔCFT	ETDRS	2	+	+			-	*ARMS2* rs10490924
Imai et al (42)	2010	Asian	83	Retrospective	unknown	BVC	1+prn	1, 3, 6 mo	Δ BCVA > 0	logMAR	11	+		+	+	*PEDF*	*VEGF* rs699947 *PEDF* rs1136287
Lee et al (43)	2010	Caucasian	156	Retrospective	Excluded	RNB	1+prn	6, 9 mo	Δ BCVA, Injection No.	logMAR	1	+				-	none
McKibbin et al (39)	2011	Caucasian	104	unknown	unknown	RNB	3+prn	6 mo	Δ BCVA ≥ 6	ETDRS	3	+		+	+	-	*CFH* Y402H *ARMS2* rs10490924 *VEGFA* rs1413711
Kloeckener-Gruissem et al (38)*	2011	Caucasian	243	unknown	Included	RNB	1+prn	12 mo	Δ BCVA	ETDRS	8	+	+	+	+	*CFB, FZE5, LRP5, KDR*	*CFH* Y402H
Nischer et al (44)	2011	Caucasian	197	Prospective	unknown	BVC	1+prn	Final FU (≥ 6 mo)	Δ BCVA	logMAR	1	+				-	*CFH* Y402H
Orlin et al (45)	2011	Caucasian	150	Retrospective	PDT, laser excluded	RNB + BVC	3+prn	Final FU (≥ 3 mo)	Δ BCVA ≥ 0	logMAR	7	+	+	+		-	none
Wickremasinghe et al (46)	2011	Caucasian	168	Retrospective	Excluded	RNB + BVC	1+prn	3, 6, 12 mo	Δ BCVA ≥ +2 line	logMAR	1					*APOE*	*APOE*
Nakata et al (63)	2011	Asian	94	Retrospective	Surgery excluded	BVC	3+prn	12 mo	Δ BCVA	logMAR	4				+		*VEGF* rs699946
Francis et al (64)	2011	Caucasian	65	Prospective	Excluded	RNB	2+prn	6, 12 mo	ΔBCVA	ETDRS	~550 K	+	+	+	+	SNP chip	Referece
Wang et al (65)	2012	Caucasian	106	unknown	Anti-VEGF in 20%	RNB + BVC	1+prn	12 mo	ΔBCVA ≥ -15 + no fluid on OCT	ETDRS	21				+	*PLA2G12A, IL-23R, STAT3, KDR, HIF-1A*	*PLA2G12A* rs2285714
Smailhodzic et al (53)	2012	Caucasian	420	Pro+Retrospective	unknown	RNB	3 inj	3 mo	ΔBCVA	logMAR	8	+	+		+	*KDR, FZD4, LRO5*	*CFH* Y402H *ARMS2* rs10490924 *VEGFA* rs699947
Menghini et al (56)	2012	Caucasian	204	Retrospective	No Tx other than RNB	RNB	1+prn	12, 24 mo	ΔBCVA ≥ +5	ETDRS	1	+				-	*CFH* Y402H

Boltz et al (48)	2012	Caucasian	185	Prospective	No Tx within 6mo	BVC	1 inj	various	Final BCVA	Decimal	7				+	-	none
Yamashiro et al (61)	2012	Asian	78	Retrospective	unknown	RNB	3+prn	3, 12 mo	ΔBCVA	logMAR	3	+	+				none
Park et al (66)	2012	Asian	51	Retrospective	Excluded	PDT+ BVC	every 3 mo	12 mo	Final BCVA, GLD	logMAR	2		+	+		-	*ARMS2* rs10490924 *HTRA1* rs11200638
Tian et al (54)	2012	Asian	144	Prospective	Excluded	BVC	2 inj	3 mo	ΔBCVA, ΔCFT	ETDRS	12	+	+	+	+	*SERPING1, C3*	CFH rs800292 *ARMS2* rs10490924 *HTRA1* rs11200638
Kang et al (67)	2012	Asian	75	Retrospective	No Tx within 6mo	BVC	3+prn	6, 12 mo	ΔBCVA, ΔCFT	logMAR	3	+	+	+		-	*ARMS2* rs10490924
Chang et al (68)	2013	Asian	102	Retrospective	Excluded	RNB	3+prn	3, 6 mo	ΔBCVA, ΔCFT	logMAR	5	+	+	+	+	*KDR*	*VEGF* rs833069 with CFT
Hautamaki et al (69)	2013	Caucasian	96	Retrospective	Excluded	BVC	3 inj	3.5 mo	OCT criteria	ETDRS	7	+	+		+	*C3, EPO, IL8*	*IL8* rs4073
CATT study group (49)*	2013	Caucasian	834	RCT	Excluded	RNB + BVC	Monthly or 1+prn	12 mo	ΔBCVA	ETDRS	4	+	+	+		*C3*	none
Abedi et al (47)*	2013	Caucasian	201	Prospective	Excluded	RNB + BVC	3+prn	12 mo	ΔBCVA	ETDRS	7				+	-	*VEGF* rs3025000
Abedi et al (41)*	2013	Caucasian	224	Prospective	Excluded	RNB + BVC	3+prn	12 mo	ΔBCVA	ETDRS	17	+	+	+		*CFHR1-5, C2,C3,CFB, F13B*	*ARMS2* rs10490924 *HTRA1* rs11200638
Park et al (57)*	2013	Asian	273	Prospective	Excluded	RNB	3+prn	5 mo	ΔBCVA ≥ +8	ETDRS	23	+	+	+	+	*C2, CFB, CFI, SKIV2L, APOE, PEDF, SCARB1, SYN3/TIMP3*	None
Kitchens et al (70)	2013	Caucasian	101	Retrospective	Excluded	RNB + BVC	3+prn	9 mo	OCT criteria ΔBCVA ≥ 3 lines	Snellen	10	+	+		+	-	*ARMS2* rs10490924 with OCT criteria

AMD = age-related macular degeneration; VEGF = vascular endothelial growth factor; VA = Visual acuity; BCVA = Best-corrected visual acuity; FU = follow-up; BVC = Bevacizumab; RNB = Ranibizumab; PDT = Photodynamic therapy; ETDRS = Early treatment diabetic retinopathy study; logMAR = logarithm of Minimum Angle of Resolution; CFT = central foveal thickness; SNP = single nucleotide polymorphism; OCT = optical coherence tomography; GLD = greatest linear dimension
*: Correction for multiple testing was performed

Table 12. Results of meta-analysis for the association between rs1061170, rs10490924, rs11200638 polymorphisms and visual acuity change after for anti-vascular endothelial growth factor treatment for neovascular age-related macular degeneration

	Model (No. of patients)			Number of included study	Difference in mean BCVA change*	95% CI	Significance (Z test)		Heterogeneity (Q test)		
							Z	*P* - value	Q	*P* - value	I^2 (%)
***CFH* rs1061170**	CC (203)	vs	TT+TC (848)	5	2.181	- 0.223 to 4.585	1.778**	0.075	3.582	0.465	0
	CC (203)	vs	TT (466)	5	2.056	- 2.275 to 6.386	0.930†	0.352	7.981	**0.092**	49.9
***ARMS2* rs10490924**	GG (160)	vs	TT+TG (577)	4	- 3.604	- 5.935 to - 1.274	- 3.031**	**0.002**	0.415	0.937	0
	GG (301)	vs	TT (390)	5	- 4.327	- 6.527 to - 2.126	- 3.853**	**< 0.001**	4.611	0.330	13.3
***HTRA1* rs11200638**	GG (143)	vs	AA+AG (537)	3	- 1.286	- 5.010 to 2.438	- 0.677†	0.498	4.687	**0.096**	57.3
	GG (143)	vs	AA (257)	3	- 4.669	- 8.390 to - 0.948	- 2.460**	**0.014**	4.099	0.129	51.2

