Case Reports in Ophthalmology

Case Rep Ophthalmol 2011;2:314–318
DOI: 10.1159/000332830

Published online: September 23, 2011



ISSN 1663–2699
www.karger.com/cop





# Macular Edema Formation and Deterioration of Retinal Function after Intravitreal Bevacizumab Injection for Proliferative Diabetic Retinopathy

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**Key Words**
Anti-vascular endothelial growth factor · Bevacizumab · Avastin · Multifocal electroretinography · Full-field electroretinography · Diabetic retinopathy · Optical coherence tomography · Macular edema

**Abstract**
***Purpose:*** To report a case of proliferative diabetic retinopathy (PDR) showing transient macular edema (ME) and deteriorated retinal function after intravitreal bevacizumab injection (IVB).
***Methods and Results:*** A 53-year-old man received IVB (1.25 mg/0.05 ml) in both eyes for the treatment of PDR. There was no treatment-related complication. However, he complained of photopsia in both eyes 6 h after the injection. Slit-lamp examination revealed mild cellular infiltrations (1+) in the anterior chamber in both eyes. Optical coherence tomography showed ME formation in the left eye. Both full-field and multifocal electroretinography (ERG) revealed the deterioration of all parameters in both eyes compared with pretreatment. The inflammation in the anterior segment and ME disappeared 1 day after the injection. ERG parameters were improved 9 days after the injection, except for the N1 and P1 amplitude of multifocal ERG in the left eye.
***Conclusion:*** We propose that patients who undergo IVB should be carefully informed and followed up for possible complications including temporal ME formation and retinal function deterioration.

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## Introduction

A number of reports described the benefit of intravitreal bevacizumab (Avastin®; Genentech, Inc., South San Francisco, Calif., USA) injection (IVB) for proliferative diabetic retinopathy (PDR) [1–3]. Excessive fibrosis of fibrovascular membrane (FVM) and tractional retinal detachment after IVB are reported to date [4–6]. In this report, we present a case of PDR showing transient macular edema (ME) and deteriorated retinal function after IVB.

## Case Report

A 53-year-old man consulted our hospital for the treatment of PDR in both eyes. At the first visit, the best corrected visual acuity measured by Landolt ring chart was 1.0 in both eyes. No remarkable finding was observed in the anterior segment. Fundoscopic examination revealed normal macular appearance and apparent FVM on the disc and elsewhere. Scattered chorioretinal scars from retinal photocoagulation performed more than 3 months before were also found. Fluorescein angiography showed remarkable leakages of dye from the FVM (fig. 1a). However, the optical coherence tomography (OCT) findings showed normal macular appearances (fig. 2). To reduce the activity of FVM, we performed off-label IVB (1.25 mg/0.05 ml) in both eyes with the patient's written informed consent, the approval of the institutional review board of Kobe University of Medicine and the tenets of the Declaration of Helsinki. Although no complication occurred during the surgical procedures, the patient complained of photopsia in both eyes 6 h after the injection. Slit-lamp examination revealed mild cellular infiltrations (1+) in the anterior chamber in both eyes. Although the fundoscopic findings in both eyes did not reveal any retinal change, the OCT showed ME formation in the left eye (fig. 2). Both full-field and multifocal electroretinography (ERG) revealed the deterioration of all parameters in both eyes compared with pretreatment (fig. 3). The next day, the inflammation in the anterior segment and ME disappeared (fig. 2), and after 9 days, the patient's complaint had totally disappeared. Best corrected visual acuity was maintained at 1.0 in both eyes during the event. Fluorescein angiography revealed leakage reduction (fig. 1b). Moreover, 9 days after the injection, ERG parameters were improved, except for the N1 and P1 amplitude of multifocal ERG in the left eye (fig. 3).

## Discussion

Although Fung et al. [7] published the incidence of ocular inflammation related to IVB, they did not evaluate the structural change of the retina after the injection. To our knowledge, this is the first report describing ocular inflammation accompanied by ME shortly after IVB. It is known that ME usually results from accelerated breakdown of the blood-retinal barrier due to PDR. However, in our case, apparent ME occurred quite rapidly after IVB, which suggests that ME was induced by the surgical procedure or by bevacizumab itself. Forooghian et al. [8] reported aqueous vascular endothelial growth factor (VEGF) downregulation and the upregulation of inflammatory cytokines (TGF-β2 and IL-8) after IVB. They discussed that acute elevation of these inflammatory cytokines may mediate a rapid contraction of FVM, thereby causing tractional retinal detachment. Since there is no report demonstrating such a rapid appearance of ME after IVB, we suggest that the acute alteration of the intraocular cytokine concentration might have had a predominant role in inducing inflammatory ME in the present case.

The ERG findings showed a functional deterioration after IVB. Changes of all ERG parameters were determined after only 6 h following IVB, corresponding to the slit-lamp and OCT findings, but the recovery of the ERG parameters was delayed compared to the improvement seen in the slit-lamp and OCT findings. This result indicates that

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subclinical dysfunction of the retina lasts relatively longer than morphological changes. Peters et al. [9] showed the reduction of choriocapillaris endothelial cell fenestrations after IVB, and Heiduschka et al. [10] showed the penetration of bevacizumab through the retina and diminished immunoreactivity of VEGF in the retina 14 days after IVB. They suggested that these ultrastructural changes may lead to functional and morphologic damage of retinal pigment epitheliums and photoreceptors. We considered that the VEGF withdrawal after IVB suppressed the physiological neurotrophic effect of VEGF [11], and caused temporal retinal dysfunction accompanied by the abnormal ERG parameters.

In summary, we propose that patients who undergo IVB should be carefully informed and followed up for possible complications including temporal ME formation and retinal function deterioration.

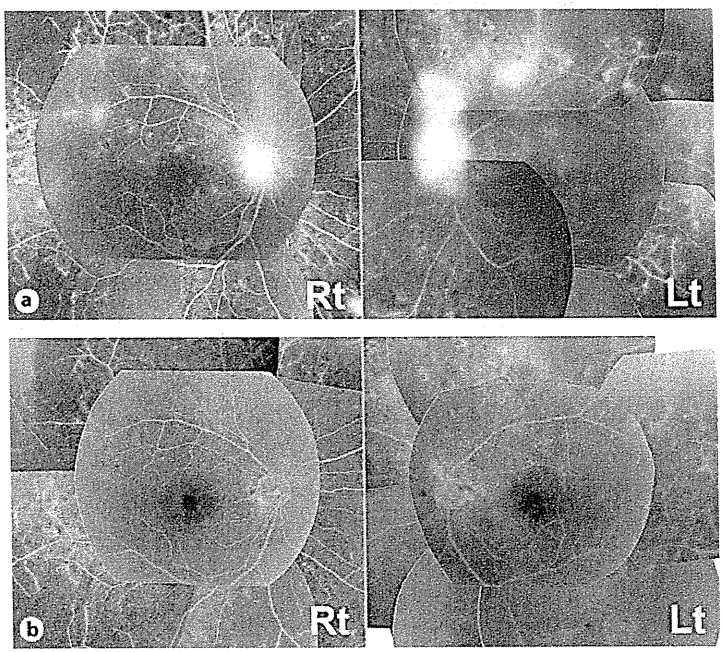


**Fig. 1.** Late-phase fluorescein angiography findings. **a** Leakages from neovascularizations on the disc and elsewhere were seen before IVB in both eyes. **b** Leakages from neovascularizations were reduced 9 days after the injection.

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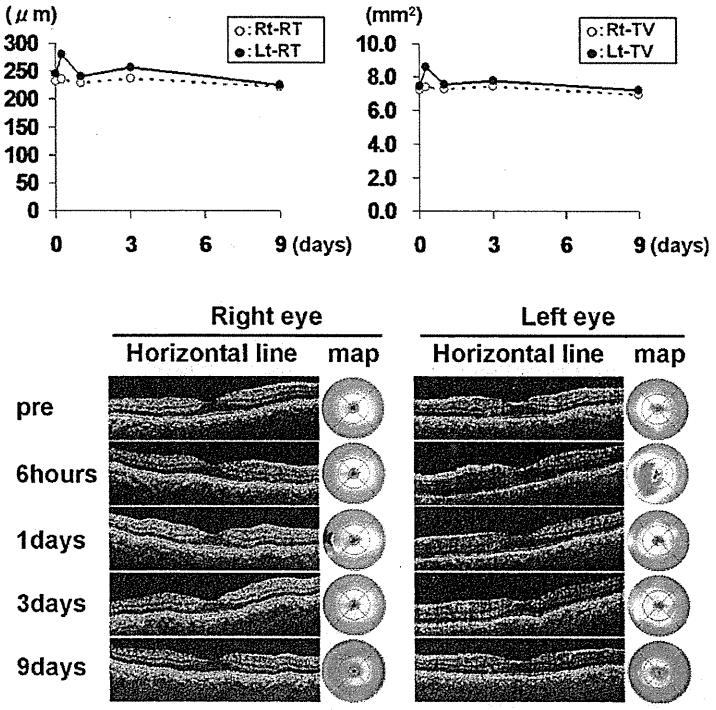


**Fig. 2.** Upper panel: transition of retinal thickness and total macular volume in OCT. Lower panel: transition of the horizontal line and the color map image of retinal thickness in OCT. Before the injection, OCT findings showed normal macular appearances in both eyes. The retinal thickness and total macular volume in the left eye were increased 6 h after the injection. OCT findings in the left eye improved after 1 day and were kept normal after that. OCT findings in the right eye were kept normal during the follow-up period.

