Genetic factors associated with response to as-needed aflibercept therapy for typical neovascular age-related macular degeneration and polypoidal choroidal vasculopathy

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In the present study, we investigated the association between susceptible genetic variants to age-related macular degeneration (AMD) and response to as-needed intravitreal aflibercept injection (IAI) therapy for exudative AMD including both typical neovascular AMD and polypoidal choroidal vasculopathy (PCV) over 12-months. A total of 234 patients with exudative AMD were initially treated with 3 monthly IAI and thereafter as-needed IAI over 12 months. Seven variants of 6 genes including ARMS2 A69S (rs10490924), CFH (I62V:rs800292 and rs1329428), C2-CFB-SKIV2L (rs429608), C3 (rs2241394), CETP (rs3764261) and ADAMTS-9 (rs6795735) were genotyped for all participants using TaqMan technology. After adjusting for age, gender, baseline BCVA and AMD subtype, A (protective) allele of C2-CFB-SKIV2L rs429608 was associated with visual improvement at 12-month (p = 0.003). Retreatment was associated with T(risk) allele of ARMS2 A69S (P = 2.0 × 10−4; hazard ratio: 2.18:95%CI: 1.47–3.24) and C(risk) allele of CFH rs1329428 (P = 2.0 × 10−3; hazard ratio: 1.74:95%CI: 1.16–2.59) after adjusting for the baseline confounders. The need for additional injections was also associated with t allele of ARMS2 A69S (P = 1.0 × 10−5) and c allele of CFH rs1329428 (P = 3.0 × 10−3) after adjusting for the baseline confounders. The variants of ARMS2 and CFH are informative for both physicians and patients to predict recurrence and to quantify the need for additional injections.

Advanced age-related macular degeneration (AMD), a leading cause of blindness in the industrialized countries of Asia1, is subdivided into choroidal neovascularization secondary to exudative AMD or geographic atrophy (GA). A recent clinic-based study reported that exudative AMD accounts for almost 95% of advanced AMD in the Japanese2. It has been reported that more than 20 genetic variants are associated with AMD through genome-wide association studies3–5. Of the genetic variants which make one more susceptible to AMD, variants in ARMS2 and CFH genes have been reported to be strongly associated with AMD, followed by variants in C2-CFB-SKIV2L and C3 genes3.

To date, intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents have been first-line therapy for exudative AMD worldwide. VIEW1/2 study demonstrated that three monthly intravitreal aflibercept injection (IAI) followed by bimonthly IAI was effective for treating exudative AMD and was non-inferior to monthly intravitreal ranibizumab injection regarding best-corrected visual acuity (BCVA)6. Pro re nata (PRN) regimen is also referred to as-needed regimen, and several prospective studies have demonstrated that PRN regimen is almost equivalent as monthly dosing regimen in terms of visual improvement7,8. PRN may also be a more practical treatment strategy due to the burdens of treatment cost and frequent clinic visits.

In a recent genome-wide association study investigating the response to ranibizumab for exudative AMD among Japanese patients using a PRN regimen, no specific gene variants were associated with a significant

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improvement of visual acuity at 12 months. However, the ARMS2 A69S variant was associated with need for retreatment. To date, there have been no reports investigating differences in response to aflibercept for exudative AMD among genotypic variants.

In the present study, we investigated the genetic associations of response to as-needed IAI with visual outcomes and the need for additional injections after initial 3 monthly IAI and followed by as-needed IAI for exudative AMD during 12-month follow-up.

Results

Table 1 shows baseline demographic and genetic characteristics of 234 patients composed of 118 patients with typical neovascular AMD and 116 patients with polypoidal choroidal vasculopathy (PCV). Compared with patients with PCV, patients with typical neovascular AMD were older ($p = 3.5 \times 10^{-6}$) and had higher T allele frequency of ARMS2 A69S ($p = 3.5 \times 10^{-3}$). The fellow eye condition was no drusen ($n = 96$), pachydrusen ($n = 20$), soft drusen ($n = 49$), pseudodrusen ($n = 32$), and exudative AMD/scarring ($n = 37$).

Table 2 shows the demographic and genetic characteristics of patients with or without requiring retreatment. Of 234 patients, 157 (67.1%) required the additional injection. Compared with patients without requiring the retreatment, patients requiring the retreatment were significantly older ($p = 5.2 \times 10^{-9}$) and had higher risk allele
frequency of ARMS2 A69S and CFH rs1329428 (p = 2.0 × 10^{-4} and 2.0 × 10^{-3}, respectively). Table 3 shows the number of additional intravitreal aflibercept injections according to genotypes in 7 variants of 6 genes. Number of additional injections was significantly different among ARMS2 A69S and CFH rs1329428 (p = 1.2 × 10^{-5} and 4.0 × 10^{-3}, respectively). Number of additional injections was significantly associated with the risk allele of ARMS2 A69S (risk allele: T allele) and CFH rs1329428 (risk allele: C allele) (p = 1.0 × 10^{-6} and 3.0 × 10^{-3}, respectively). Figure 1 shows Kaplan-Meier estimator demonstrating retreatment-free period from the initial injection according to ARMS2 A69S genotypes and CFH rs1329428 genotypes. A statistically significant difference was seen in retreatment-free period among ARMS2 A69S genotypes (P = 6.0 × 10^{-4}, log-rank test).

