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ARMS2 variants may predict the 3-year outcome of photodynamic therapy for wet age-related macular degeneration

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Purpose: To determine the association of *age-related maculopathy susceptibility 2* (*ARMS2*) gene polymorphisms with the 3-year outcomes of photodynamic therapy (PDT) in wet age-related macular degeneration (wet AMD).
Methods: The single nucleotide polymorphism (SNP) at rs10490924 in the *ARMS2* gene of 65 patients with wet AMD who underwent PDT was genotyped using the TaqMan assay. The clinical characteristics and the outcomes of PDT were compared among the three genotypes at rs10490924. A multivariate regression analysis was performed to evaluate the influence of the clinical cofactors on the association of rs10490924 with the visual outcome at 36 months after the first PDT.
Results: A significant difference was found among the genotypes in the age and the baseline lesion size. The patients with the GG genotype showed a significant improvement in vision, and the patients with the TT genotype showed a significant worsening of vision at all time points measured after the initial PDT. In the multivariate regression analysis, the number of the G allele at rs10490924 was associated with a significantly greater improvement in the baseline best-corrected visual acuity (BCVA) at 36 months after the first PDT.
Conclusions: *ARMS2* variants are likely associated with the 3-year outcomes of PDT in patients with wet AMD.

Age-related macular degeneration (AMD) is a leading cause of central vision loss in the elderly in industrialized countries [1]. The number of patients with AMD has increased remarkably over the years, and further increases in patients with severe visual impairment due to AMD are a concern [2]. Advanced AMD is clinically classified into dry (atrophic) AMD and wet (exudative) AMD, and in current clinical practice, wet AMD is mostly treated with antivascular endothelial growth factor (VEGF) agents [3]. However, anti-VEGF therapy requires frequent injections to maintain the patients' vision, which may burden the patients for expenses and side effects represented by secondary geographic atrophy [3,4]. Photodynamic therapy (PDT) with verteporfin is an older modality than anti-VEGF for wet AMD but is known to be effective in certain cases that have beneficial factors for this therapy and now is being reconsidered as an adjunctive therapy for patients who show poor response to anti-VEGF therapies [5,6]. For example, a case of wet AMD with polypoidal choroidal vasculopathy (PCV) is a good candidate for PDT [7], but it is not always possible to distinguish those cases from other wet AMD because fine images of indocyanine green angiography (ICGA) are necessary, and expert reading of images by AMD specialists is required for precise diagnosis [8]. Recently, several genetic association studies were conducted to predict the outcomes of several interventions for wet AMD, including PDT [9-12]. Since genetic information is case-specific and can be used by every physician without any diagnostic biases, it is important to find reliable genetic variants associated with the outcomes of therapies. We previously reported that genetic variants of rs10490924 (A69S) in the *age-related maculopathy susceptibility 2* (*ARMS2*; ID: 387715, OMIM: 611313) gene were associated with 12-month visual outcomes in wet AMD cases [12]. Similar results were reported by other groups over 12 months of follow-up [9,11], but no studies have been published to date regarding the genetic association with the outcomes of PDT over a 3-year follow-up period. In this study, we investigated the association of rs10490924 in *ARMS2* with the 3-year visual outcomes of PDT in patients with wet AMD.

METHODS

Study participants: This study was approved by the Institutional Review Board at the Kobe University Graduate School of Medicine and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. All cases in this study were Japanese individuals recruited from the Department of Ophthalmology at Kobe University Hospital in Japan.

