TABLE 5. Age-Specific Prevalence of Late Age-Related Macular Degeneration in Various Populations

	Nagahama[a]	Hisayama[10]	Los Angeles[23]	Singapore[7]	Blue Mountains[28]	Beaver Dam[29]	Baltimore[27]	Barbados[30]
N	6065	1486	6357	3280	3632	4752	1843	3444
Ethnicity	Japanese	Japanese	Latino	Malay	White	White	Black	Black
Years	2008-2010	1998	2000-2003	2004-2006	1992-1994	1988-1990	1985-1988	1988-1992
Age, y								
50-59 (95% CI)	0.39 (0.02-0.77)	0.45	0.22	0.21	0.0	0.2	0.35	0.7
60-69 (95% CI)	0.53 (0.26-0.80)	0.88	0.26	0.39	0.5	0.8	0.42	0.4
70-79 (95% CI)	0.99 (0.35-1.63)[b]	0.51	1.50	2.49	2.6	3.7	0.00	1.0
Sex								
Male (95% CI)	0.73 (0.28-1.18)	1.2	0.53	0.46	1.3	1.2	-	0.36
Female (95% CI)	0.30 (0.13-0.48)	0.34	0.38	0.22	2.4	1.9	-	0.89

CI = confidence interval.
[a]The prevalence was standardized to the World Health Organization standard population.
[b]The last age group is 70-74 years.

The lesions of late AMD have been defined and graded similarly in most population studies. The age-specific prevalence of late AMD in various populations is shown in Table 5. Although the small number of cases in each study limits these comparisons, the age-specific prevalence of late AMD in Japanese subjects aged <70 years was comparable with that reported in other populations.[7,10,23,27–30] However, the age-specific prevalence of late AMD in subjects aged 70-79 years was relatively lower than that in the other populations. Caution should be exercised when interpreting our data for the oldest age group because we evaluated subjects aged 70-74 years, which would underestimate the prevalence of AMD in elderly Japanese people. However, considering that a recent meta-analysis in whites reported the predicted late AMD prevalence at 70 and 75 years as 1.4% and 2.8%, respectively, the current study suggests that the prevalence of late AMD is lower in elderly Japanese than in elderly whites.[31] This difference among age groups might be linked to the exceptional change in circumstances in Japan that would lead to potential differences in the lifestyles of these groups; for example, participants aged 66 or younger were born after the end of World War II.

In the present study, the prevalence of early and late AMD was higher in men than in women ($P = .0007$ and $P = .025$, respectively). These results are consistent with those of previous studies in Asian populations, which reported a higher prevalence of AMD among men than among women.[7,11,32,33] Although it is speculated that the reason for this disparity is the higher smoking rate in Asian men compared to women, these sex differences remained in this study even after adjusting for smoking status ($P = .0128$). A similar association was found in LALES.[23] The reason for the higher prevalence of AMD in Japanese men is unclear. A previous genetic study in Japanese subjects[34] may provide insight into this observation because this study suggested that sex had the greatest effect on the development of PCV. In this study, we found sex differences in the prevalence of RPE abnormalities in all age groups. Similar results have been consistently found in Asians[7,10,11] but not in whites.[28,29] Given that RPE atrophy was a prevailing finding in the fellow eyes of patients with PCV,[35] this difference between Asians and whites regarding the background of RPE abnormalities may be associated with the higher prevalence of the particular phenotype of AMD, such as PCV, in Asian populations. In contrast, we did not find a sex difference in the prevalence of drusen. These results are consistent with those of many studies in white populations[6,28,36,37] but are inconsistent with those of previous Japanese studies[11,24] that reported a sex difference in the prevalence of drusen.