Table 2. Comparison of genetic characteristics between patients with or without retreatment. (*) adjusting for gender, baseline BCVA, AMD subtype. (**) adjusting for age, gender, baseline BCVA, AMD subtype.
Table 4 shows baseline BCVA and BCVA improvement according to genotypes in 7 variants of 6 genes. Although there was not a significant difference in baseline BCVA among genotypes in the 7 variants, A allele in rs429608 of C2-CFB-SKIV2L was significantly associated with BCVA improvement at 12-month.

Discussion
To date, anti-VEGF therapy is a first-line treatment for exudative AMD worldwide; however, for each eye there may be a wide range of clinical responses as well as number of injections needed. In some patients, BCVA greatly improved without additional injections, while in other patients, BCVA deteriorated in spite of monthly injection over 12-months. Given that several genetic factors are associated with clinical phenotype in exudative AMD, it would be reasonable to consider that differences in response to the treatment might contribute to genetic factors. Our study has found some genetic basis in the variable response as well as treatment burden among Japanese.
patients with macular neovascularization from typical neovascular AMD and PCV. We found that the A (protective) allele of SKIV2L rs429608 was associated with visual improvement at 12-months. Further, we found that the need for retreatment was associated with T(risk) allele of ARMS2 A69S and the C(risk) allele of CFH rs1329428 after adjusting for the baseline confounders.

There have been several reports investigating the association between genetic variants and response to ranibizumab or bevacizumab for exudative AMD. In CATT study, none of the investigated variants (CFH, ARMS2/HTRA1, C3) were associated with clinical outcomes including visual outcomes, OCT parameters, number of additional injections44. A recent Japanese prospective study using ranibizumab PRN regimen demonstrated that risk allele of ARMS2 A69S was associated with retreatment5. Several retrospective studies using ranibizumab also reported that risk allele of ARMS2 was associated with retreatment or number of additional injections45,46. In the present study using aflibercept, our results were consistent with previous reports, but our study is the first to investigate the effect of aflibercept response among the aforementioned genetic variants. In addition to ARMS2 A69S, risk (C) allele of CFH rs1329428 was associated with retreatment and number of additional injections47,48. In the present study, we genotyped major risk allele of ARMS2 risk allele of CFH rs1329428 is a susceptible variant to AMD; on the other hand, it has been reported that T allele of A69S, risk (C) allele of CFH rs1329428 was associated with retreatment and number of additional injections15,16. In BCV A, and AMD subtype.

Table 4. Visual improvement according to genotypes at 12-month. (∗)adjusting for age, gender, baseline BCVA, and AMD subtype.

| Genotype  | Baseline BCVA | p value | BCVA improvement at 12month | p value | Adjusted p value |
|-----------|---------------|---------|-----------------------------|---------|-----------------|
| ARMS2 A69S (rs10490924) | TT 0.42 ± 0.37 | 0.42 ± 0.28 | 0.12 ± 0.28 | 0.34 | NA |
|            | TG 0.49 ± 0.40 | 0.20 ± 0.33 | 0.34 | NA |
|            | GC 0.30 ± 0.24 | 0.14 ± 0.20 | 0.34 | NA |
| CFH I62V (rs800292) | GG 0.41 ± 0.37 | 0.15 ± 0.26 | 0.39 | NA |
|            | GA 0.43 ± 0.41 | 0.16 ± 0.32 | 0.39 | NA |
|            | AA 0.54 ± 0.45 | 0.25 ± 0.43 | 0.39 | NA |
| CFH (rs1329428) | CC 0.42 ± 0.37 | 0.17 ± 0.27 | 0.48 | NA |
|            | CT 0.43 ± 0.38 | 0.15 ± 0.30 | 0.48 | NA |
|            | TT 0.40 ± 0.45 | 0.14 ± 0.33 | 0.48 | NA |
| C2-CFB-SKIV2L (rs429608) | AA 0.70 ± 0.42 | 0.50 ± 0.14 | 1.0 × 10⁻³ | 3.0 × 10⁻³(∗) |
|            | AG 0.49 ± 0.44 | 0.30 ± 0.37 | 0.30 | NA |
|            | GG 0.41 ± 0.36 | 0.13 ± 0.27 | 0.30 | NA |
| C3 (rs2241394) | GG 0.7 | 0.18 | 0.18 | NA |
|            | GC 0.52 ± 0.39 | 0.18 ± 0.29 | 0.70 | NA |
|            | CC 0.41 ± 0.37 | 0.15 ± 0.29 | 0.70 | NA |
| CETP (rs3764261) | TT 0.27 ± 0.09 | 0.04 ± 0.40 | 0.04 | NA |
|            | TG 0.39 ± 0.34 | 0.18 ± 0.23 | 0.34 | NA |
|            | GG 0.45 ± 0.39 | 0.16 ± 0.30 | 0.42 | NA |
| ADAMTS9 (rs6795735) | CC 0.33 ± 0.30 | 0.14 ± 0.28 | 0.057 | NA |
|            | CT 0.35 ± 0.28 | 0.09 ± 0.26 | 0.057 | NA |
|            | TT 0.45 ± 0.39 | 0.18 ± 0.30 | 0.057 | NA |
In summary, the A allele of C2-CFB-SKIV2L rs429608 was associated with visual improvement while the T allele of ARMS2 A69S and C allele of CFH rs1329428 were associated with the need for retreatment and a greater number of additional injections during the first 12 months of PRN aflibercept therapy among treatment naive exudative AMD of Japanese descent.