Sixty-five eyes of 65 consecutive patients with wet AMD (53 males and 12 females, the mean age 75.8±6.6 years) who underwent PDT from 2005 to 2008, then were followed up for 3 years, and accepted DNA sampling were retrospectively included in this study. The detailed information about the

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state of health of the patients were not available. All subjects in this study were part of our previous study regarding 12 months of results for PDT in wet AMD [12]. As ranibizumab and aflibercept was not available until 2009 and 2012 in Japan, respectively, all patients with wet AMD had received PDT as the initial therapy during this recruitment period. All patients underwent ophthalmic examinations, including visual acuity measurements, slit-lamp biomicroscopy of the fundi, color fundus photography, optical coherence tomography, fluorescein angiography (FA), and ICGA. The diagnosis of PCV was made by choroidal polypoidal lesions with/without vascular networks detected on ICGA [13]. Visual acuity was determined using a Landolt C chart and was converted to a logarithm of the minimum angle of resolution (logMAR) for calculations. Patients who had received any previous treatment for AMD were not included in this study. Patients with retinal angiomatous proliferation (RAP) were not included because of the failure of follow-up more than a year after the initial PDT.

Photodynamic therapy: All patients in this study were followed up for at least for 36 months after their first session of PDT. PDT was performed with standard procedures described previously [14]. The lesion status was assessed every 3 months, and treatments were performed again when serous retinal detachment, hemorrhage, or macular edema was recognized accompanied by a leakage on FA, or a defined lesion was observed on ICGA. No patients in this study received other treatments or combined therapy during the follow-up period except two patients who received a single session of intravitreal ranibizumab at 30 months and 32 months after the initial PDT.

Genotyping: Genomic DNA was extracted from the peripheral blood using QIAmp DNA Blood Maxi Kit (Qiagen, Hilden, Germany). Genotyping was performed using TaqMan® SNP Genotyping Assays or Custom TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ Real-Time PCR System (PCR conditions: 95 °C×20 s, 60 °C×20 s, 25 °C×30 s, 45 cycles; Applied Biosystems) in accordance with the manufacturer's recommendations.

Comparison of indices: Age, sex, lesion size (greatest linear dimension, GLD) based on FA findings, and the baseline best-corrected visual acuity (BCVA) were compared among the three genotypes of rs10490924 in *ARMS2*. These parameters were measured for each case under masked conditions for the genotype. The primary outcome measure was the change in the BCVA until 36 months after the initial PDT. The number of cases who showed a recurrence of the lesion over the 36-month follow-up period and the time to recurrence were also evaluated. To identify the factors useful for predicting the change in BCVA at 36 months post-PDT, stepwise multivariate regression analysis with backward elimination methods was performed. The explanatory variables included sex, age, the baseline BCVA, GLD, the presence or absence of PCV, and the number of the T (risk) allele at rs10490924 in *ARMS2*. Dummy variables were applied for sex (female = 1, male = 0), the presence of PCV (yes = 1, no = 0), and the recurrence of the lesion within 3 years (yes = 1, no = 0).

Statistical analysis: All statistical analyses were performed using R version 3.1.1 software. The parameters were compared among the genotypes using the chi-square test or the Kruskal–Wallis test where applicable. For the time-course analysis, two time points in each genotype were compared using a paired *t* test (two-tail). A p value of less than 0.05 was considered to be statistically significant.

RESULTS

The clinical characteristics of the patients with wet AMD stratified by the genotypes of rs10490924 in *ARMS2* are presented in Table 1. The mean age and the GLD were significantly different among the genotypes, and the post-hoc test (Steel-Dwass) revealed that patients with the TT genotype showed a significantly higher age and a larger GLD than those with the GT genotype (p = 0.025 and 0.038, respectively). The patients with the GG genotype tended to show fewer recurrences of lesions and a longer time to the recurrence than the GT and TT genotypes although there was no statistical significance.