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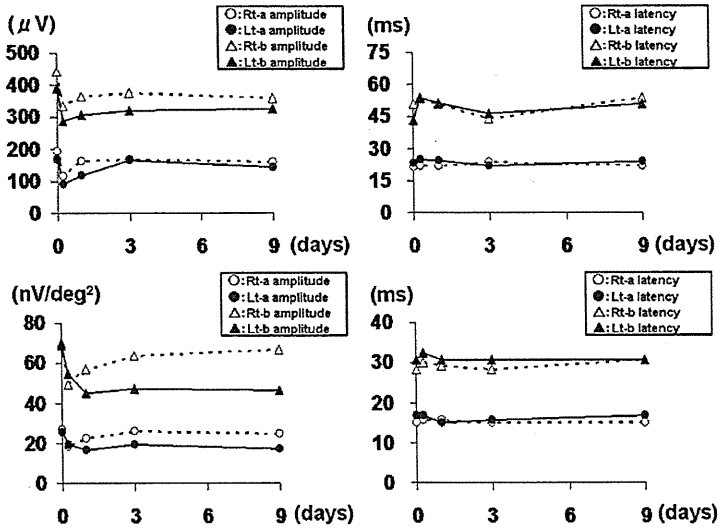


**Fig. 3.** Transition of ERG parameters. Upper panel: transition of full-field bright-flash ERG. Lower panel: transition of multifocal ERG at 3 central rings of 103 hexagon analysis. In both methods, all ERG parameters worsened 6 h after the injection and improved with time, except for the N1 and P1 amplitudes of the left eye.

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nature genetics

# Genome-wide association study identifies two susceptibility loci for exudative age-related macular degeneration in the Japanese population

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**Age-related macular degeneration (AMD), the leading cause of irreversible blindness in the world, is a complex disease caused by multiple environmental and genetic risk factors. To identify genetic factors that modify the risk of exudative AMD in the Japanese population, we conducted a genome-wide association study and a replication study using a total of 1,536 individuals with exudative AMD and 18,894 controls. In addition to *CFH* (rs800292, $P = 4.23 \times 10^{-15}$) and *ARMS2* (rs3750847, $P = 8.67 \times 10^{-29}$) loci, we identified two new susceptibility loci for exudative AMD: *TNFRSF10A-LOC389641* on chromosome 8p21 (rs13278062, combined $P = 1.03 \times 10^{-12}$, odds ratio = 0.73) and *REST-C4orf14-POLR2B-IGFBP7* on chromosome 4q12 (rs1713985, combined $P = 2.34 \times 10^{-8}$, odds ratio = 1.30). Fine mapping revealed that rs13278062, which is known to alter *TNFRSF10A* transcriptional activity, had the most significant association in 8p21 region. Our results provide new insights into the pathophysiology of exudative AMD.**

AMD is a major cause of severe visual impairment among the elderly population in developed countries[1,2]. Late AMD is divided into exudative AMD and geographic atrophy, and the prevalences of these two types of AMD are different between the European and Asian populations[3,4]. Exudative AMD is a major type of late AMD in the Asian population and is characterized by abnormal vasculopathies arising from the choroidal vasculature, which may lead to recurrent serous exudation and hemorrhages[3]. In contrast, geographic atrophy is a common type of late AMD in the European population and is characterized by retinal pigment epithelium (RPE) atrophy and thinning of the retina without exudative or hemorrhagic changes. Although the inflammation of the RPE-choroid interface and the apoptosis of both photoreceptor and RPE cells have crucial roles in the development of AMD, the precise pathogenesis of AMD has not been fully elucidated[5,6].

Previous genome-wide association studies (GWAS) have identified many common variants in AMD risk[7–12]. Landmark GWAS identified *CFH* (complement factor H) and *ARMS2* (age-related maculopathy susceptibility 2) as the susceptibility genes for AMD[7,8]. Recent advances in genetic research have clarified that the variants of several complement pathway-associated genes have important roles in the pathogenesis of AMD[13–17]. Although previous GWAS have identified eight susceptibility loci for AMD, most of these findings included only the results from European populations[7–11]. Regardless of the differences in the prevalence of AMD type between European and Asian populations, there is scarce information for the susceptibility genes of AMD in the Asian population.

To investigate the genetic background of exudative AMD in the Japanese population, we conducted a GWAS to identify genes related to exudative AMD susceptibility using 827 cases and 3,323 controls. We genotyped these samples using the Illumina Human610-Quad BeadChip for cases and the Illumina HumanHap550v3 BeadChip for controls. Genotype concordance between these two BeadChips was 99.99% among 182 duplicate samples, indicating a low possibility of genotype error. After we applied stringent quality control criteria, we carried out an association analysis in 457,489 autosomal SNPs that were available on both BeadChips. Principal component analysis (PCA) showed no population substructure, and the quantile-quantile plot showed that the inflation factor was 1.057 (**Supplementary Fig. 1a,b**). To further examine the possibility of population substructure and its influence on our GWAS results, we performed PCA again using the HapMap JPT and CHB populations as the references. Almost all subjects fell into the known two main clusters of the Japanese population

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Received 16 March; accepted 17 August; published online 11 September 2011; doi:10.1038/ng.938

**Table 1 Summary of the GWAS and replication study**

| SNP | Allele | Minor allele | Chr. | Chr. location | Gene | Study | No. of samples Case | No. of samples Control | MAF Case | MAF Control | Age and sex adjusted $P$ | OR | 95% CI | $P_{het}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| rs13278062 | T/G | T | 8 | 23,138,916 | *TNFRSF10A-LOC389641* | GWAS | 827 | 3,323 | 0.417 | 0.343 | $2.46 \times 10^{-6}$ | 0.71 | 0.62–0.82 | |
| | | | | | | Replication | 701 | 15,565 | 0.417 | 0.346 | $8.19 \times 10^{-8}$ | 0.74 | 0.66–0.82 | |
| | | | | | | Combined | | | | | $1.03 \times 10^{-12}$ | 0.73 | 0.67–0.80 | 0.68 |
| rs1713985 | T/G | G | 4 | 57,481,207 | *REST-C4orf14-POLR2B-IGFBP7* | GWAS | 827 | 3,323 | 0.333 | 0.286 | $9.03 \times 10^{-5}$ | 1.34 | 1.16–1.56 | |
| | | | | | | Replication | 708 | 15,569 | 0.329 | 0.282 | $5.71 \times 10^{-5}$ | 1.27 | 1.13–1.43 | |
| | | | | | | Combined | | | | | $2.34 \times 10^{-8}$ | 1.30 | 1.19–1.42 | 0.56 |

The age- and sex-adjusted $P$ values were calculated by logistic regression analysis under an additive model. The combined $P$ values were calculated by the inverse variance method. The $P$ values of heterogeneities ($P_{het}$) across the population were estimated formally using a Cochran's $Q$ test. Chr., chromosome; No., number; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

(**Supplementary Fig. 1c**). When we evaluated the quantile-quantile plot only using the samples in the main (Hondo) cluster, the inflation factor was 1.076 (**Supplementary Fig. 1d**). Therefore, we considered that genotype misclassification or population substructure might not be the cause of the difference in the inflation factor.