Cigarette smoking is a consistently identified risk factor for AMD.[38–40] Although several previous reports confirmed a link between current smoking and AMD in the Japanese,[10,32] this association has not been studied in detail. In this study, we showed that smoking is associated with the development of both early and late AMD in the Japanese, and this is particularly dependent on the total amount of cigarettes smoked. This observed association for smoking is consistent with many previous studies that reported a dose-response effect in whites.[36,39,40] In addition, a strong association between smoking and RPE pigment abnormalities has been revealed. This association is consistent with the Beaver Dam Eye Study, which suggested that smoking is associated with the incidence and progression of RPE pigment abnormalities.[39] However, because this association failed to reach significance when we divided the subjects by sex, it must be evaluated in a larger cohort to conclude whether an association exists between smoking and pigment abnormalities. In contrast to late AMD, the association between cigarette smoking and drusen remains controversial because of the limited number of previous studies. In the present study, we did not find any association between smoking and the incidence of drusen, which is consistent with the result of the LALES.[41]

One of the potential limitations of our study is that it included a low percentage of the overall population, which may have introduced selection bias. It is speculated that women who did not work full time were more likely to participate, resulting in the high female-to-male ratio of this study. Because this study recruited persons who were able to participate on their own, the participants may have been highly health conscious. Further, people working in government and citizen organizations may have been more likely to participate in this study. Finally, people who could not read or move on their own would have experienced difficulty participating in this study, and this bias may have resulted in an underestimated prevalence of late AMD in the Japanese population. However, because the symptoms of early AMD are usually not obvious[2] and would not affect study participation, the magnitude of the selection bias on early AMD prevalence should be negligible. Another limitation was the lack of a detailed evaluation for the subtypes of late AMD (ie, PCV) because of the limited examination in our cohort. A study in which further ophthalmic examinations are performed in the general population is required to identify the prevalence and rate of AMD subtypes in the Japanese population.

Previous reports revealed that early signs of AMD are strong predictors of subsequent advanced stage. The reported 5-year-risk estimates for the development of advanced AMD for each of the scores from 0 to 4 are 0.4%, 3.1%, 11.8%, 25.9%, and 47.3%, respectively.[3] In our study, 1.2% of men aged 70-74 years had a score of 4. If our data are generalizable to all Japanese people, we anticipate that an increased number of Japanese individuals, particularly men, will have late AMD (see Supplemental Figure, available at AJO.com). Applying the reported estimates to our data indicates that a total of 3.1% of men aged 70-74 years may develop advanced AMD in 5 years.

In summary, our study involving >6000 participants aged ≥50 years provides the first evidence of the age-specific prevalence and detailed characteristics of phenotypes of AMD in the Japanese population. We found that the rates of early AMD in the Japanese population are comparable to those of white populations and that the rates of late AMD were comparable to those of white populations aged <70 years but were relatively lower in those aged ≥70 years. Further, we found a male-dominant prevalence of RPE pigment abnormalities associated with cigarette smoking. In the Nagahama study, follow-up examination will be carried out 5 years after the baseline survey. Further studies with longitudinal progression of phenotypes of AMD are needed to estimate the relative risk of developing late AMD in the Japanese.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST and none were reported. This study was partly supported by grants-in-aid from the following organizations: the Ministry of Education, Culture, Sports, Science and Technology of Japan (2006-2012); the Japan Society for the Promotion of Science (Nos. 19390442, 22791706, and 22791653); the Japanese National Society for the Prevention of Blindness; and the Takeda Science Foundation (2008-2012). The funding agency had no role in the design or conduct of the research presented in this paper. Contributions of authors: conception and design of the study (I.N., K.Y., N.Y.); analysis and interpretation (I.N., K.Y., N.Y.); writing of the article (I.N.); critical revision of the article (I.N., K.Y., A.T., F.M., N.Y.); final approval of the article (I.N., K.Y., H.N., Y.K., M.M., A.T., F.M., N.Y.); and data collection (I.N., K.Y., H.N., Y.K., M.M., A.T., N.Y.).

The authors thank the participants of the Nagahama Study, the Nagahama City Office, and the nonprofit organization "Zero-ji Club for Health Promotion."