BCVA = Best-corrected visual acuity; CI = Confidence interval

*: Negative value implies better visual outcome in minor allele homozygotes (CC for rs1061170, GG for both rs10490924 and rs11200638)

†: Random-effect model was used

**: Fixed-effect model was used

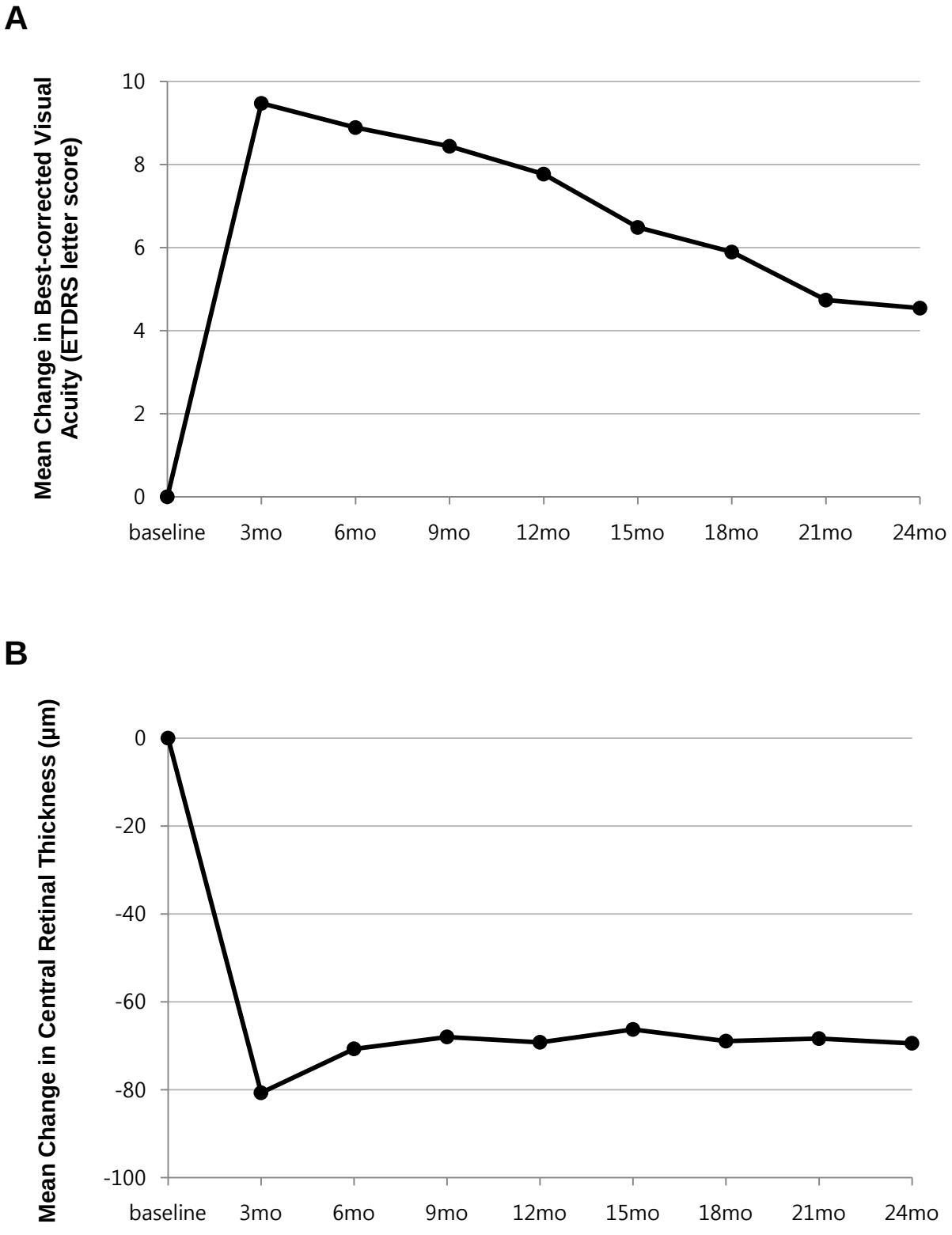

Figure 1. Treatment outcome during 24-month follow-up. (A) Mean change in visual acuity. (B) Mean change in central retinal thickness.

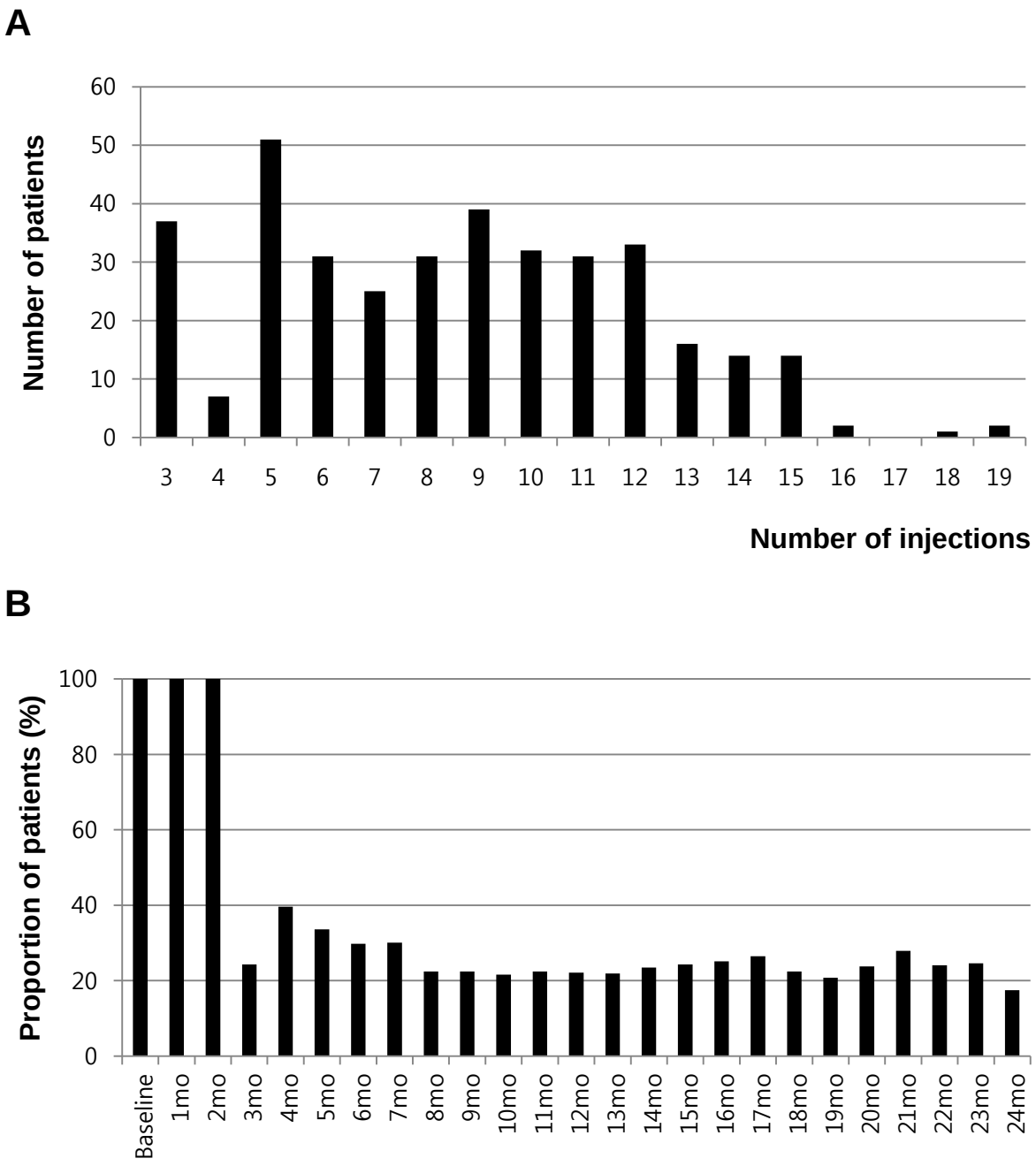

Figure 2. The anti-vascular endothelial growth factor (VEGF) injection pattern of the present study cohort. (A) Distribution of patients according to the number of anti-VEGF injections during 24 months. (B) Proportion of patients who received anti-VEGF treatment at each monthly follow-up visit.