In our GWAS, two loci reached a genome-wide significant level of association ($P < 5 \times 10^{-8}$; **Supplementary Fig. 1g**). These two loci have already been reported in previous GWAS[7–12]: *ARMS2* (rs3750847, $P = 8.67 \times 10^{-29}$) and *CFH* (rs800292, $P = 4.23 \times 10^{-15}$). Results from these loci are shown in **Supplementary Figure 2**. We also checked the association of previously reported susceptibility loci for AMD (**Supplementary Table 1**). We found a significant association for three loci (*CFI*, *C2* and *CFB*), whereas we did not replicate susceptibility loci identified in recent GWAS of the European population (*TIMP3* and *LIPC*), probably because of the lower statistical power in our study. Notably, rs2230199, a marker SNP at the *C3* locus, was not polymorphic in the Japanese population. Although exudative AMD is a major type of AMD in Japanese compared to European individuals, these results indicate that the underlying disease mechanisms of AMD are largely similar for both populations, and the differences in genetic modifiers or environmental factors may represent the differences in the prevalence of a specific late-stage AMD type.

To identify additional susceptibility loci, we conducted a replication study using an independent set of 709 Japanese exudative AMD cases and 15,571 controls. Among 146 SNPs that showed $P < 1.0 \times 10^{-4}$ in GWAS, we selected 77 SNPs for the replication study after excluding 47 SNPs within the same locus ($r^2 > 0.8$) and 22 SNPs located at previously reported loci. We successfully genotyped all 77 SNPs using the multiplex PCR-based Invader assay and found significant association in two SNPs after Bonferroni correction (corrected $P < 6.49 \times 10^{-4}$; **Supplementary Table 2**). When we combined the results of the GWAS and a replication study by the inverse variance method, two SNPs reached a genome-wide significance level of association: rs13278062 on chromosome 8p21 (combined $P = 1.03 \times 10^{-12}$, odds ratio (OR) = 0.73, 95% confidence interval (CI) 0.67–0.80) and rs1713985 on chromosome 4q12 (combined $P = 2.34 \times 10^{-8}$, OR = 1.30, 95% CI 1.19–1.42; **Table 1**). The ORs between the GWAS and replication study were quite similar, and we did not observe any heterogeneity across the studies. A previous linkage study has also indicated an association on chromosome 8p21 (ref. 18).

Because exudative AMD is classified into typical AMD (t-AMD) and polypoidal choroidal vasculopathy (PCV), we found both SNPs showed a similar effect on susceptibility to each AMD subtype (**Supplementary Table 3**). Although we performed age-adjusted analysis, there is a possibility that aging or cohort effect might distort

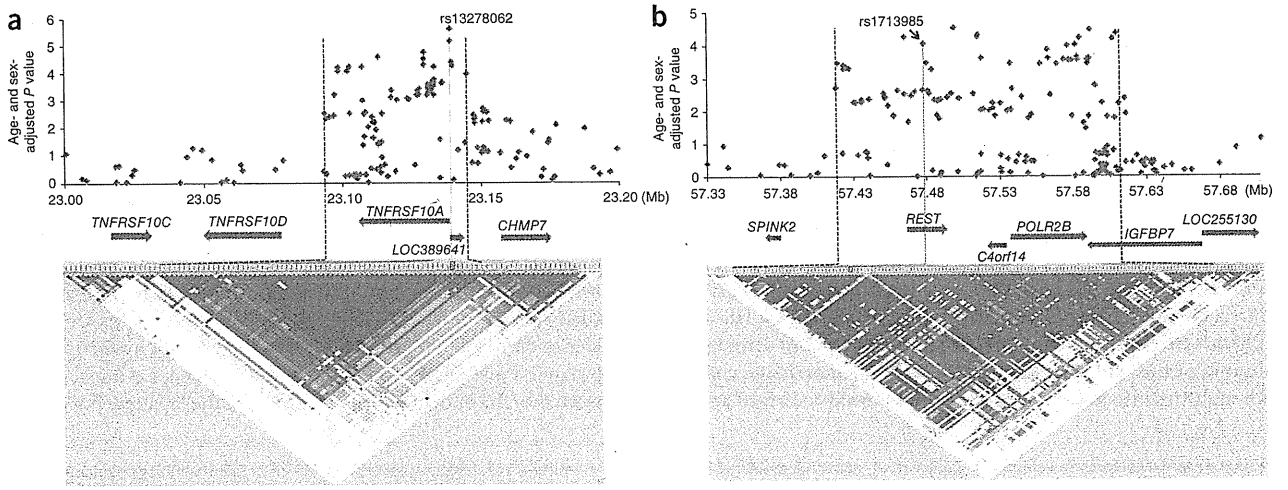


**Figure 1** Case-control association plots, LD map and genomic structure of the *TNFRSF10A- LOC389641* region in chromosome 8p21 (**a**) and the *REST-C4orf14-POLR2B-IGFBP7* region in chromosome 4q12 (**b**). The candidate region is shown between the two black dashed lines. We performed fine mapping in the regions from 23.09–23.14 Mb in chromosome 8p21 and 57.42–57.61 Mb in chromosome 4q12. The blue diamonds represent $-\log_{10} P$ values obtained from GWAS and fine mapping. We drew the LD map based on $D'$ values using the genotype data from the cases and controls in the GWAS samples. The blue lines indicate the position of the marker SNPs (rs13278062 (**a**) and rs1713985 (**b**)).

the findings of our study because the controls were younger than the cases, overall. However, we did not find marked differences in the minor allele frequencies (MAFs) of the two SNPs in each 10-year age group (**Supplementary Table 4**).

To narrow down the candidate regions and to identify susceptibility genes for exudative AMD, we carried out fine mapping using GWAS case-control samples. We first defined a linkage disequilibrium (LD) block and constructed a $-\log_{10} P$ plot of chromosome 8p21 using GWAS data. We found that the most highly associated SNP (rs13278062) represented an LD block that spanned from 23.078–23.152 Mb. Then, we selected and genotyped 18 SNPs around this LD block based on the data from the HapMap phase 2 Japanese population with a MAF ≥ 0.05. Next, we resequenced a 51-kb region from 23.094–23.145 Mb using 48 individuals with exudative AMD. After excluding repeat sequences, we identified 9 new SNPs in addition to the 88 known SNPs registered in the dbSNP database. After excluding 23 SNPs already genotyped, we genotyped 74 SNPs with MAF ≥ 0.05, and we successfully genotyped 73 of these. However, no SNPs showed stronger association than rs13278062 (**Fig. 1a** and **Supplementary Table 5a**). We also performed haplotype analysis using the four highly associated SNPs (rs2235126, rs7820465, rs13278062 and rs13281363), however, no haplotype showed stronger association than the single-marker association of rs13278062 (**Supplementary Fig. 3**).

rs13278062 is located in *LOC389641* and is also 397 bp upstream of *TNFRSF10A*, the tumor necrosis factor receptor superfamily 10a gene, which encodes one of the TRAIL receptors, TRAILR1. TRAILR1 is broadly expressed in human adult RPE[19] and rod photoreceptors in mice[20], whereas the expression of LOC389641 was low or absent according to the Gene Expression Omnibus (GEO) database. Binding of TRAIL to TRAILR1 is known to induce apoptosis through caspase 8 activation[21]. In addition, the TRAIL-TRAILR1 complex has a nonapoptotic pathway that induces the production of inflammatory cytokines and the promotion of inflammation through activation of nuclear factor B[22,23]. A previous study showed that the activator protein 1 will bind the sequence around rs13278062 and directly regulate TRAILR1 mRNA expression[24]. Moreover, the G allele of rs13278062 has been reported to enhance the transcriptional activity of TRAILR1 by 1.2- to 1.5-fold as compared to the T allele[25]. Although further functional studies are needed, these results speculate that *TNFRSF10A* is the susceptibility gene for exudative AMD and that rs13278062 may be the candidate of functional importance.

We observed the second most significant association at rs1713985 on chromosome 4q12. Based on the LD block and the $-\log_{10} P$ plot of chromosome 4q12 obtained by the GWAS data, we found that rs1713985 represents an LD block that spans from 57.421–57.611 Mb and includes four genes, *REST*, *C4orf14*, *POLR2B* and *IGFBP7* (**Fig. 1b**). Then, we selected and genotyped 120 SNPs around this LD block based on the data from the HapMap phase 2 Japanese population with MAF ≥ 0.05 (**Supplementary Table 5b**). However, this analysis could not narrow down the candidate region because of the long-range LD. According to the global expression profiles in GEO database, all four genes were expressed in human adult RPE[19] and rod photoreceptors in mice[20]. *REST* is a transcriptional repressor that may act as a master negative regulator of neurogeneisis. Overexpression of the *C4orf14* gene product has been reported to induce apoptosis by regulating mitochondrial nitric oxide and calcium[26]. The *IGFBP7* gene product, angiomodulin, is reported to bind chemokines and growth factors including vascular endothelial growth factor A, whose expression is high in RPE cells of AMD.