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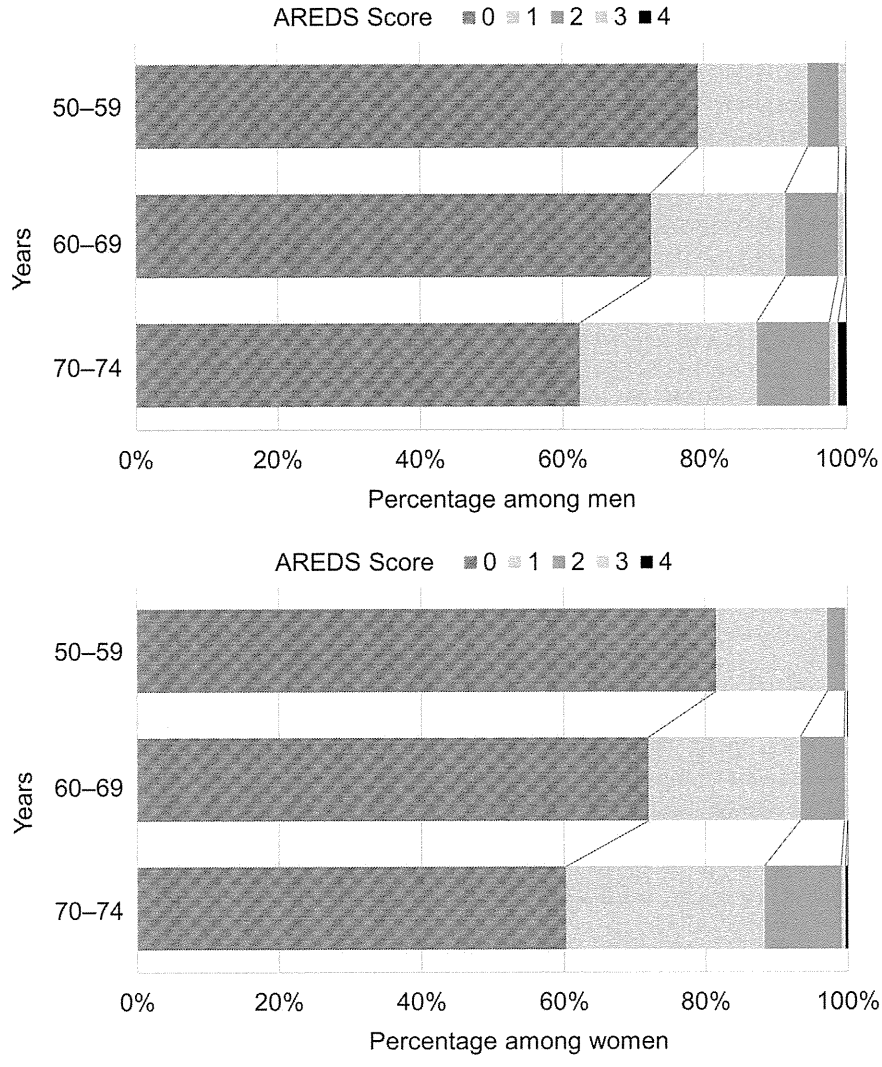

SUPPLEMENTAL FIGURE. Percentages of persons with a risk score for the development of late age-related macular degeneration among men (Top) and women (Bottom) in the Japanese population. Each risk score was calculated by following the severity scale for age-related macular degeneration in the Age-Related Eye Disease Study (AREDS).[3]

SUPPLEMENTAL TABLE 1. Association Between the Brinkman Index and the Risk of Age-Related Macular Degeneration in Japanese

	Brinkman Index[a]			
	Under 500	Over 500	*P* Value	OR (95% CI)
Early AMD	21.7%	25.5%	.011	1.24 (1.05–1.45)
Late AMD	0.43%	0.97%	.042	2.27 (1.03–5.00)

AMD = age-related macular degeneration; CI = confidence interval; OR = odds ratio.

[a]The Brinkman index was calculated by the daily number of cigarettes × years.

SUPPLEMENTAL TABLE 2. Association Between the Brinkman Index and the Phenotype of Age-Related Macular Degeneration in Japanese by Sex

	Male			Female		
	N	Mean BI[a]	*P* Value	N	Mean BI[a]	*P* Value
Total	1977	466.0 ± 451.3		3618	18.6 ± 91.3	
No AMD	1470	461.3 ± 449.6		2849	19.6 ± 94.9	
Early AMD	491	478.7 ± 454.1	.459	756	14.4 ± 76.0	.165
Late AMD	16	511.9 ± 533.7	.655	13	43.5 ± 106.3	.365
Soft drusen			.402			.216
Absent	1212	459.3 ± 447.6		2181	20.1 ± 95.5	
Present	765	476.8 ± 457.1		1437	16.3 ± 84.6	
Large drusen			.414			.260
Absent	1620	462.1 ± 450.8		3004	19.3 ± 94.6	
Present	357	483.7 ± 453.7		614	14.8 ± 73.1	
Pigment abnormality			.500			.145
Absent	1785	463.8 ± 451.3		3419	19.1 ± 92.2	
Present	192	486.9 ± 451.7		199	9.4 ± 74.6	

AMD = age-related macular degeneration; BI = Brinkman index.
[a]The Brinkman index was calculated by the daily number of cigarettes × years.