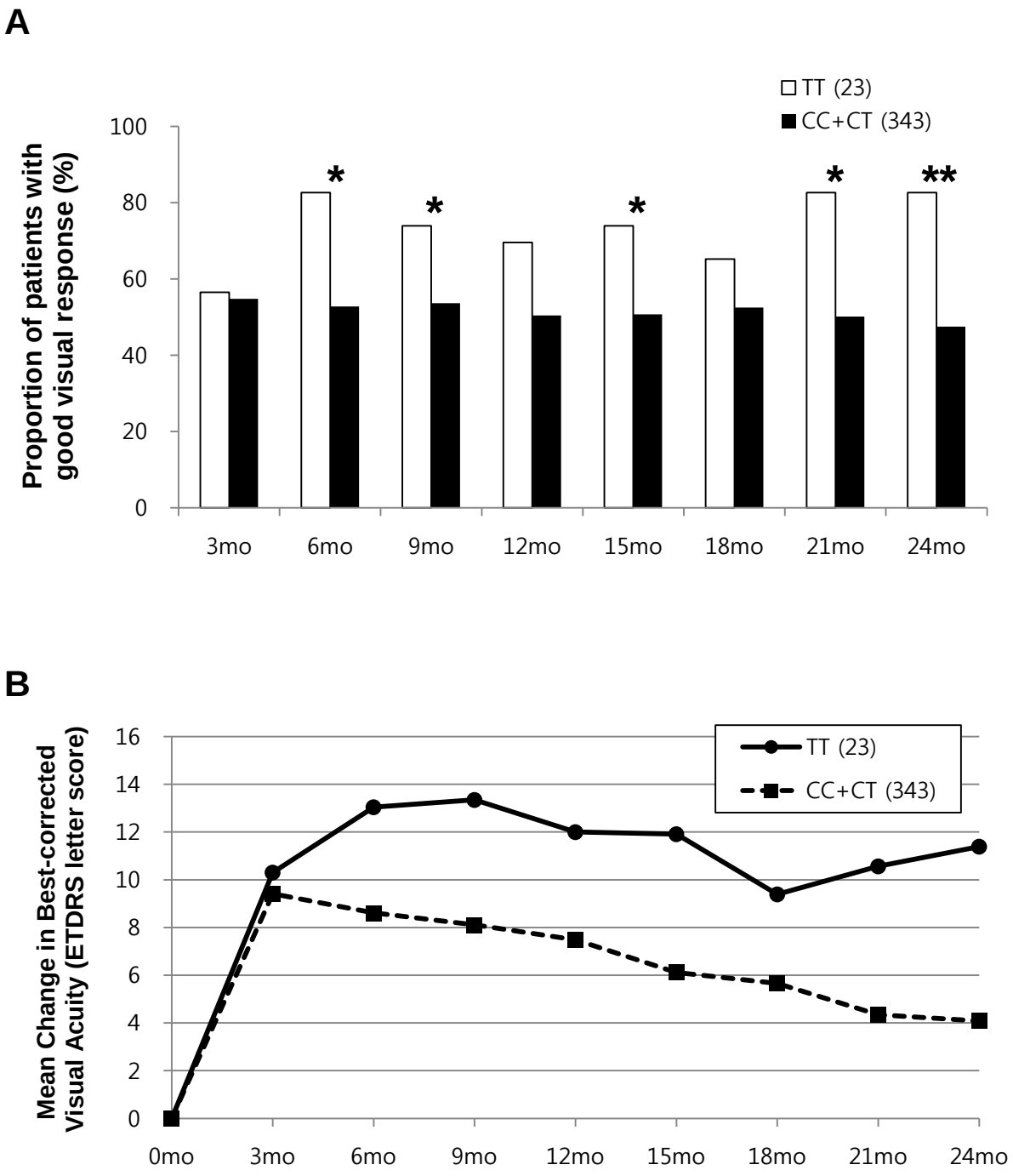

Figure 3. Visual outcome during 24-month follow-up according to the *VEGFA* gene rs3025039 genotype under recessive genetic model. (A) The proportion of patients who showed good visual response. Significant differences are indicated with a single asterisk (uncorrected $P < 0.05$) or a double asterisk (corrected $P < 0.05$). (B) Mean change in visual acuity. Significant difference (uncorrected $P < 0.05$) was not found at any time points.

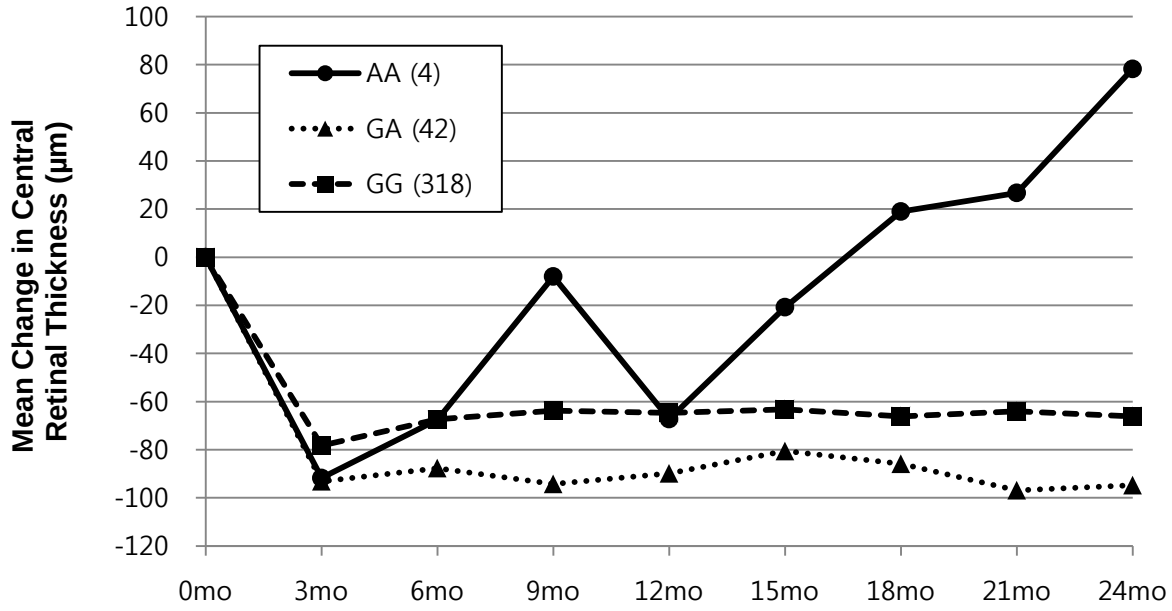

Figure 4. Mean change in central retinal thickness during 24-month follow-up according to the *SKIV2L* gene rs429608 genotype.