In conclusion, our data showed that *TNFRSF10A-LOC389641* on chromosome 8p21 and *REST-C4orf14-POLR2B-IGFBP7* on chromosome 4q12 are new susceptibility loci for exudative AMD in the Japanese population. Further functional studies are necessary to clarify the mechanisms of these loci on the susceptibility to exudative AMD.

**URLs.** GEO, http://www.ncbi.nlm.nih.gov/geo/; R statistical environment version 2.7.0, http://www.r-project.org/; PLINK 1.05, http://pngu.mgh.harvard.edu/~purcell/plink/; Genetic Power Calculator, http://pngu.mgh.harvard.edu/~purcell/gpc/; EIGENSTRAT, http://genepath.med.harvard.edu/~reich/Software.htm.

## METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

**Accession codes.** The expression microarray data on the human adult RPE and rod photoreceptors in mice have been deposited in the GEO database under accession numbers GSE18811 and GSE22317.

*Note: Supplementary information is available on the Nature Genetics website.*

ACKNOWLEDGMENTS

We thank all of the subjects who participated in this study. We are grateful to A. Yoshida, K. Kano, S. Kawahara, R. Arita, K. Ishikawa, E. Hasegawa, R. Asato, S. Notomi, T. Asakuma and A. Kuni of the Kyushu University, K. Horie-Inoue, S. Inoue and T. Awata of the Saitama Medical University, H. Bessho, N. Kondo and W. Matsumiya of the Kobe university and M. Inoue of the Yokohama City University Medical Center for collecting samples. We thank the staff of the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, the staffs of the BioBank Japan project and the members of the Rotary Club of Osaka-Midosuji District 2660 Rotary International in Japan. We want to express special thanks to F. Miya for the support of gene expression data. This work was conducted as a part of the BioBank Japan Project and supported by the Ministry of Education, Culture, Sports, Sciences and Technology of the Japanese government.

AUTHOR CONTRIBUTIONS

S.A., T.I., Y.N. and M.K. designed the study. S.A., N.H., K.A., T.A. and M.K. performed genotyping. S.A. and M.K. wrote the manuscript. A.T. performed statistical analysis at the genome-wide phase. Y.N. and M.K. managed DNA samples belonging to BioBank Japan. T.I. and Y.N. obtained funding for the study. M.Y., Y.O., S.Y. and H.E. collected GWAS samples. T.T., K.M., S.H., A.N., A.A. and K.K. collected case samples for the replication study. Y.K., N.K., Y.N. and M.K. supervised the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Samples.** Characteristics of the study subjects are shown in **Supplementary Table 6**. For the GWAS, 827 individuals with exudative AMD were collected at Kyushu University. The diagnosis of exudative AMD was based on comprehensive ophthalmic examination, including fluorescein angiography and indocyanine green angiography findings and optical coherence tomography after pupil dilation. We classified exudative AMD into four subtypes under established criteria[27–30]: typical neovascular AMD (t-AMD), polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP) and unclassified. In 827 individuals from the GWAS, we found 298 cases with t-AMD, 480 with PCV, 14 with RAP and 23 unclassified cases. Among these, we found 12 cases that had different subtypes in each eye. Cases with other macular diseases such as high myopia, angioid streaks and central serous chorioretinopathy were excluded. For the control subjects, we used genome-wide screening data of BioBank Japan samples, which consists of 2,421 individuals with thirteen diseases and 902 healthy volunteers recruited from Osaka-Midousuji Rotary Club, Osaka, Japan[31]. Disease status in the control group did not affect the MAFs of the SNPs (**Supplementary Table 7**).

For the replication study, 709 individuals with exudative AMD were recruited at Saitama Medical University ($n = 396$), Kobe University ($n = 212$) and Yokohama City University Medical Center ($n = 101$) under the same criteria as the GWAS cases. The numbers of exudative AMD subtypes were $n = 325$ for t-AMD, $n = 358$ for PCV, $n = 3$ for RAP and $n = 23$ for an unclassified subtype. We also used genome-wide screening data of BioBank Japan data, which consists of 15,571 individuals with one of ten diseases (colorectal cancer, breast cancer, prostate cancer, lung cancer, stomach cancer, diabetes, arteriosclerosis obliterans, atrial fibrillation, cerebral infarction and myocardial infarction) as controls.

All control individuals had not had a recent eye examination, and therefore it is unknown what ocular conditions, including AMD, were present in these individuals. Moreover, controls were significantly younger than cases, which indicates that some of the controls will go onto develop AMD as they age, although the incidence of late AMD is low in the Japanese population[32]. Because these limitations will underestimate the impact of SNPs on the development of late AMD, the true associations may be stronger than those shown in this study.

All participants provided written informed consent to participate in this study. This study was approved by the ethical committees of Kyushu University, Saitama Medical University, Kobe University, Yokohama City University Medical Center, Institute of Medical Science, the University of Tokyo and the RIKEN Yokohama Institute.

**SNP genotyping.** For the GWAS, we genotyped 832 individuals with exudative AMD using an Illumina Human610-Quad BeadChip, and we genotyped 3,323 controls using the Illumina HumanHap550v3 BeadChip. Although the call rate was ≥0.98 for all cases and controls, five cases were excluded because of paired closely related samples. Among the common SNPs in both BeadChips, 457,489 SNPs in the autosomal chromosomes passed the quality control filters (call rate ≥0.99 in both cases and controls and Hardy-Weinberg equilibrium $P \geq 1.0 \times 10^{-6}$ in controls) and were further analyzed. For the replication study, we selected 146 SNPs that showed an age- and sex-adjusted $P < 1.0 \times 10^{-4}$ in the GWAS. Among these SNPs, 22 were located at previously reported loci[7–12] and were excluded from further study. We calculated the LD coefficient ($r^2$) between the remaining SNPs and selected the 77 SNPs with the lowest $P$ value within each region of $r^2 \geq 0.8$. In the replication study, we genotyped an additional panel of 709 individuals with exudative AMD using the multiplex PCR-based Invader assay[33] (Third Wave Technologies). We regarded genotyping as having been successful when the number of undetermined samples was less than 10 in a 384-well plate.

**Fine mapping and resequencing.** For the fine mapping, we used all case and control samples in the GWAS. Tagging SNPs were selected from those with MAF ≥ 5% in the region of interest based on the HapMap phase 2 JPT population. Resequencing of the candidate regions was performed in 48 exudative AMD cases by using an ABI3730 Genetic Analyzer.

**Statistical analysis.** In all stages, the associations of each SNP were assessed by age- and sex-adjusted logistic regression analysis under an additive model. The combined analysis of the GWAS and the replication study was conducted using the inverse variance method. Heterogeneities across the population were estimated formally by using a Cochran's $Q$ test. To characterize the population structure, we computed genome-wide average identity by state and performed PCA using the EIGENSTRAT program (see URLs). GWAS and replication data were calculated using R statistical environment version 2.7.0 or PLINK 1.05 software[34]. Haploview software was used to analyze LD values[35].


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*Molecular Vision* 2011; 17:977-982 <http://www.molvis.org/molvis/v17/a108>
Received 7 December 2010 | Accepted 15 April 2011 | Published 20 April 2011



# The association of age-related maculopathy susceptibility 2 polymorphisms with phenotype in typical neovascular age-related macular degeneration and polypoidal choroidal vasculopathy

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**Purpose:** To determine the association of age-related maculopathy susceptibility 2 *(ARMS2)* gene polymorphisms with the phenotype of typical neovascular age-related macular degeneration (tAMD) and polypoidal choroidal vasculopathy (PCV) and the effects of photodynamic therapy (PDT).

**Methods:** The single nucleotide polymorphisms at rs10490924 (A69S) in *ARMS2* of 68 tAMD and 119 PCV patients who underwent PDT were genotyped using the TaqMan assay. The baseline best corrected visual acuity (BCVA) and lesion size were compared among the three genotypes at rs10490924. A multivariate regression analysis was performed to evaluate the influence of the baseline BCVA, greatest linear dimension (GLD), and lesion phenotype (tAMD or PCV) on the association of rs10490924 with the BCVA 12 months after the first PDT.