Association Between the Cholesteryl Ester Transfer Protein Gene and Polypoidal Choroidal Vasculopathy

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See the Appendix for the members of the Nagahama Study Group.

Submitted: January 4, 2013
Accepted: August 8, 2013

Citation: Nakata I, Yamashiro K, Kawaguchi T, et al; for the Nagahama Study Group. Association between the cholesteryl ester transfer protein gene and polypoidal choroidal vasculopathy. *Invest Ophthalmol Vis Sci.* 2013;54:6068–6073. DOI:10.1167/iovs.13-11605

Purpose. To determine whether genetic variants in the lipid-associated genes are related to the risk of developing polypoidal choroidal vasculopathy (PCV) in a Japanese population.

Methods. Five hundred eighty-one patients with PCV and 793 controls were enrolled in the study. Association analysis of allele and genotype frequencies was performed for the following single-nucleotide polymorphisms (SNPs) that are associated with high-density lipoprotein cholesterol levels in blood: rs493258 at the hepatic lipase gene (*LIPC*), rs3764261 at the cholesteryl ester transfer protein gene (*CETP*), and rs12678919 at the lipoprotein lipase gene (*LPL*). A further model adjusting for age-related maculopathy susceptibility 2 (*ARMS2*) A69S, complement factor H (*CFH*) I62V, age, sex, and smoking status was used to confirm the independent association of these SNPs with other covariates.

Results. *CETP* rs3764261 was significantly associated with the development of PCV; the frequency of the minor allele *A* was higher in the PCV cases (24.0%) than in the control subjects (18.5%) ($P = 0.0025$; odds ratio [OR], 1.41; 95% confidence interval, 1.13–1.75). Furthermore, we found an independent association of *CETP* variants with age, sex, smoking status, and genetic background of *ARMS2* A69S, *CFH* I62V, *LIPC* rs493258, and *LPL* rs12678919 ($P = 0.0013$; OR, 1.50). *LIPC* rs493258 and *LPL* rs12678919 did not show significant associations with the development of PCV ($P > 0.05$).

Conclusion. *CETP* variants are associated a risk of developing PCV among the Japanese population.

Keywords: PCV, lipid, *CETP*, case-control study

Polypoidal choroidal vasculopathy (PCV) is characterized by aneurysmal dilations with interconnecting vessels that are best demonstrated by indocyanine green angiography.[1–3] Clinically, PCV is classified into a specific subtype of age-related macular degeneration (AMD), and the incidence of PCV in Asian populations has been reported to be higher than that in Caucasians.[4–6] Controversies exist about the pathogenesis of PCV; whether this condition represents inner choroidal vascular abnormalities or a particular variety of choroidal neovascularization (CNV) remains undetermined. However, because there are apparent differences in the demographic risk profile, clinical course, and visual prognosis, PCV is thought to be a distinct clinical entity.[7] For example, the response to treatment, particularly in photodynamic therapy for PCV, is completely different from that for typical AMD and CNV.[8,9]

Cholesterol and lipids are reported to accumulate underneath the retinal pigment epithelium (RPE) with age. When sufficient debris, including lipids, accumulates and forms a mound between the RPE cell and its basement membrane, it can be seen clinically as drusen. Because many population-based studies have shown the association between drusen and the progression of AMD, drusen is thought to be one of the determinants of both early and late AMD. In fact, an association between high-density lipoprotein (HDL) cholesterol level and the development of AMD has been reported in several studies.[10–12]

Previous studies[13–15] showed that the prevalence of drusen under RPE was reported to be lower in PCV than in AMD. Therefore, the absence of drusen was thought to be one of the criteria necessary to diagnose PCV.[6,15,16] However, the results of a clinical study[16] suggested that drusen is frequently seen in PCV eyes, and several studies[6,17,18] reported that drusen were observed in 20% to 27% of unaffected, fellow eyes in patients with unilateral PCV. Therefore, whether drusen has a functional role in the development of PCV remains controversial.