### Methods

#### Subjects.
We retrospectively reviewed the medical charts of 234 eyes (from 234 patients) with treatment naive exudative AMD receiving 3 monthly intravitreal aflibercept (0.05 ml/2 mg) injections at the University of Yamanashi Hospital between 2013 January and 2018 September and completed 12-months of follow-up. We included the patients with exudative AMD including PCV and typical neovascular AMD. Eyes with typical neovascular AMD show type 1 or type 2 NV on SD-OCT and absence of polyoidal lesion on ICGA. This study was approved by the institutional review board at the University of Yamanashi and adhered to the tenets of Declaration of Helsinki. Written informed consent was obtained from each patient before treatment.

#### Treatment and follow-up.
Prior to the treatment, all patients had undergone comprehensive ophthalmic examinations including best-corrected visual acuity (BCVA) measurement using Landolt C chart, intraocular pressure measurement, slit-lamp biomicroscopy with 78 diopter contact lens, fundus color photography, spectral-domain optical coherence tomography (OCT) using Spectralis version 5.4 HRA + OCT (Heidelberg Engineering, Dossenheim, Germany) and fluorescein angiography (FA) and indocyanine green angiography (ICGA) using a confocal laser scanning system (HRA-2; Heidelberg Engineering, Dossenheim, Germany). The fellow eye condition was classified into 5 groups: 1) no drusen, 2) pachychoroid, 3) soft drusen, 4) pseudodrusen with or without soft drusen, 5) exudative AMD/scarring based on the criteria as we previously described.

All patients received 3-monthly intravitreal aflibercept injection (IAI) followed by monthly follow-up. BCVA and IOP measurement, biomicroscopy with or without 78D lens and a crosshair OCT scan were examined for all study eyes at each visit. If intraretinal/subretinal fluid was detected on SD-OCT or new macular hemorrhage were found on ophthalmoscopy, single additional intravitreal aflibercept injection was administered.

#### Genotyping.
A peripheral blood sample (5 ml) was collected when baseline FA/ICGA was performed. Genomic DNA was purified using PureGene Isolation Kit (Gentra Systems, Minneapolis, US). The variants of ARMS2 A69S (rs10490924), CFH I62V (rs800292), CFH rs1329428, C2-CFB-SKIV2L rs429608, C3 rs2241394, CETP rs3764261, ADAMTS9 rs6795735 were selected to genotype in the present study because these variants were reported to have a strong association with AMD in the Japanese population. Because the T allele of CFH rs1329428 was reported to be associated with central serous chorioretinopathy and choroidal vascular hyperpermeability in eyes with PCV, the CFH I62V variant was also included in our genotyping analysis. Genotyping was performed using TaqMan genotyping assays with 7300/7500 Real-Time PCR Systems (Applied Biosystems, Foster City, CA) with manufacturers’ recommendation as we previously described.

#### Statistical analysis.
Statistical analysis was performed using DR. SPSS for Windows (IBM, Tokyo, Japan). BCVA measured with decimal format using Landolt chart was converted to the logarithm of minimum angle of resolution (log MAR) for statistical analysis. Differences in categorical variables were examined using chi-square test. Difference in continuous variables between 2 groups and 3 groups were examined using Mann-Whitney U test and analysis of variance, respectively. Adjusted p-value was obtained using multivariate linear regression analysis. P-value less than 0.05 was considered statistically significant. In addition, we genotyped 7 genetic variants and assumed that these 7 genetic variants were statistically independent, we used the Bonferroni correction, which require less than 0.0071 (0.05/7) to reach a statistical significance.

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Competing interests
The authors declare no competing interests.

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