The patients with the GG genotype showed a significant improvement of vision at 12, 24, and 36 months post-PDT (−0.30, −0.30, and −0.28 logMAR and p = 0.0074, 0.012, and 0.023, respectively), and the patients with the TT genotype showed a significant worsening of vision at 12, 24, and 36 months after the initial PDT (0.23, 0.25, and 0.34 logMAR and p = 0.0097, 0.0029, and 0.00013, respectively; Figure 1). The result at 36 months post-PDT was not changed after we excluded the data of two patients with the GG genotype who received intravitreal ranibizumab at 30 months and 32 months after the initial PDT (−0.29 logMAR and p = 0.019 after exclusion). The patients with the GT genotype did not show any significant change in BCVA over the 36-month follow-up period. The results of the stepwise multivariate regression analysis conserved the significance of the association of rs10490924 (A69S) variants with the improvement in 36-month BCVA after the initial PDT (Table 2) although the presence of PCV showed the strongest association with the improvement in BCVA. Namely, the patients with more G alleles at rs10490924 showed greater improvement in BCVA after PDT. As a complication, two patients with the

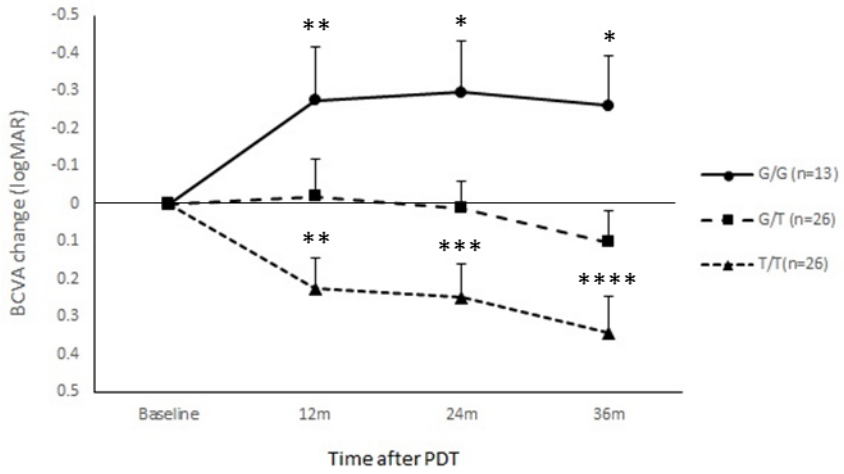

Figure 1. Influence of the genotype at rs10490924 (A69S) in *ARMS2* on the chronological change in the BCVA (logMAR) in patients with wet AMD treated with PDT. All values are presented as means ± standard error of the mean (SEM). Time points in each genotype were compared with the baseline. * p<0.05, ** p<0.01, *** p<0.005, **** p<0.0005.

Table 1. Clinical characteristics stratified by the genotype of A69S in ARMS2 gene.

Factors	GG (n=13)	GT (n=26)	TT (n=26)	P value	Statistics
Sex (male/female)	12/1	22/4	19/7	0.30	†
Age (years)	75.6±6.5	73.9±5.6	77.9±7.2	0.029	*
GLD (μm)	3192.3±1532.5	3327.3±1418.7	4405.4±1820.6	0.029	*
PCV (present/absent)	10/3	17/9	16/10	0.63	†
Baselne BCVA (logMAR)	0.86±0.40	0.62±0.37	0.65±0.42	0.13	*
Recurrence within 3 years (yes/no; ratio of "yes")	5/8 (38%)	14/12 (54%)	14/12 (54%)	0.61	†
Time to recurrence (months)	25.4±11.5	15.9±8.7	18.6±9.9	0.18	*

Values are presented as mean±SD where applicable. Abbreviations: GLD represents greatest linear dimension, PCV represents polypoidal choroidal vasculopathy, BCVA represents best-corrected visual acuity, logMAR represents logarithm of minimum angle resolution. † χ^2 test, * Kruskal–Wallis test.

Table 2. Results of the stepwise multiple regression analysis. Prognostic factors for the improvement of the BCVA (logMAR) at 36 months after PDT.

Prognostic factors	SPRC	SEM	t-value	P value
Number of T (risk) allele at rs10490924 in ARMS2	0.22	0.060	3.72	0.00045
Baseline BCVA (logMAR)	−0.41	0.12	−3.37	0.0013
The presence of PCV (yes=1, no=0)	−0.37	0.095	−3.93	0.00022
Recurrence within 3 years (yes=1, no=0)	0.27	0.097	2.83	0.0063

Multiple R-squared: 0.55, Adjusted R-squared: 0.50 Abbreviations: SPRC represents standardized partial regression coefficient, SEM represents standard error of the mean, BCVA represents best-corrected visual acuity, PCV represents polypoidal choroidal vasculopathy.