While previous investigations showed a lower prevalence of drusen among patients with PCV, lipid deposits that distribute from the inner retina to the outer retina are known to be the paramount features of PCV (Figure). Some recent investiga-

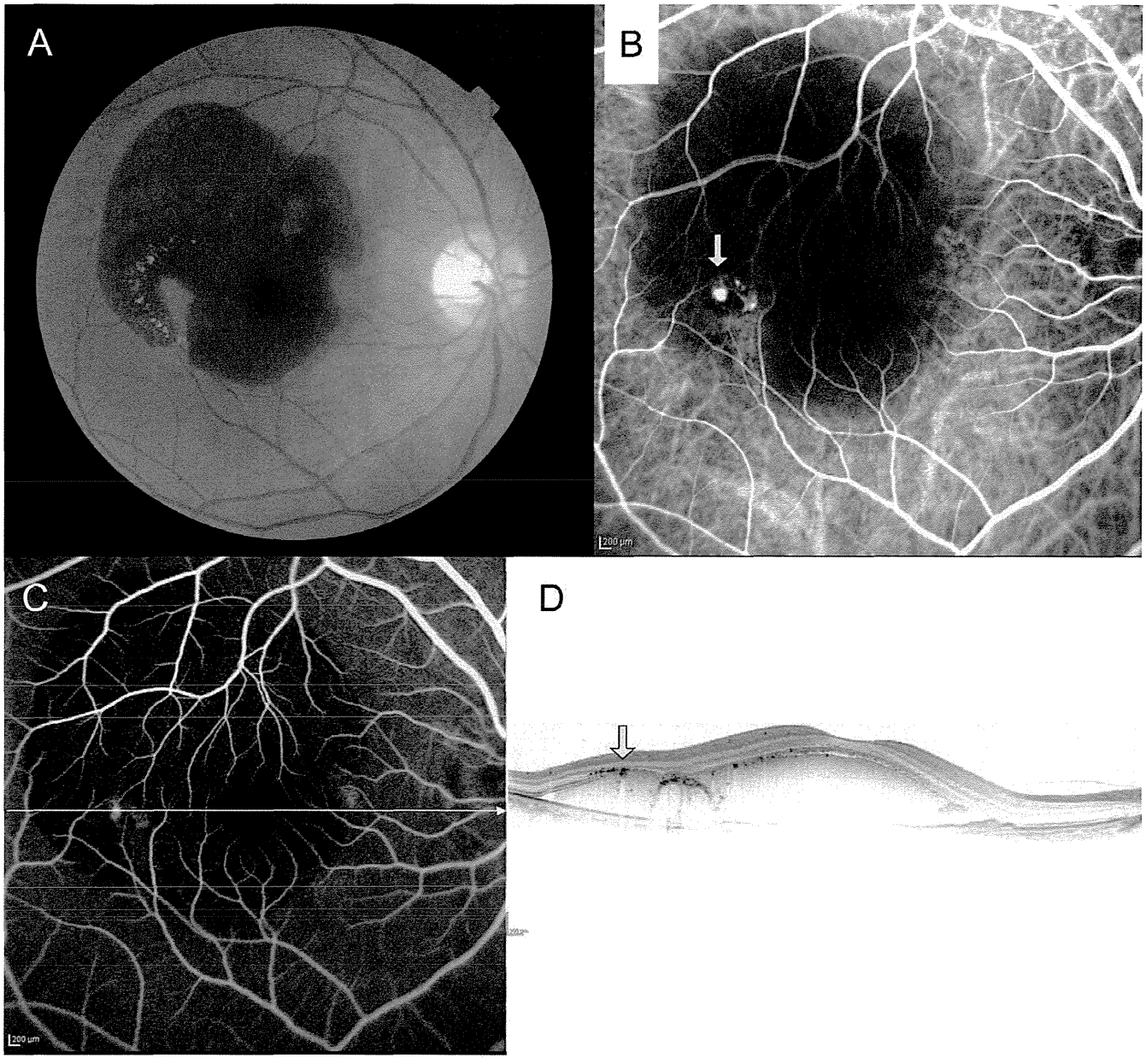

FIGURE. A 64-year-old woman with a typical case of PCV in the right eye. (**A**) Fundoscopic examination shows massive subretinal hemorrhage, lipid deposits, and reddish orange nodules. (**B**) Indocyanine green angiography demonstrates a small branching vascular network terminating in polypoidal lesions (*white arrow*). The speckle noise-reduced spectral-domain optical coherence tomography image of a horizontal section corresponding to the arrow indicated in fluorescein angiography (**C**) shows hyperreflective foci, indicating lipids ([**D**], *arrowhead*), in the outer retina beside the polyp ([**D**], *white arrow*).

tions, including a study[19] in a large cohort of Caucasians, showed significant associations between the lipid-associated genes and the development of AMD. These discoveries of genetic variants in the lipid pathway provided new insight into the pathogenesis of AMD. However, there are limited reports evaluating the association between the lipid-associated genes and the development of PCV. Although several genes are thought to be involved in regulating susceptibility to the development of PCV,[20–23] almost all are identical to those involved in the development of AMD, including the age-related maculopathy susceptibility 2 and high-temperature requirement factor A1 genes (*ARMS2/HTRA1*) locus[24,25] and the complement factor H gene (*CFH*).[26–29] Considering that several studies[13–15] reported a difference in the clinical features of drusen between AMD and PCV, there could be different roles of the lipid-associated genes in these subtypes. Thus, we aimed in this study to determine whether genetic variants in the lipid-associated genes, including variants affecting HDL cholesterol levels, are related to the risk of developing PCV in a Japanese population.

METHODS

All procedures in this study adhered to the tenets of the Declaration of Helsinki, and the ethics committee of each institution involved approved the study protocols. All patients were fully informed about the purpose and procedures of this study, with each patient providing written consent.

Investigative Ophthalmology & Visual Science