**Results:** The mean lesion size was significantly different among the GG, GT, and TT genotypes at rs10490924 in the PCV group, although no significant differences were detected in the tAMD group. PCV patients with a G allele had significantly better vision at 3 months after the initial PDT. tAMD patients with a TT genotype had significantly poorer vision at 12 months after the first PDT. In the multivariate regression analysis, the additive model of the G allele at rs10490924 was associated with a significantly better BCVA 12 months after the first PDT in tAMD and PCV patients.

**Conclusions:** *ARMS2* variants are likely associated with the phenotype and the effects of PDT in tAMD and PCV.

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 anticipated [2]. Advanced AMD is clinically classified into atrophic AMD and exudative AMD. Exudative AMD is further sub-classified into typical neovascular AMD (tAMD), polypoidal choroidal vasculopathy (PCV), and retinal angiomatous proliferation [1]. These phenotypes are known to have different characteristics in their natural courses and responses to interventions, such as photodynamic therapy [3,4] and antivascular endothelial growth factor (VEGF) therapy [5], although the reasons are unknown. Recently, several genetic association studies were conducted to explain the different characteristics among the phenotypes of exudative AMD [6, 7]and their susceptibility to several interventions, including photodynamic therapy [8]. A report suggested that genetic variants of rs10490924 (A69S) in the age-related maculopathy susceptibility 2 (*ARMS2*) gene were associated with lesion size in PCV cases [9]; however, no replication studies have been reported to date.

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PCV is known to have a better response to photodynamic therapy (PDT) than tAMD [3,4], but the reason for this is not understood. Moreover, there is some heterogeneity in the response to PDT among PCV patients [10]. Recent reports have evaluated the association of genetic variants of rs10490924 (A69S) in the *ARMS2* gene with the effects of PDT in neovascular AMD, but a positive association was not found [11,12]. However, a more recent report demonstrated the association of rs10490924 variants with the effects of PDT in Japanese PCV patients [13,14]. They suggested that more studies are needed to confirm their findings.

In this study we investigated the association of rs10490924 (A69S) in *ARMS2* with the phenotypes and visual outcomes of PDT in tAMD and PCV patients in our Japanese cohort.

## 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.

One hundred and eighty-seven consecutive patients with 68 tAMD (mean age±standard deviation [SD], 76±7 years; ratio of men to women, 53:15) and 119 PCV (mean age±SD, 73±8 years; ratio of men to women, 96:23) who underwent

TABLE 1. CLINICAL DETAILS OF THE TYPICAL NEOVASCULAR AGE-RELATED MACULAR DEGENERATION (tAMD) PATIENTS STRATIFIED BY THE GENOTYPE OF RS10490924 IN THE AGE-RELATED MACULOPATHY SUSCEPTIBILITY 2 *(ARMS2)* GENE.

| n=68 | G/G | G/T | T/T | P |
|---|---|---|---|---|
| Gender (female/male) | 0/8 | 9/25 | 6/20 | 0.26† |
| Age (mean±SD, years) | 76±5 | 76±6 | 76±8 | 0.87* |
| GLD (mean±SD, μm) | 3063±972 | 3358±1206 | 3950±1715 | 0.37* |
| Number of PDT sessions/year (mean±SD) | 1.6±0.9 | 1.9±1.0 | 2.1±1.0 | 0.57* |
| Baseline BCVA log MAR (mean±SD) | 0.87±0.51 | 0.76±0.36 | 0.78±0.42 | 0.96* |
| 12Mo BCVA log MAR (mean±SD) | 0.66±0.40 | 0.84±0.49 | 1.03±0.39 | 0.09* |
| 12Mo-Baseline BCVA log MAR (mean±SD) | −0.21±0.32 | 0.07±0.51 | 0.25±0.42 | 0.01* |

Abbreviations: GLD represents greatest linear dimension, PDT represents photodynamic therapy, BCVA represents best-corrected visual acuity, Mo represents month. † $\chi^2$ test, * Kruskal–Wallis test.

PDT and accepted DNA sampling were retrospectively included in this study. All patients received ophthalmic examinations, including visual acuity measurements, slit-lamp biomicroscopy of the fundi, color fundus photography, optical coherence tomography, fluorescein angiography, and indocyanine green angiography (ICG). The visual acuities were determined using a Landolt C chart and were converted to a logarithm of the minimum angle of resolution for calculations. To classify the patients clearly into tAMD and PCV subgroups, the differential diagnoses were based on ICG [15]. All AMD patients had clear images of choroidal neovascular networks on ICG. The PCV cases showed a choroidal origin of their polypoidal lesions, typically with vascular networks in the posterior poles on ICG and subretinal orange-reddish protrusions corresponding to the polypoidal lesions on ICG. Those patients who received any prior treatment for AMD were not included in this study.

*Photodynamic therapy:* All patients in this study were followed up for at least 12 months after their first session of PDT. PDT was performed with standard procedures described previously [16]. The lesion status was assessed every three months, and treatments were performed again when serious retinal detachment, hemorrhage, or macular edema was recognized and accompanied by a leakage on fluorescein angiography (FA) or a defined lesion was observed on ICG. No patients in this study received other treatments or combined therapy during the follow-up period.

*Genotyping:* Genomic DNA was extracted from the peripheral blood using QIAmp DNA Blood Maxi Kit (Qiagen, Hilden, Germany). Genotyping was performed using TaqMan® single nucleotide polymorphism (SNP) genotyping assays or Custom TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems) in accordance with the supplier's recommendations.

*Indices compared:* The mean age, gender, 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 genotype. As outcomes of the PDT, the BCVA until 12 months after the initial PDT and the number of PDTs performed during the first 12 months of the treatment term were evaluated.

*Statistical analysis:* The parameters were compared among three genotypes using an $\chi^2$ test for gender and a Kruskal–Wallis test for the indices. For the time-course analysis, two time points in each genotype were compared using a paired *t* test (two tailed). P values<0.05 were considered to be statistically significant.

## RESULTS

The clinical details of the tAMD and PCV patients stratified by the genotypes of rs10490924 in *ARMS2* are listed in Table 1 and Table 2, respectively. Patients with a TT genotype showed a significantly larger mean lesion size than those with GG and GT genotypes in the PCV group. In the tAMD patients, the mean lesion size tended to increase with the number of T alleles, although there was no statistical significance.

The mean age, gender, and pretreatment BCVA were not different among the three genotypes in the tAMD and PCV groups. The number of PDT sessions per year tended to increase with the number of T alleles in the tAMD and PCV groups, but no statistical significance was detected. The mean BCVA at 12 months after the first PDT was significantly better for the GG and GT genotypes than the TT genotype in the PCV patients. In the tAMD patients, the mean BCVA tended to be better with an increasing number of G alleles at 12 months after the first PDT, although there was no statistical significance. However, the change of BCVA from the baseline was significantly better for the GG and GT genotypes than the TT genotype in the tAMD and PCV patients. In the time-course analysis, the PCV patients with GG and GT genotypes showed a significant improvement in their mean BCVA at 3, 6, and 12 months post-initial PDT (Figure 1). In contrast, the tAMD patients with a TT genotype showed a significant worsening of their mean BCVA at 12 months post-

TABLE 2. CLINICAL DETAILS OF POLYPOIDAL CHOROIDAL VASCULOPATHY (PCV) PATIENTS STRATIFIED BY THE GENOTYPE OF RS10490924 IN THE AGE-RELATED MACULOPATHY SUSCEPTIBILITY 2 *(ARMS2)* GENE.

| n=119 | G/G | G/T | T/T | P |
|---|---|---|---|---|
| Gender (female/male) | 3/21 | 8/38 | 12/37 | 0.43† |
| Age (mean±SD, years) | 73±9 | 73±7 | 72±8 | 0.88* |
| GLD (mean±SD, μm) | 3475±1615 | 3051±1550 | 4342±1737 | 0.00073* |
| Number of PDT sessions/year (mean±SD) | 1.5±0.7 | 1.5±0.5 | 1.6±0.7 | 0.75* |
| Baseline BCVA log MAR (mean±SD) | 0.79±0.41 | 0.62±0.32 | 0.65±0.34 | 0.27* |
| 12Mo BCVA log MAR (mean±SD) | 0.59±0.50 | 0.47±0.44 | 0.71±0.41 | 0.014* |
| 12Mo-Baseline BCVA log MAR (mean±SD) | −0.20±0.39 | −0.15±0.45 | 0.06±0.41 | 0.012* |

Abbreviations: GLD represents greatest linear dimension, PDT represents photodynamic therapy, BCVA represents best-corrected visual acuity, Mo represents month. † $\chi^2$ test, * Kruskal–Wallis test.