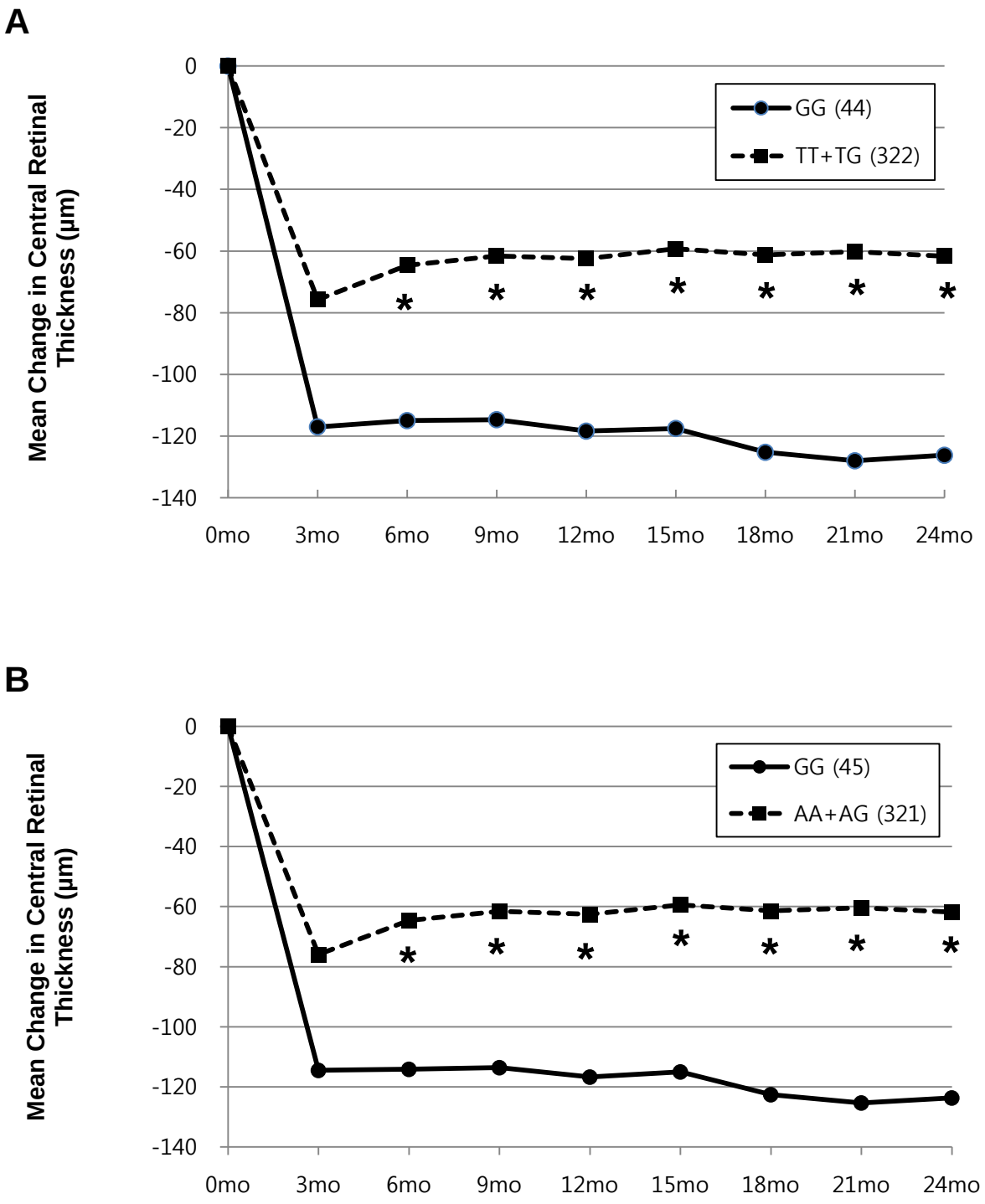

Figure 5. Mean change in central retinal thickness according to the genotype of 10q26 locus polymorphisms under recessive genetic model. Significant differences (uncorrected $P < 0.05$) are indicated with an asterisk. (A) *ARMS2* gene rs10490924. (B) *HTRA1* gene rs11206038.

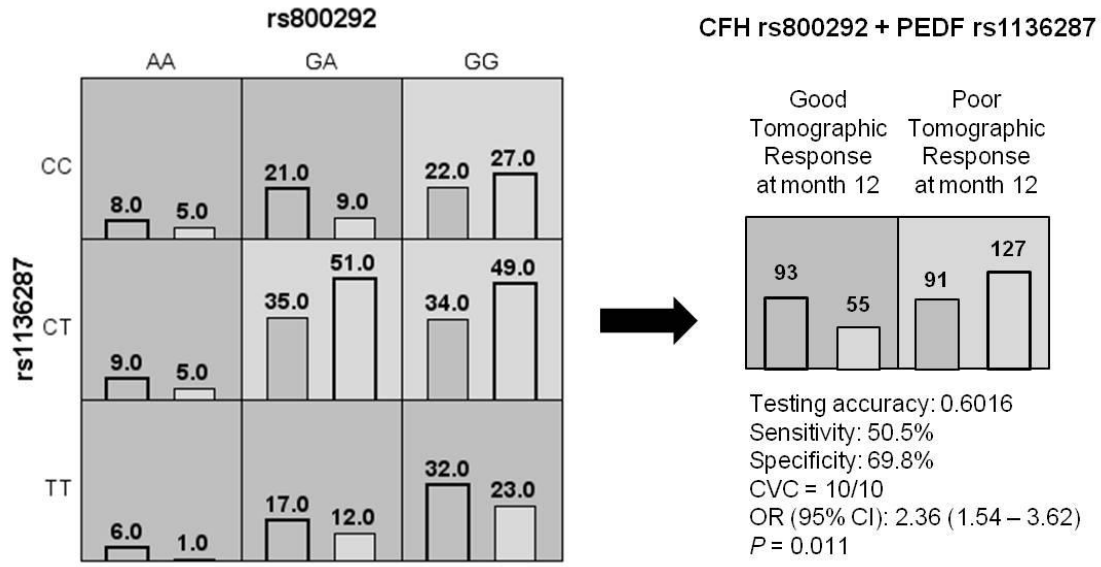

Figure 6. Distribution of tomographic responders and non-responders at month 12 for each genotype combination from *CFH* rs800292 and *PEDF* rs1136287. For each cell, left bar represents responders and right bar represents non-responders. Genotype combinations with high probability for good response are shaded gray. The new variable constructed by multifactor dimensionality reduction method is shown on the right.