TABLE 1. Characteristics of the Study Population

Variable	Cases, $n = 581$	Controls, $n = 793$	P Value
Age, y			
Mean ± SD	72.59 ± 8.13	65.99 ± 4.33	<0.0001
Range	48–92	60–75	
Sex, n (%)			
M	420 (72.3)	326 (41.1)	<0.0001
F	161 (27.7)	467 (58.9)	
Smoking status, n (%)			
Never	200 (38.5)	509 (64.3)	<0.0001
Former	195 (37.6)	176 (22.3)	
Current	124 (23.9)	106 (13.4)	

Five hundred eighty-one patients with PCV were recruited from the departments of ophthalmology at Kyoto University Hospital, Fukushima Medical University Hospital, and Kobe City Medical Center General Hospital. The diagnosis of PCV was based on indocyanine green angiography, which showed a branching vascular network terminating in polypoidal swelling (Figure), and was confirmed by three retina specialists (KY, AT, AO); a fourth specialist (NY) was consulted when the diagnosis could not be agreed on by the initial three reviewers. Patients who had both typical CNV and polypoidal lesions were excluded from this study. The control group consisted of 793 unrelated individuals 60 years or older recruited in the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (the Nagahama Study).[30] Fundoscopic photographs of both eyes confirmed the absence of any signs of AMD (large drusen or pigment change) using the Age-Related Eye Disease Study[31] severity scale, with grading by two independent ophthalmologists (IN, YAK), followed by grading by a senior reviewer (KY).

We targeted three single-nucleotide polymorphisms (SNPs) of three genes reported to be associated with HDL cholesterol levels in blood, including rs493258 at the hepatic lipase gene (*LIPC*), rs3764261 at the cholesteryl ester transfer protein gene (*CETP*), and rs12678919 at the lipoprotein lipase gene (*LPL*).[32] Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). All case samples were genotyped using the Taqman SNP assay with an ABI PRISM 7700 system (Applied Biosystems, Foster City, CA). Controls were genotyped using Human610-Quad BeadChips and HumanOmni2.5 BeadChips (Illumina, Inc., San Diego, CA). *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292) were also genotyped in the same manner. Fasting serum samples from the control subjects were analyzed for HDL cholesterol level, measured using a direct assay system with the selective inhibitory method on an automatic analyzer (LABOSPECT 008; Hitachi, Ltd., Tokyo, Japan). We did not have HDL cholesterol data for the case samples.