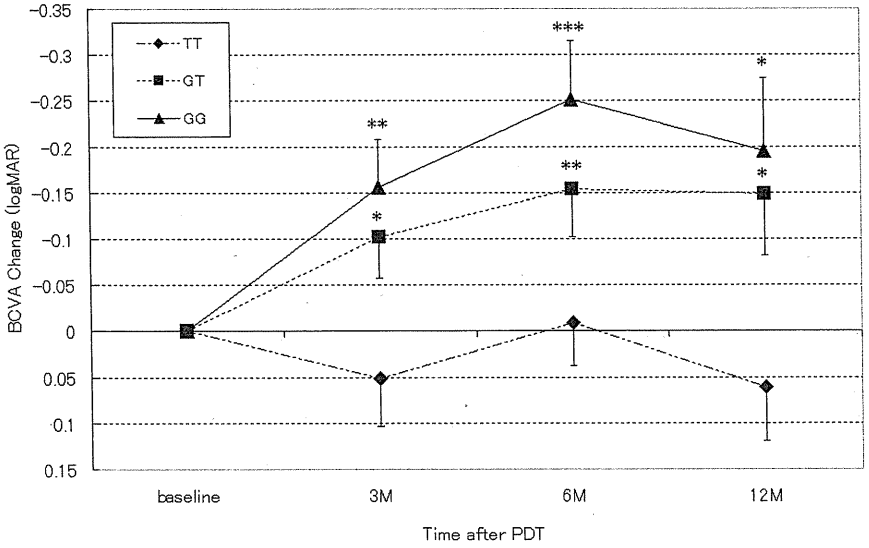


Figure 1. Influence of the genotype at rs10490924 (A69S) in age-related maculopathy susceptibility 2 on the time course of the logMAR baseline best corrected visual acuity (BCVA) value was evaluated in all polypoidal choroidal vasculopathy patients treated by photodynamic therapy (PDT). All values are presented as means±standard error of the mean (SEM). The GG and GT genotypes show significant improvements in their mean BCVA at 3, 6, and 12 months ($p<0.05$, ** $p<0.01$, *** $p<0.001$).

initial PDT (Figure 2). To evaluate the effects of age, pretreatment BCVA, GLD, PDT frequency, and lesion phenotype (tAMD or PCV), which may influence the effects of PDT [7], we performed stepwise multiple regression analyses with backward elimination methods, including those factors in addition to the number of risk alleles as explanatory variables. The results of this analysis conserved the significance of the association of rs10490924 (A69S) with the improvement of the 12 month BCVA after the first PDT (Table 3).

## DISCUSSION

We genotyped the well recognized SNP in *ARMS2* in tAMD and PCV patients who underwent PDT and found that the genotype at rs10490924 (A69S) in *ARMS2* was significantly associated with the lesion size in PCV patients and the visual outcome in tAMD and PCV patients at 12 months after their first PDT. Namely, patients with more G alleles at rs10490924 showed better BCVA values until 12 months after the first PDT.

Recent genetic association studies have performed comparative assessments for the association of rs10490924 (A69S) in *ARMS2* among three different phenotypes of exudative AMD: tAMD, PCV [17-20], and retinal angiomatous proliferation [18]. These studies found that the association of rs10490924 (A69S) in *ARMS2* was consistently higher in tAMD patients than in PCV patients, which suggested heterogeneities in the association of this SNP within the AMD phenotype spectrum. The association of *ARMS2* with the lesion size in PCV has been reported previously, and that study emphasized the necessity of replication studies with other cohorts [9]. In the present study we also found an association of *ARMS2* with the lesion size in PCV that supported the previous report. Although a few previous reports from Western countries failed to demonstrate an association of *ARMS2* with the effects of PDT in neovascular AMD [11,12], we found that the coding variants

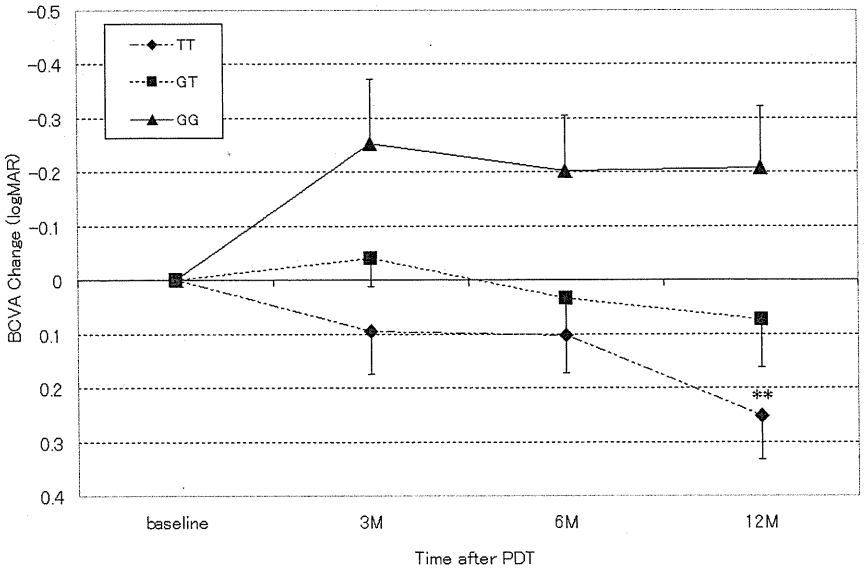


Figure 2. Influence of the genotype at rs10490924 (A69S) in age-related maculopathy susceptibility 2 on the time course of the logMAR best-corrected visual acuity (BCVA) value was evaluated in all typical neovascular age-related macular degeneration (tAMD) patients treated by photodynamic therapy (PDT). All values are presented as means±standard error of the mean (SEM). The TT genotype shows a significant worsening of their mean BCVA at 12 months (** $p<0.01$).

Table 3. Results of the stepwise multiple regression analysis. Prognostic factors for the baseline best-corrected visual acuity (BCVA) at 12 months after photodynamic therapy (PDT) including all phenotypes.

| Prognostic factors | SPRC | SEM | p value | 95%CI |
|---|---|---|---|---|
| Number of risk (T) alleles at rs10490924 in *ARMS2* | 0.17 | 0.041 | 0.0095 | 0.027–0.19 |
| Number of PDTs | 0.16 | 0.036 | 0.0184 | 0.015–0.16 |
| GLD | 0.15 | 0.00 | 0.0266 | 0.000–0.0001 |
| Lesion phenotype (tAMD=0, PCV=1) | −0.21 | 0.062 | 0.0017 | −0.32–0.075 |
| Baseline BCVA logMAR | −0.4 | 0.080 | <0.0001 | −0.65–0.33 |

Abbreviations: SPRC represents standardized partial regression coefficient, SEM represents standard error of the mean, CI represents Confidence interval, GLD represents greatest linear dimension, BCVA represents best-corrected visual acuity.

at rs10490924 (A69S) in *ARMS2* were significantly associated with the visual outcome at 12 months post-PDT in Japanese tAMD and PCV patients. This may be explained by differences in the proportion of AMD phenotypes (i.e., neovascular AMD versus PCV) in different ethnic groups or explained by differences in the baseline BCVA between the studies, which may influence the post-PDT BCVA [4]. Our results were consistent with previous reports that suggested a positive association of this SNP with the outcome of PDT in PCV [13,14], a major subtype of Japanese AMD [21]. Although we could not detect a significant association of the genotype at rs10490924 with the lesion size in tAMD patients, there was a tendency for the lesion size to increase with an increasing number of T alleles. We hypothesized that the statistical power was not sufficient to detect a significant association of rs10490924 (A69S) in *ARMS2* with the lesion size of tAMD patients in our cohort and that the association of *ARMS2* may be independent of the AMD phenotype (tAMD or PCV). In fact, the multivariate regression analysis revealed that the number of risk alleles at rs10490924 was an independent factor significantly associated with an improved BCVA at 12 months after PDT. A recent study demonstrated the significant association of SNP rs11200638 in the promoter region of the high-temperature requirement factor A1 (*HTRA1*) gene with the visual outcome after PDT in Japanese exudative AMD patients [8]. Their results were similar to our findings, probably because SNP rs11200638 has a very high linkage disequilibrium with rs10490924 in *ARMS2* [17].