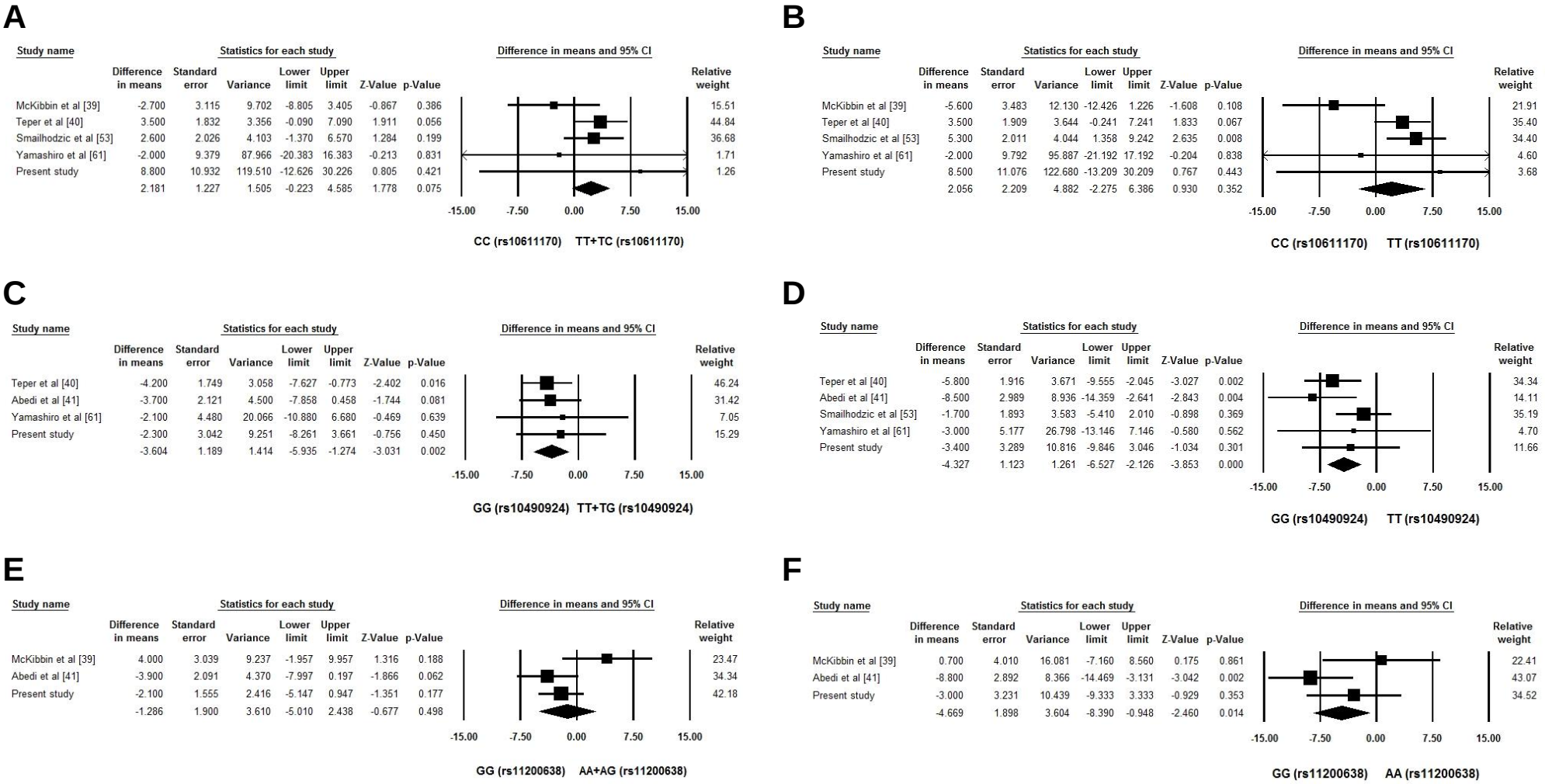

Figure 7. Forest plots of meta-analyses for the association between mean change in visual acuity and three major polymorphisms. Meta-analyses were performed under recessive genetic model or between the homozygotes for major and minor allele for *CFH* rs1061170 (A, B), *ARMS2* rs10490924 (C, D), and *HTRA1* rs11200638 (E, F).

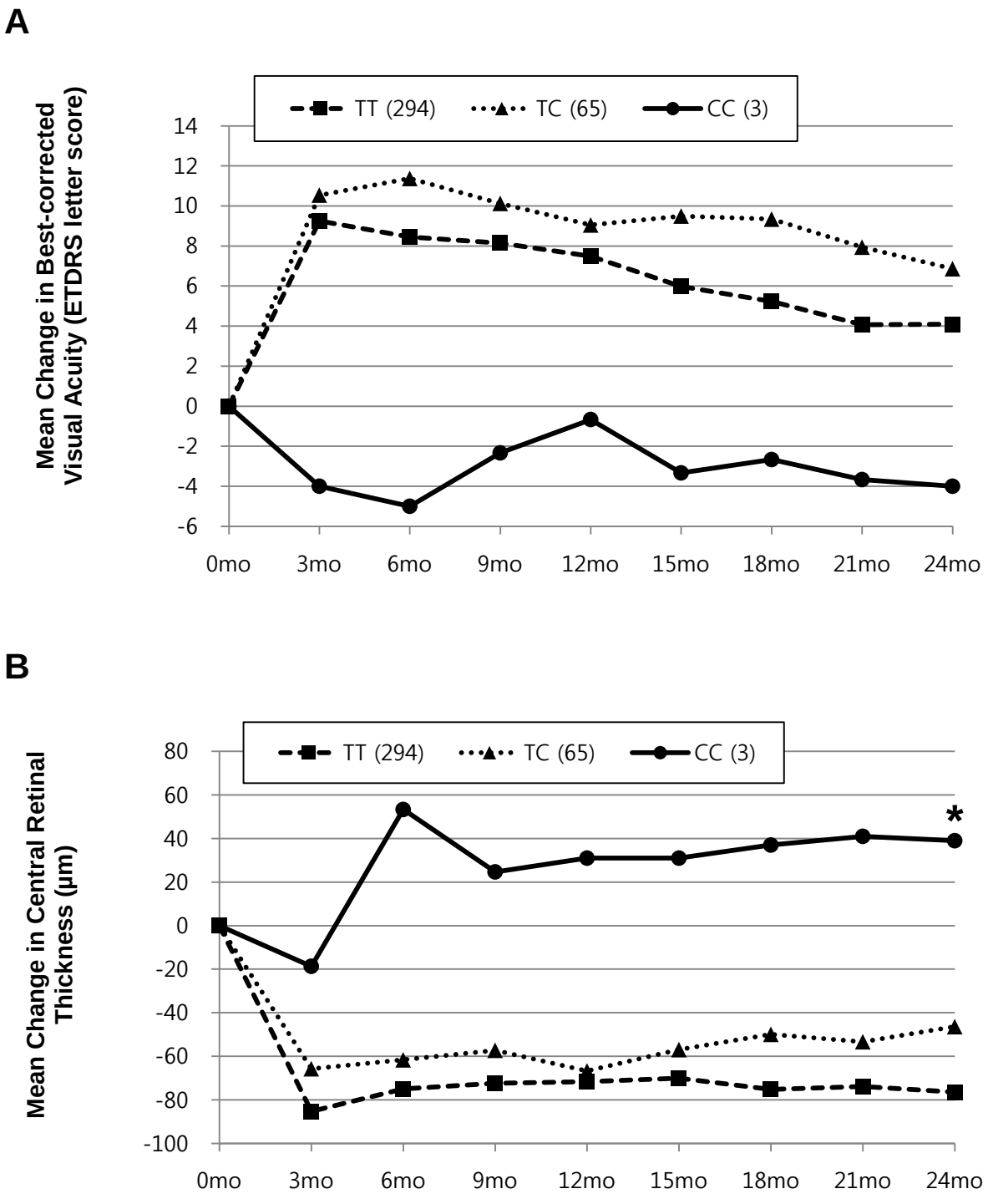

Figure 8. Treatment outcome during 24-month follow-up according to the *CFH* gene rs1061170 genotypes. (A) Mean change in visual acuity. Under dominant model (TT versus TC + CC), significant difference (uncorrected $P < 0.05$) was not found at any time points. (B) Mean change in central retinal thickness. Significant difference (uncorrected $P < 0.05$) under dominant model is indicated with an asterisk.

DISCUSSION

This is the first large-scale, long-term pharmacogenetic study on anti-VEGF treatment for neovascular AMD in East Asians. In the cohort consisted of 366 Korean neovascular AMD patients, we found genetic biomarkers associated with the outcome variables after 2-year anti-VEGF treatment with as-needed protocol. We evaluated the treatment outcome multilaterally from visual, tomographic, and angiographic aspects. The prospective design with a standardized treatment protocol for treatment-naïve patients minimized the possible bias from variation in the treatment regimen. Detailed criterion for good visual response was used to prevent the ceiling or flooring effect, and genetic heterogeneity was minimized by forming the study cohort only with unrelated Koreans.

In addition, this study confirmed the long-term therapeutic effect of anti-VEGF treatment in Korean neovascular AMD cohort. Mean visual acuity gain of 4.5 letters at month 24 is comparable to the two-year results from two recent randomized clinical trials of anti-VEGF for neovascular AMD treatment, which were 5.87 and 3.5 letters for as-needed regimen groups.(36, 71) In Asian population, a retrospective study reported visual improvement of 0.14 logMAR unit (equivalent to 7 ETDRS letter) at 2 years after as-needed treatment with ranibizumab, but its sample size was small.(72)

In genotype analysis, *C3* rs2230199 showed no variant and was excluded from the pharmacogenetic analysis. Although the association between rs2230199 and AMD has been reported in many studies of western populations, its variation was extremely rare or absent in East Asian studies.(15, 18, 19, 73, 74) In the Hapmap data, minor allele frequency (MAF) of rs2230199 was 0 in Japanese in Tokyo or Chinese in Beijing, while 0.192 in Europeans (http://hapmap.ncbi.nlm.nih.gov/biomart).