Information on smoking status was obtained via a self-reported questionnaire with three categories of never smoker, former smoker, and current smoker. The never smokers were those who had smoked fewer than 100 cigarettes in the past, current smokers were those who had smoked in the past year, and former smokers were those who had quit smoking more than 1 year earlier.

TABLE 3. Logistic Regression Analysis, Including Major Factors Associated With PCV

Variable	P Value*	OR (95% CI)
Age	<0.0001	1.18 (1.16–1.21)
F:M sex	<0.0001	3.16 (2.20–4.52)
ARMS2 rs10490924 (*G/T*)	<0.0001	2.27 (1.86–2.77)
CFH rs800292 (*A/G*)	<0.0001	1.77 (1.43–2.19)
LIPC rs493258 (*G/A*)	0.689	1.05 (0.82–1.35)
CETP rs3764261 (*C/A*)	0.0013	1.50 (1.17–1.92)
LPL rs12678919 (*A/G*)	0.948	0.99 (0.72–1.35)
Smoking (never, former, or current)	0.0107	1.35 (1.07–1.69)

* A logistic regression model was used for covariate adjustment.

Deviations in genotype distributions from the Hardy-Weinberg equilibrium (HWE) of the controls were assessed with the HWE exact test. Statistical differences in the observed allelic distribution were identified using logistic regression analyses with age and sex adjustments, under the assumption of an additive genetic effect where the genotypes of each SNP are coded numerically as 0, 1, and 2 for the number of minor alleles carried. A linear regression analysis was performed to assess the association between HDL cholesterol level and genotype. R software (http://www.r-project.org/ in the public domain) was used for statistical analyses. $P < 0.05$ was considered statistically significant.

RESULTS

Demographics of the study population are given in Table 1. Genotype and allele frequencies of the three SNPs were analyzed in 581 patients with PCV and compared with those of 793 age-matched individuals without any signs of AMD or PCV. The genotyping of all evaluated SNPs had a success rate exceeding 99.4%.

Table 2 gives details of genotype and allele frequencies and summary statistics. The distributions of the genotypes for all evaluated SNPs were in HWE ($P > 0.05$). We found that *CETP* rs3764261 was significantly associated with the development of PCV; the frequency of the minor allele *A* in the patients with PCV (24.0%) was higher than that in the controls (18.5%) ($P = 0.0025$; odds ratio [OR], 1.41; 95% confidence interval [CI], 1.13–1.75). This significant association remained even after a correction for multiple testing ($P = 0.0075$). *LIPC* rs493258 and *LPL* rs12678919 did not show significant associations with the development of PCV ($P > 0.05$).

Next, we conducted a logistic regression analysis that included the effects of the most robust Japanese variants associated with AMD and PCV, *ARMS2* A69S (rs10490924) and *CFH* I62V (rs800292), as well as age, sex, smoking status, *LIPC*

TABLE 2. Distribution of Genotypes and Results of the Association Tests

		Allele		Cases, $n = 581$				Controls, $n = 793$				Association Results*	
Gene	SNP	1	2	11	12	22	MAF	11	12	22	MAF	P Value	OR (95% CI)
LIPC	rs493258	*G*	*A*	32	185	354	0.22	37	259	497	0.21	0.706	1.04 (0.84–1.30)
CETP	rs3764261	*C*	*A*	332	210	33	0.24	528	237	28	0.19	0.0025	1.41 (1.13–1.75)
LPL	rs12678919	*A*	*G*	439	135	3	0.12	602	179	12	0.13	0.883	1.02 (0.77–1.35)

MAF, minor allele frequency.
* Adjusted for age and sex.

rs493258, and *LPL* rs12678919 in the regression model. Table 3 gives the results of the logistic regression analysis. *CETP* rs3764261 remained significant for the development of PCV even after including the effects of these covariates ($P = 0.0013$; OR, 1.50; 95% CI, 1.17–1.92).