The role of *ARMS2* in PDT is unknown. Recent reports demonstrated that *ARMS2* can affect the lesion size in PCV [9], which may influence the visual outcome at 12 months post-PDT [4]. However, in the present study the results of the multivariate analysis showed that the association of the coding variants in *ARMS2* with the effects of PDT was independent of the pretreatment GLD, BCVA, and lesion phenotype. Kanda et al. reported that ARMS2 distributes to the outer membrane of the mitochondria and may be involved in the regulation of oxidative stress [22]. Reactive oxygen species play a key role by which PDT affects neovascular endothelial cells, followed by thrombosis and the occlusion of

neovascular tracts [23]. However, further studies will be needed to elucidate the role of *ARMS2* in the pathogenesis of tAMD and PCV.

The limitation of the present study is the relatively smaller sample size and retrospective nature. A prospective study for the outcome of PDT with a larger population will be needed to disclose further associations of *ARMS2* variants with the effect of PDT in tAMD and PCV patients.

Since PDT is known to induce several gene expression changes in the retina–choroidal complex [23,24], the detailed mechanisms by which multiple genes interact with each other close to the choroidal neovascularization (CNV) is poorly understood. However, the present genetic association study suggests some clinical possibilities that can be applied for personalized therapies in individual tAMD and PCV patients.

## ACKNOWLEDGMENTS

This study was supported by a Grant-in Aid (C) 20592042 (S.H.) from the Ministry of Education, Science and Culture, Tokyo, Japan. The funding organization had no role in the design or conduct of this research.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 15 April 2011. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

# NATURAL COURSE AND FUNDUSCOPIC FINDINGS OF POLYPOIDAL CHOROIDAL VASCULOPATHY IN A JAPANESE POPULATION OVER 1 YEAR OF FOLLOW-UP

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**Purpose:** To evaluate the natural course and possible funduscopic risk factors for polypoidal choroidal vasculopathy in a Japanese population.

**Methods:** The records of 42 eyes from 41 patients (27 men and 14 women) diagnosed as having polypoidal choroidal vasculopathy located in the macula between November 1999 and October 2005 were retrospectively reviewed. The funduscopic findings at the first visit were evaluated. The changes in the best-corrected visual acuity (BCVA) from the baseline to 12 months were analyzed. The lesion types (clustered vs. nonclustered) found on indocyanine green angiography were compared for changes in the BCVA from the initial visit to 12 months.

**Results:** The mean age of the subjects was 73.8 ± 8.0 years. The mean logarithm of the minimum angle of resolution (LogMAR) BCVA was 0.48 ± 4.0 at baseline and deteriorated to 0.75 ± 5.7 after 12 months, which was statistically significant ($P = 0.00075$). The mean LogMAR BCVA in the patients showing "nonclustered" polypoidal choroidal lesions on indocyanine green angiography was maintained for 12 months, while that of the "clustered" group decreased significantly during the same period ($P = 0.0014$).

**Conclusion:** Polypoidal choroidal vasculopathy did not show a favorable outcome in terms of the mean BCVA 12 months after the initial visit. The clustered polypoidal choroidal lesions on indocyanine green angiography may be related to poor prognosis of polypoidal choroidal vasculopathy over the natural course.

**RETINA** 31:1598–1602, 2011

Age-related macular degeneration (AMD) is the leading cause of visual loss in the elderly population in industrial countries.[1] The number of patients with AMD has increased remarkably over the years, and further increases in the number of patients with severe visual impairment because of AMD are anticipated.[2] In particular, the exudative type of AMD showed a poor prognosis that is the main focus of current clinical management. Exudative AMD is subcategorized into three phenotypes: typical AMD, polypoidal choroidal vasculopathy (PCV), and retinal angiomatous proliferation.[1] Polypoidal choroidal vasculopathy has been characterized by orange-colored protrusions on funduscopic examinations,[3,4] which show a polyplike pooling of the dye at the terminals of the choroidal branching vascular network on indocyanine green angiography (ICG).[3–6] Polypoidal choroidal vasculopathy accounts for approximately 8% to 13% of white patients and more than 50% of Japanese patients diagnosed with exudative AMD.[7,8]

The incidence and natural course of PCV have been described previously, in which various outcomes were reported.[9–12] For example, some patients showed spontaneous resolutions without any treatment, but others showed acute deterioration of subretinal hemorrhage and lost their visions. However, no

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The authors have no conflicts of interest to disclose.

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statistical evidence about the visual prognosis of PCV over its natural course has been reported to date. Although Uyama et al[13] reported that clusters of grapelike collections of small polypoidal choroidal neovascularizations found by ICG were a prognostic risk factor in PCV, the statistical assessment has not been performed yet. In this study, we assessed the clinical outcomes of PCV over its natural course using statistical analysis and evaluated whether ICG findings may be related to the prognosis of PCV.

## Subjects and Methods

The institutional clinical records of patients diagnosed as having subfoveal PCV lesion at the Kobe University Graduate School of Medicine from November 1999 to October 2005 were reviewed. Because photodynamic therapy was not available before April 2004 in Japan, all patients before this period were just observed for the first 12 months from their initial visit. No patient received photocoagulation therapy for subfoveal PCV for the first 12 months in our institute. Fourteen patients after this period were included in the study because they declined photodynamic therapy for personal reasons. All patients received detailed ophthalmic examinations, including visual acuity measurements, slit-lamp biomicroscopy, and color fundus photography. Indocyanine green angiography was performed on 37 patients (38 eyes). The visual acuities were determined using a Landolt C chart and were converted to logarithm of the minimum angle of resolution (logMAR) values for calculation. All PCV subjects included in this study met the criteria for definite cases of PCV, as proposed by the Japanese Study Group of Polypoidal Choroidal Vasculopathy,[14] namely, the patients showed 1) subretinal reddish orange protrusions in fundus examination and/or 2) the characteristic polypoidal choroidal lesions on ICG. Four eyes were matched to the first criterion, and the others were matched to both criteria. The polypoidal choroidal lesions on ICG were further classified as "clustered" and "nonclustered" types, as previously described.[12] Patients with histories of high myopia, retinal vessel occlusion, or uveitis were excluded.

In total, 42 eyes from 41 patients (27 men and 14 women) were included in this study. The age of patients was 73.8 ± 8.0 years (mean ± SD, range, 51–93 years), and the follow-up period was 20.5 ± 6.0 months (mean ± SD, range, 12–36 months). Funduscopic findings at the initial visit were evaluated for the state of retinal pigment epithelial detachment (PED), serous retinal detachment (SD), hard exudate, subretinal hemorrhage, macular edema, subretinal fibrinous material, retinal pigment epithelial abnormality, and macular drusen.

For statistical analysis, we first evaluated the changes in the best-corrected visual acuity (BCVA) from baseline to 12 months over the natural course. The funduscopic findings that might be related to the changes in the BCVA were summarized. Next, the lesion types (clustered vs. nonclustered) found on ICG were compared for changes in the BCVA from the initial visit to 12 months.

All statistical analyses were performed using Stata/SE-11 software (StataCorp LP, College Station, TX). A two-tailed $t$-test was performed to compare any two groups. $P$ values of $\leq 0.05$ were considered to be statistically significant.

## Results

The mean initial logMAR BCVA values of the patients was 0.48 ± 4.0. The summary of the funduscopic findings in the patients with PCV at the initial visit is shown in Table 1. The PED and SD were found in most patients, whereas macular edema and drusen were found in a few patients. On ICG, clustered lesions (19 eyes, 50%) were more frequent than nonclustered lesions (15 eyes, 40%), while polypoidal choroidal lesions were not detectable in 4 eyes (10%). Plots of the baseline and 12-month logMAR BCVA values for the patients with PCV are shown in Figure 1. A number of patients maintained their initial BCVA after 12 months; however, some patients with good BCVA values at the initial visit had their vision deteriorate remarkably. Consequently, the proportion of the BCVA changed during the 12 months (Figure 2). Although the number of cases with logMAR BCVA < 0.3 were same between baseline and 12 months later, what with logMAR BCVA > 0.7 were increased twice. Looking at the changes in the logMAR BCVA, 48% of the cases deteriorated >0.2 logMAR BCVA

Table 1. Funduscopic Findings at the Initial Visit

| Findings | Eyes, n (%) |
|---|---|
| PED | 40 (95) |
| Serous | 12 (29) |
| Hemorrhagic | 6 (14) |
| SD | 38 (91) |
| Subretinal hemorrhage | 26 (62) |
| Hard exudate | 20 (48) |
| Macular edema | 5 (12) |
| Subretinal fibrinous material | 7 (17) |
| Retinal pigment epithelial abnormality | 31 (74) |
| Macular drusen | 6 (14) |

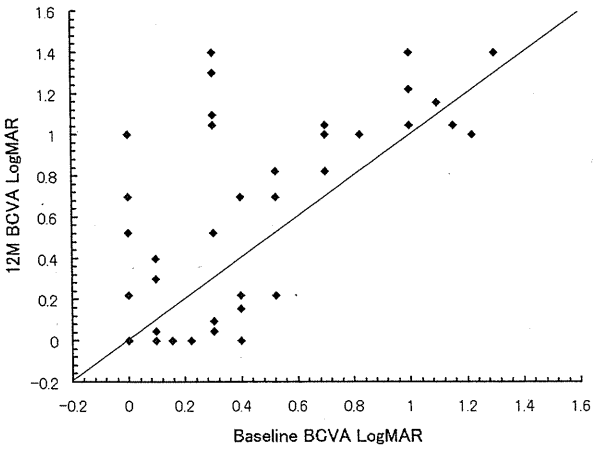


**Fig. 1.** Distribution of the BCVA of patients with PCV at the initial visit and after 12 months. The BCVA values were determined using the Landolt C chart and are presented as decimal visual acuities.