Deviation from HWE was observed in two polymorphisms in 10q26 locus, but this was not thought to result from common causes of deviation such as genotyping error, selection bias, or population

stratification. All of subjects in this study are neovascular AMD patients and the deviation might indicate the genetic association between the locus and the disease.(75) For this reason, testing for HWE for each study included in meta-analysis was not performed.

In this study, injection frequency was not included in analyses as an outcome measure. Number of injection is thought to be less representative of the treatment responsiveness than other parameters, because retreatment was determined at the clinician's discretion on the basis of visual acuity change and OCT finding. In addition, anti-VEGF treatment was discontinued in some patients who developed fovea-involving neurosensory retinal atrophy or subretinal fibrosis and was thought to have no more therapeutic effect with anti-VEGF treatment. These patients would show poor treatment results either for categorical or continuous outcome variables with fewer injections, while there are some patients who maintain relatively good visual acuity and dry macula with frequent injections.

The major weakness of this study is that the influence of patients who failed to follow-up till month 24 was not evaluated. Because patients who experienced poor response to anti-VEGF treatment might be more likely to stop their follow-up visits compared to good responders, final cohort of this study would represent relatively better performance compared to initial cohort.

Another limitation is that we could not evaluate the intraocular or systemic expression or transcription level of each candidate polymorphism and its relevance to anti-VEGF responsiveness. Single nucleotide polymorphism and resultant amino acid change often alters the expression level or activity of the target protein. Instead, functional relevance to AMD development or anti-VEGF response is discussed in the following sections for major candidate polymorphisms. Among the rest candidate polymorphisms, rs1410996, rs429608, and rs9621532 are located in the intron region and are less likely to be functional. A synonymous variant *SCARB1* gene rs5888 has been reported to be associated with SR-BI protein expression level or altered serum lipid level, but its functional association with AMD development or anti-VEGF response is unknown.(76, 77) As for *APOE* gene,

ε4 allele was reported to be protective against AMD, and susceptibility to AMD seems to result from different binding affinity of three isoforms of APOE to the low-density lipoprotein (LDL) receptor or other LDL receptor-related proteins.(78-81) APOE haplotypes are also related to VEGF expression. *APOE* ε4 allele showed suppression of VEGF expression in cultured human RPE, while transgenic mice expressing human *APOE* ε2 showed overexpression of VEGF and basic fibroblast growth factor suggesting contribution of ε2 allele to neovascularization by altering angiogenic cytokines.(82, 83) However, *APOE* genotype showed no association with anti-VEGF response in this cohort.

1. Pharmacogenetics of exudative AMD

Since the association between genetic variants and AMD has been verified, investigators have attempted to determine the role of an individual's genetic background in the response to AMD treatment. In a recent study, benefit of antioxidants or zinc supplementation which was reported to decrease progression to advanced AMD was different according to the presence of *CFH* or *ARMS2* risk allele in moderate AMD patients.(84) Influence of genetic variants has been also investigated in PDT, although there remains controversy. As for *CFH* Y402H, Brantley et al (85) reported a worse visual outcome in PDT-treated patients with *CFH* Y402H TT genotype than other genotypes, whereas other studies reported that the proportion of PDT responder was comparable among the genotypes.(86, 87) For *VEGF* gene, Immonen et al (62) reported that two SNPs (rs699947, rs2146323) were strong determinants of the anatomic outcomes after PDT. However, Tsuchihashi et al (87) denied an association between the response to PDT and three SNPs in *VEGF* gene including rs699947.

2. Complement factor H (CFH) Y402H variant and anti-VEGF response

The CFH gene encodes a protein acting as a regulator of the alternative pathway activation in the complement cascade. Y402H (rs1061170) polymorphism, which is located in the short consensus

repeat 7, results in an amino acid substitution of histidine for tyrosine in a particular domain of factor H that contains binding sites for C-reactive protein (CRP), heparin, and streptococcal M6 protein.(88) Risk variant *CFH* 402H was reported to have reduced binding properties to CRP, heparin, and RPE cells, suggesting abnormal or inefficient complement regulation at the cell surface.(89, 90) Human donor eyes homozygous for C allele (402HH) showed higher levels of CRP in the RPE-choroid compared to 402YY but without significant differences in the level or expression of CFH.(91) In addition, recent study showed that systemic level of pro-inflammatory cytokines such as tumor necrosis α or interleukin-6 is higher in CC genotype AMD patients compared to TT genotype.(92) In *CFH* risk allele carrier, chronic inflammation state resulted from attenuated inhibitory regulation of CFH may contribute to AMD pathogenesis. On the other hand, biologic effect of anti-VEGF agent in neovascular AMD does not seem to be mediated by inflammatory change in local retinal environment. In animal model, systemic anti-VEGF significantly reduced the expression and level of inflammatory cytokine and local inflammation.(93, 94) However, inflammatory cytokine level in aqueous humor did not change significantly after intravitreal anti-VEGF injection in patients with neovascular AMD or diabetic macular edema due to branch retinal vein occlusion.(95, 96)

Although some studies in the Asian population found no association of *CFH* Y402H with AMD, this could be attributable to the lower MAF in Asians compared to Caucasian populations.(21, 22) The C allele frequency is 0.067 in Han Chinese in Beijing and 0.057 in Japanese in Tokyo, while 0.282 in Europeans (http://hapmap.ncbi.nlm.nih.gov/biomart). In this cohort, MAF for Y402H variant was 0.098. Recently, meta-analyses of Asian studies found substantial evidence for an association between the Y402H variant and AMD.(97, 98)

With widespread use of anti-VEGF agents for neovascular AMD, the pharmacogenetic association of anti-VEGF outcome has been increasingly investigated. *CFH* Y402H (rs1061170) was one of the most frequently investigated polymorphism as shown in Table 11, but the findings have been

inconsistent among the studies. In some reports, patients homozygous for the risk allele (CC genotype) showed worse visual outcomes compared to the CT or TT genotypes after ranibizumab or bevacizumab treatment.(37, 38, 44, 56) When combined with other associated genetic variants, carrying risk allele of CFH Y402H raised the probability for poor visual outcome.(53) In contrast, other studies reported no significant influence of *CFH* Y402H genotype on anti-VEGF treatment outcome.(39-41, 43, 45, 49, 57, 68, 69) Because MAF of *CFH* Y402H is less than 0.1 In East Asian populations, subjects with CC genotype are extremely rare compared to Caucasians. Imai et al (42) reported that the CT genotype of rs1061170 was more frequently presented with visual worsening than TT genotype while there was no CC genotype patient among the 83 patients that received bevacizumab for neovascular AMD.

In our Korean cohort, only three patients (0.8%) had CC genotype and they showed poor treatment outcome. There was no significant association under recessive genetic model. One patient showed a severe visual loss from 44 letters at baseline to hand motion due to development of massive subretinal hemorrhage at month 5. Other two patients with CC genotype experienced visual improvement of 19 to 43 and 27 to 33 letters respectively, but tomographic findings were not good showing persistent subretinal fluid both at month 12 and 24. TC and TT genotype showed comparable changes in BCVA and CRT during 24-month follow-up, but further evaluation under dominant model revealed the significant association in CRT change only at month 24 (TT genotype -77.1 μm vs. TC+CC genotype -42.6 μm, nominal P = 0.0428; Figure 8). In a recent pharmacogenetic study with 102 Korean neovascular AMD patients treated with ranibizumab, 2 patients (2.0%) had the CC genotype and their mean visual acuity change at 3 and 6 months were also much worse than other genotypes, but it was not significant.(68)

3. Other CFH gene variants and genes related to complement pathway

Other common *CFH* variants besides Y402H have been reported to be significantly associated with AMD including rs800292, rs1410996.(8, 74, 99) Among these, significant pharmacogenetic association with rs800292 was reported in a recent Chinese study.(54) Patients homozygous for minor allele showed larger amount of visual improvement compared to other genotypes. Also in the present study, rs800292 minor allele homozygotes (AA genotype) showed better visual and tomographic performance under recessive model both at month 12 and 24, but did not reach statistical significance (Table 7 and 8). However, AA genotype had significantly lower chance of good angiographic response compared to other genotypes. Although valid explanation for this discrepancy may be elusive, rs800292 is thought to affect the anti-VEGF response in any way.