TT genotype showed a retinal pigment epithelial tear during the follow-up period.

DISCUSSION

We evaluated the association of a well-recognized SNP in *ARMS2* and the 3-year outcomes of PDT in patients with wet AMD and found that the genotype at rs10490924 (A69S) in *ARMS2* was significantly associated with the visual outcome of patients with wet AMD at 36 months after their first PDT. Namely, patients with the GG genotype at rs10490924 showed significant improvement in BCVA at 12 months after the initial PDT, which was sustained over the 36-month follow-up period.

Recent genetic association studies have performed comparative assessments for the association of rs10490924 (A69S) in *ARMS2* among three phenotypes of wet AMD [15-18], which suggested heterogeneities in the association of this SNP within the AMD phenotype spectrum. The association of *ARMS2* variants with the outcomes of established therapies for wet AMD has also been reported with several cohorts [9-12,19]. Several studies indicated a significant association of *ARMS2* variants with the 12-month visual outcome of PDT in patients with wet AMD [9,11,12], but the studies mentioned the importance of replication studies with a longer follow-up period. The present study demonstrated that the beneficial effect of the G allele at rs10490924 (A69S) in *ARMS2* on the visual outcome was sustained up to 3 years after the initial PDT in wet AMD. Although we could not detect a significant association of the variants at rs10490924 with the chance of recurrence of the lesion or the time to the recurrence, they might be due to insufficient statistical power to detect a significant association. In fact, the multivariate regression analysis revealed that the recurrence of the lesion was an independent risk factor significantly associated with the change in BCVA at 36 months after PDT. The presence of PCV showed the strongest association with the greater improvement in BCVA at 36 months post-PDT in the multivariate regression analysis, but finding PCV lesions is not always possible depending on the availability of ICGA and the expert reading of images by AMD specialists [8]. Recent commercial genotyping services enable patients and physicians to obtain individual genotypes with a shorter time period and a lower cost (ScienceExchange). Using genetic information for choosing interventions is anticipated to be more common in future clinical practice [20].

The role of *ARMS2* in PDT is unknown. Recent reports demonstrated that *ARMS2* can affect the progression of wet AMD [21,22], which may influence the visual outcome at 36 months post-PDT. Kanda et al. reported that ARMS2 distributes to the outer membrane of the mitochondria and may be involved in the regulation of oxidative stresses [23]. Reactive oxygen species play a key role by which PDT affects neovascular endothelial cells, followed by thrombosis and the occlusion of neovascular tracts [24]. It is also possible that the *ARMS2* genotype is associated with a wet AMD subtype (i.e., typical AMD, PCV, and RAP) resulting in the differential response than a direct effect on PDT in itself [15-18]. However, the result of the stepwise multiple regression analysis indicated the *ARMS2* genotype is an independent contributor to the visual outcomes of PDT. Further studies will be needed to disclose the certain role of *ARMS2* in the pathogenesis of wet AMD and the mechanism in which PDT works to treat choroidal neovascularization. Nevertheless, the present study demonstrated that the patients with wet AMD with the GG genotype would be good candidates for PDT, which suggests the assessment of genetic information is likely to be useful for evaluating the applicability of PDT in patients with wet AMD.

The limitations of the present study are the relatively small sample size and retrospective nature. A prospective study for the outcome of PDT with a larger population is needed to disclose the further association of *ARMS2* variants with the effect of PDT in patients with wet AMD.

As PDT is known to induce several changes in gene expression in the retina-choroidal complex [24,25], the detailed mechanisms by which multiple genes interact with each other to close the CNV is poorly understood. However, the present results suggest that genetic association studies provide clinical possibilities that can be applied for personalized therapies in individual patients with wet AMD.

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