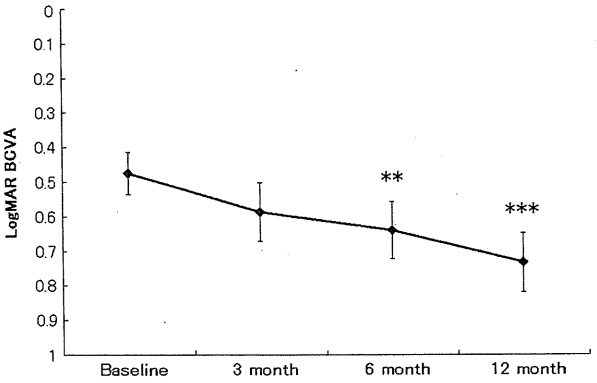


**Fig. 3.** Time course of the logMAR BCVA value in patients with PCV. All values are presented as mean ± standard error of mean. $^{**}P = 0.0063$, $^{***}P = 0.00075$. *P* values are contrasts of the logMAR BCVA at the indicated time to baseline logMAR BCVA.

points after 12 months, while 24% of the cases improved >0.2 logMAR BCVA points during the same period over the natural course. The mean BCVA of all patients gradually decreased and was significantly worse than the baseline BCVA at 6 months and 12 months (Figure 3). The reductions in the BCVA were because of sustained or deteriorating submacular exudation and hemorrhage. In particular, sudden occurrence or deterioration because of subretinal hemorrhage was observed in approximately 33% (14 eyes) of the total patients with PCV in the present study. In these patients, approximately 57% (8 eyes) showed serous PED at their initial funduscopic examination. In contrast, 50% (5 eyes) of the subjects who gained >0.2 logMAR BCVA (10 eyes) showed subretinal fibrinous material on PED at the initial examination. Interestingly, no patients with subretinal fibrinous material (7 eyes) showed a sudden occurrence or deterioration because of submacular hemorrhage. The mean BCVA in the patients showing nonclustered polypoidal choroidal lesions on ICG was maintained for 12 months, while that of the clustered group decreased significantly during the same period (Figure 4).

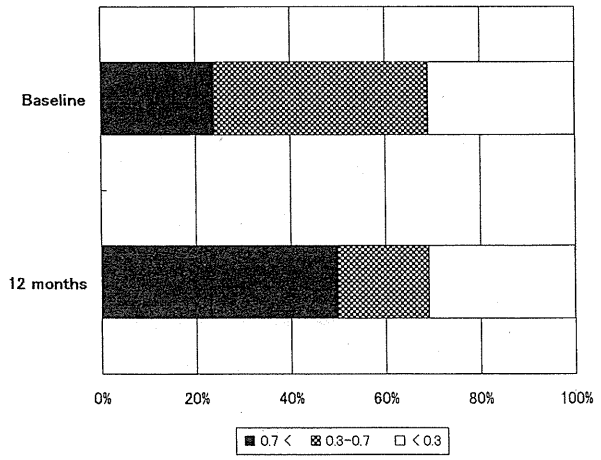


**Fig. 2.** Proportion of the logMAR BCVA values in patients with PCV at the initial visit and after 12 months.

## Discussion

We investigated the natural course of PCV as the changes in the BCVA after 12 months of observation and found that the mean BCVA of the patients with PCV worsened significantly over the natural course. We also found that the clustered polypoidal choroidal lesions on ICG may be related to poor prognosis of PCV over the natural course.

Previous studies have reported that the natural course of PCV is preferable because it often shows spontaneous regression.[5,11,13] However, there was no evidence to support their conclusions because no

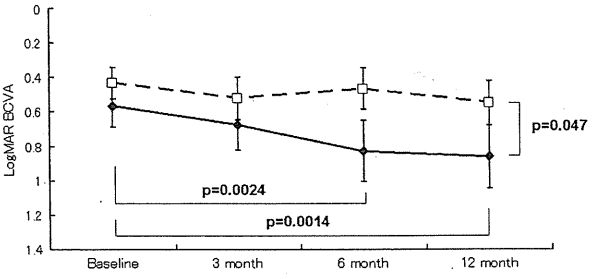


**Fig. 4.** Time course of the logMAR BCVA value in patients with PCV with "clustered" (solid line) and "nonclustered" (dashed line) polypoidal lesions on ICG. All values are presented as mean ± standard error of mean.

statistical analysis was performed. Our results revealed that the prognosis of PCV is variable, but the overall outcome was relatively poor over its natural course. Although more than 20% of patients with PCV gained visual acuities >0.2 logMAR, approximately 50% of the patients lost their visual acuity by >0.2 logMAR during the 12 months after the initial visits. Consequently, the mean BCVA of patients with PCV was significantly reduced after 6 months over its natural course. The presence of serous PED is a suspected risk factor, whereas the presence of subretinal fibrinous material is a possible beneficial factor for the 12-month BCVA in patients with PCV. A previous study has reported that polyp lesions are mostly present at the margin and inside of PED, and serous PED suggests a high infiltrating activity of the PCV.[15] We speculated that the accelerated infiltration from the polyp lesions into the subretinal pigment epithelial space increases the tension on the PED flap and causes PED microlips, followed by an acute decompression of the PED and increased blood flow in the polyp lesions, which then results in the rupture of these polyp lesions and acute severe hemorrhage. In fact, PCV is often associated with systemic hypertension.[16] Two previous studies also reported that subretinal fibrinous material on the PED is often observed in PCV, which exhibits classic choroidal neovascularization on FA.[17,18] Tamura et al[18] reported better outcomes for patients with PCV having subretinal fibrinous material adjacent to polypoidal lesions treated with photodynamic therapy. Although the detailed mechanism of how subretinal fibrinous material contributes to a better prognosis of PCV over its natural course is unknown, we suspect the involvement of an acute inflammatory pathway to produce the fibrinous material in certain cases of PCV[19–21] that often resolves spontaneously, like common inflammatory conditions.[22]

The funduscopic findings at the initial visit may vary depending on when the patients visit the hospital. Although Uyama et al[13] reported that approximately 83% (10 of 12 patients) of patients with PCV showed SD at the first visit, Sho et al[11] reported that approximately 52% of patients with PCV showed SD. We found SD in 91% of the patients and subretinal hemorrhage in 62% of the patients with PCV at our first examination. In addition, clustered grapelike polypoidal lesions on ICG were found more frequently in our cases (50%) than by Uyama et al[13] (25%, 3 of 12 patients). Because most of our patients were first examined by local physicians and then referred to the university hospital on demand, there might be a time lag from the onset of disease to our first consultation. Hence, more advanced cases than in the previous reports might be involved in the present study. In addition, our results supported the previous findings that the clustered lesions found on ICG induced a disadvantage to the visual outcome in PCV.[13]

Recently, a number of therapies for AMD have been developed, and some of them have been reported to be effective for PCV.[23–27] Hence, it is no longer possible to observe untreated patients with PCV in Japan unless their BCVA is not affected. However, understanding the natural course is one of the most important elements in the evaluation of a therapy. We therefore summarized and statistically analyzed the natural course of PCV in our hospital. Hopefully, the present data will be useful to explain the necessity of therapy to patients with PCV.

In conclusion, the mean outcome of PCV, a predominant phenotype of AMD in the Japanese population, is poor. Appropriate therapies should be considered in certain cases with the correct timing.

**Key words:** polypoidal choroidal vasculopathy, natural course, clustered polypoidal lesion, statistical analysis, Japanese.

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