Other than complement factor H, complement genes such as factor B, factor I, component 2, and 3 have been reported to be associated with AMD.(16-20, 28) However, no pharmacogenetic association of these polymorphisms with anti-VEGF treatment in neovascular AMD has been reported. In this study, rs429608 in *SKIV2L* gene, which is located adjacent to *C2* and *CFB* and reported to be the strongest AMD-associated variant across *C2-CFB-RDBP-SKIV2L* region, showed significant difference in CRT change at month 24 under recessive model. However, number of AA genotype patients was too small and it seems that more patients are necessary to verify the influence of minor allele homozygosity of rs429608. Furthermore, longitudinal evaluation revealed that the increase in mean CRT compared to other genotypes was after month 15, and it was attributable to a patient who developed massive subretinal hemorrhage.

4. 10q26 locus variants and anti-VEGF response

Meta-analysis for six genome wide scans revealed that the strongest evidence for AMD susceptibility was found on 10q26 locus, which contains the genes *ARMS2* (formerly known as LOC387715) and *HTRA1*.(100) Genetic variants within this region are reported to be in strong LD,

and *ARMS2* rs10490924 and *HTRA1* rs11200638 in the present study also showed similar genetic distribution and treatment response reflecting high correlation between them (r^2 value = 0.949, D′ = 0.987).(101, 102) Non-synonymous polymorphism rs10490924 is located in the exon1 of *ARMS2* and rs11200638 is in the promoter region of *HTRA1*. Although 10q26 locus variants demonstrated strong evidence of genetic association with AMD, their biologic relevance to AMD pathogenesis is still unclear, and likewise for the responsiveness of anti-VEGF for neovascular AMD.(103, 104) ARMS2 is poorly characterized protein, and there are ongoing debates on its cellular localization and expression.(102, 105, 106) HTRA1 is a member of the high temperature requirement A family of serine protease and is thought to have nonspecific protease activity. Recently, human recombinant HTRA1 was reported to cleave RPE-secreted proteins involved in the regulation of complement cascade or amyloid deposition, suggesting a role of HTRA1 in AMD pathogenesis.(105) However, there have been inconsistent results on the level of *HTRA1* expression according to its promoter polymorphism rs11200638 genotype. Initial study showed that the allele A was associated with elevated expression levels of HTRA1 mRNA and protein in lymphocytes or RPE from AMD patients.(103) This correlation was replicated in AMD donor eyes, placental tissues, and in cultured human RPE cells.(105, 107, 108) In contrast, several studies have shown that rs11200638 or other AMD susceptibility variants at 10q26 did not significantly alter the *ARMS2* and *HTRA1* expression level in the human retina or various cell lines.(104, 109-111)

The genes at 10q26 locus also have shown controversial results for the association with responsiveness to anti-VEGF treatment for neovascular AMD. In some studies, risk allele homozygote (TT genotype) at *ARMS2* A69S (rs10490924) was significantly associated with worse visual outcome after anti-VEGF.(40, 41, 53, 54) Risk allele homozygote (AA) at *HTRA1* rs11200638, which is in high LD with ARMS2 A69S in different populations, was also associated with poor visual outcome.(41, 54) After combined PDT and intravitreal bevacizumab for PCV treatment, these

genotypes also showed poorer outcome in vision and greatest linear dimension than other genotypes.(66) However, other reports denied genetic association of 10q26 variants with response to anti-VEGF.(37, 38, 42, 45, 49, 57, 68, 69) In this study, two 10q26 variants showed possible association with treatment response. Under recessive model, minor allele homozygotes of both polymorphisms showed significantly larger CRT reduction throughout the follow-up except month 3 (nominal $P < 0.05$). Visual improvements were also larger in minor allele homozygotes, although insignificant. The function of *ARMS2* and *HTRA1* genes in human retina and their role in the AMD pathogenesis are still vague, and their influence on anti-VEGF responsiveness is also need to be elucidated.

5. *VEGFA* gene and anti-VEGF response

The association of *VEGFA* gene, which acts as a regulator of angiogenesis, with AMD development is not well established as *CFH* gene or 10q26 locus, but a number of studies supports its contribution to AMD susceptibility.(17, 31, 112) Pharmacogenetic association of anti-VEGF agent in neovascular AMD has been reported in several polymorphisms in *VEGFA* gene. Recently, Abedi et al (47) evaluated 7 tagged SNP within *VEGFA* gene and found that patients who carry T allele of rs3025000 (TT or TC genotype) had a higher chance of gaining ≥ 5 letters after anti-VEGF treatment. In another prospective study, GG genotype of rs3024997 and rs2010963 were associated with poorer visual outcome after bevacizumab in univariate analysis, but were not significant in multivariate analysis.(48) Carrying risk allele of VEGFA rs699947, along with those of CFH Y402H or ARMS2 rs10490924, was associated with poor visual response.(53)

In this study, we identified rs3025039 as predictive for visual response after anti-VEGF treatment in neovascular AMD. Although significant association with good visual response was found only at month 24, longitudinal changes in visual outcome variables during 24-month follow-up and higher

chance of angiographic response in TT genotype indicate its relevance to treatment outcome. *VEGFA* gene rs3025039 (936C>T) in the 3´-UTR led to the loss of potential binding site for transcription factor AP-4 and seems to inhibit the transcription of VEGF.(113) Serum or plasma level of VEGF was reported to be lower in carriers of T allele compared to non-carriers, and higher therapeutic effect of anti-VEGF agent in TT genotype patients in this cohort might be attributable to their inherently lower level of VEGF production.(113, 114) Carrying the C allele of rs699947, which is located in the promoter, was also reported to be associated with higher VEGF production.(115)

In East Asian populations, significant pharmacogenetic association was found largely in *VEGFA* gene. Imai et al reported rs699947 was associated with good visual outcome at 1 month after single bevacizumab injection.(42) Chang et al (68) reported that carrying risk allele of rs833069 (GG, GA genotype) was associated with greater amount of macular thickness decrease after ranibizumab treatment. In previous report which included part of this Korean cohort, we found no genetic association with early response to ranibizumab in neovascular AMD but AA genotype of rs699947 showed significantly higher chance of good visual response at month 5 when uncorrected for multiple testing.(57) However, other studies found no significant pharmacogenetic association of anti-VEGF agent with *VEGFA* gene polymorphisms including rs699947.(38, 65, 69)

In this long-term pharmacogenetic study, *VEGFA* rs699947 did not show any relevance to treatment outcome measures at month 6, 12, and 24. We used more detailed criteria for visual response in combination of visual change from baseline plus final vision, instead of vision improvement of ≥ 8 letters which was the median improvement from baseline as in the previous report. Using the amount of visual acuity change as the only criteria for response has the chance of bias such as ceiling or flooring effect, because patients with good baseline BCVA has little room for improvement whereas those with poor baseline BCVA can only improve. Patients with and without good visual response at month 24 according to this detailed criteria showed comparable baseline BCVA, which means that

chances of ceiling or flooring effect have been minimized.

6. Gene-Gene interaction analysis

For common complex diseases, it is difficult to obtain clear causal genetic relation because they are likely to be the result of interaction among many genetic and environmental factors. Epistasis, or gene-gene interaction, is increasingly assumed to play a crucial role in the genetic architecture of common diseases and its complexity underlying drug response.(116, 117) MDR was developed as a nonparametric and genetic model-free alternative to logistic regression and is useful for detection, characterization, and interpretation of epistasis in the absence of significant effects in genetic and epidemiologic studies of complex traits such as disease susceptibility.(118, 119)

In this study, MDR analysis was performed to identify potential gene-gene interaction between the candidate polymorphisms. Epistasis between *CFH* rs800292 and *PEDF* rs1136287 showed significant association with tomographic response at month 12. Although rs1136287 did not show significant association in regression analysis when corrected for multiple testing, minor allele homozygotes (TT genotype) showed higher chance of good tomographic response at month 12 compared to other genotypes (OR, 1,82; 95% CI, 1.09 - 3.04; nominal $P = 0.022$, corrected $P = 0.352$). Considering the counteracting effect of pigment epithelium-derived factor (PEDF) against the angiogenic potential of VEGF, epistatic result suggests that *PEDF* gene variant might also have certain role in the anti-VEGF response.(120) Plasma level of PEDF was reported to be associated with *PEDF* gene polymorphism.(121, 122) TT genotype of rs1136287 showed higher PEDF plasma level compared with CC genotype, and it is likely that pharmacologic effect of anti-VEGF agent could be boosted in patients with TT genotype.

7. Meta-analysis for anti-VEGF pharmacogenetic studies

In the most previous pharmacogenetic studies on anti-VEGF treatment in neovascular AMD, patients were treated with similar protocol of one to three monthly injections followed by as-needed treatment. However, primary outcome measures for responsiveness were variable. Some studies evaluated change in visual acuity from baseline as a continuous variable, while others used categorical criteria for response based on visual acuity change but the cutoff values ranged widely from loss of fewer than 15 letters to gain of 15 or more letters.(65, 70) A recent meta-analysis of pharmacogenetic study for neovascular AMD reported that *CFH* rs1061170 is a predictor of anti-VEGF treatment response.(123) However, the definitions of good response used to calculate the pooled OR were very different among the included studies.

The visual acuity change in ETDRS letter score was pooled in this meta-analysis to minimize the heterogeneity resulted from various response criteria. Pooled visual acuity data showed that *ARMS2* rs10490924 or *HTRA1* rs11200638 homozygotes gained more letters compared to other genotypes, but *CFH* rs1061170 showed no significant association. In this meta-analysis, some relevant studies were excluded because of the limited raw data of visual acuity changes, and some analyses showed significant heterogeneity. Furthermore, genetic influence on anti-VEGF responsiveness in Asian population is hard to evaluate, because only one Asian study was included. However, results of this meta-analysis suggest the influence of 10q26 locus polymorphisms on anti-VEGF responsiveness. With more faithful data or consistent criteria for good treatment response, meta-analysis could give more robust and reliable results on pharmacogenetic association.

Conclusion

This study showed genetic factors associated with long-term outcome after anti-VEGF treatment for neovascular AMD in Korean subjects. Treatment response was evaluated from visual, tomographic, and angiographic aspects and polymorphisms in *VEGFA*, *ARMS2*/*HTRA1*, and *CFH* genes showed

relevance to long-term treatment outcome. In addition, gene-gene interaction affecting the tomographic response was found between *PEDF* and *CFH* gene. With further supporting evidences, genetic background of a patient could be used as data for individualized medicine for neovascular AMD to achieve optimal response with anti-VEGF treatment.

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국문 초록

한국인 신생혈관성 나이관련황반변성 환자에서 유전학적 위험요인과 유리체강내 항혈관내피세포성장인자항체 치료반응 간의 관련성 분석

서론: 항혈관내피세포성장인자항체의 유리체강내 주사치료가 신생혈관성 나이관련황반변성의 치료결과를 크게 호전시켰지만, 치료에 대한 반응은 개인별로 다양하며 유전적 요소가 이에 관여하는 것으로 생각된다. 이 연구는 한국인 신생혈관성 나이관련황반변성 환자에서 나이관련황반변성의 위험 유전인자와 유리체강내 항혈관내피세포성장인자항체 주사치료에 대한 반응 사이의 연관성을 보고자 하였다.

방법: 본 전향적 연구에는 이전에 신생혈관성 나이관련황반변성에 대한 치료력이 없는 366 명 366 안이 포함되었으며 13 개 유전자의 17 개 단일염기다형성에 대해 유전분석이 이루어졌다. 환자들은 4 주 간격 3 회의 유리체강내 라니비주맙 주사치료(0.5mg / 0.05 ml)를 받은 후 매월 추적 관찰하면서 재치료 기준에 합당한 경우 라니비주맙 또는 베바시주맙(1.25 mg / 0.05 ml) 주사치료를 받았다. 일차 결과지표는 시력호전 정도에 바탕을 둔 '시력반응'과 최대교정시력의 변화량이었으며, 빛간섭단층촬영 결과에 바탕을 둔 '단층반응', 플루오레신 형광안저조영 소견에 바탕을 둔 '혈관조영반응', 그리고 치료 시작 전과 비교한 중심와두께 및 맥락막신생혈관 병변 넓이의 변화량도 추가로 분석하였다. 유전자형과의 연관성은 각 변수의 6, 12, 24 개월 결과에 대해 회귀분석으로 시행하였다. 잠재적인 유전자-유전자 상호작용을 평가하기 위해 Multifactor Dimensionality Reduction (MDR) 분석을 시행하였다. 항혈관내피세포성장인자 항체주

사에 대한 기존 연구들의 일관되지 못한 결과를 다시 평가하기 위해 본 연구의 결과까지 합하여 메타 분석을 실시하였다.

결과: 치료 시작 24 개월 째에 평균 시력은 4.5 ± 22.5 글자, 평균 중심와두께는 69.4 ± 112.6 μm 감소하였다. 회귀분석 결과, *VEGFA* rs3025039 의 소수 대립유전자 동형접합체가 다른 유전자형에 비해 24 개월째에 좋은 시력반응을 보일 가능성이 유의하게 높았다 (오즈비, 5.46; 95% 신뢰구간, 1.79 – 16.7; P = 0.0029). *CFH* rs800292 의 소수 대립유전자 동형접합체는 다른 유전자형에 비해 좋은 혈관조영반응을 보일 가능성이 유의하게 낮았다 (오즈비, 0.26; 95% 신뢰구간, 0.11 – 0.62; P = 0.0021). 10q26 에 위치한 *ARMS2* rs10490924, *HTRA1* rs11200638 의 소수 대립유전자 동형접합체는 12 개월째에 다른 유전자형에 비해 더 많은 중심와두께 감소량을 보였으나 경계적 유의성만을 보였다. MDR 분석 결과, *CFH* rs800292 와 *PEDF* rs1136287 사이의 유전자–유전자 상호작용이 12 개월째 단층반응에 유의한 영향을 미치는 것이 발견되었다. 기존의 5 개 항혈관내피세포성장인자항체 주사치료 관련 약물유전학 연구의 결과와 이번 연구결과를 합해 메타 분석한 결과, *ARMS2* rs10490924 와 *HTRA1* rs11200638 의 소수 대립유전자 동형접합자는 다른 유전자형에 비해 많은 양의 시력호전을 보인 것으로 나타났다.

결론: 결론적으로 한국인 습성 나이관련황반변성 코호트에서 행해진 이번 연구에서 *VEGF* rs3025039, *CFH* rs800292, *ARMS2* rs10490924, *HTRA1* rs11200638, *PEDF* rs1136287의 단일염기다형성이 항혈관내피세포성장인자항체 주사치료 반응과 관련성을 보였다. 항혈관내피세포성장인자항체의 약물유전학적 연관성에 대한 결과가 축적되면, 앞으로 개인의 유전적 배경에 기반한 맞춤형 접근을 통해 습성

나이관련황반변성 환자에서 최적의 치료효과를 얻는데 도움이 될 것으로 생각된다.

주요어: 나이관련황반변성, 항혈관내피세포성장인자항체, 약물유전학, 단일염기다형성

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