Finally, we investigated the role of *CETP* rs3764261 in blood HDL cholesterol level using fasting serum samples from 793 control subjects. The mean ± SD HDL cholesterol level of the control samples was 61.3 ± 16.1 mg/dL. In this analysis, we found that the *A* allele of rs3764261 was associated with the following increases in HDL cholesterol: 59.3 mg/dL for the *CC* genotype, 64.8 mg/dL for the *CA* genotype, and 67.2 mg/dL for the *AA* genotype ($P < 0.0001$).

Discussion

Plasma CETP was first described as a high-molecular-weight protein stimulating the transfer of cholesteryl ester between lipoproteins in plasma of hypercholesterolemic rabbits.[33] Other studies demonstrated various roles of CETP in the lipid pathway: CETP facilitates the transfer of triglycerides and phospholipids[34]; it is an important component of reverse cholesterol transport, which is chiefly characterized by the transport of cholesterol from peripheral tissues to the liver; and it regulates the concentration of HDL cholesterol.[35,36]

After the discovery of the association between HDL cholesterol level and cardiovascular diseases,[37] studies[38,39] evaluated the functional role of the lipid-associated genes that can affect the HDL cholesterol level. Among those genes, the *A* allele of *CETP* rs3764261 was associated with an increase in HDL cholesterol by 5.6 mg/dL among the Japanese population.[40] Herein, we confirmed the role of rs3764261 in increased HDL cholesterol levels among 793 healthy Japanese individuals.

In the present study comparing the allelic distributions of *CETP* variants in a sample of 581 patients with PCV and 793 control subjects, the *A* allele of *CETP* rs3764261 was significantly associated with a risk of developing PCV (OR, 1.41; 95% CI, 1.13–1.75), which indicates a higher level of HDL cholesterol in patients with PCV. In addition, the association of *CETP* variants remained significant even when we adjusted for the effects of other established risk factors for developing AMD and PCV (age, sex, smoking status, and genetic background of *ARMS2* A69S, *CFH* I62V, *LIPC* rs493258, and *LPL* rs12678919). Although the effect of *CETP* variants (OR, 1.50) was not as large as the effects of the major genes associated with AMD and PCV (ORs, 2.27 for *ARMS2* and 1.77 for *CFH*) in this regression analysis, we were able to confirm that *CETP* variants have a significant role in the development of PCV. Our findings for *CETP* rs3764261 were similar to the associations already documented in AMD among Caucasians,[41,42] which suggests that a higher HDL cholesterol level may be a risk factor in both PCV and Caucasian AMD. The hypothesis that a higher level of HDL cholesterol is associated with the development of PCV might appear contradictory to the fact that a lower level of HDL cholesterol is associated with an increased risk of cardiovascular disease. However, despite the well-known antiatherogenic properties of HDL cholesterol, some studies[10,11,43] found elevated levels of HDL cholesterol in Caucasian patients with AMD.

Recently, Zhang et al.[44] reported an investigation of lipid-associated SNPs for PCV and neovascular AMD in a Chinese population. In that article, they showed a significant association of *CETP* with PCV, while no association was found with neovascular AMD. Thus, they concluded that the HDL cholesterol pathway in the pathogenesis of PCV likely differs from that of neovascular AMD. However, the sample size evaluated in the their article was small (204 controls, 250 patients with PCV, and 157 patients with neovascular AMD), which suggests that the negative result of the association between *CETP* and neovascular AMD could have been due to insufficient power to detect the association. To confirm whether the observed association of *CETP* with PCV exists for neovascular AMD as well, we performed an additional analysis using another Japanese cohort of neovascular AMD cases ($n = 452$). In this evaluation, we found a significant association between *CETP* and neovascular AMD ($P = 0.0246$; OR, 1.35).

Adenosine triphosphate-binding cassette, subfamily A member 1 (ABCA1) is also known to be associated with the lipid pathway. Because *ABCA1* has been reported to be another susceptible gene for the development of AMD in Caucasians,[19] we also evaluated whether *ABCA1* rs1883025 has a significant role in the development of PCV but found no significant association with PCV ($P > 0.05$). In previous genome-wide association analyses for HDL cholesterol, the strongest and most consistently associated SNPs have been reported in the *CETP* locus.[45,46] Study[32] findings also suggest that *LIPC* rs493258 and *LPL* rs12678919 are associated with HDL cholesterol level in Caucasians, so the lack of association in the present study could be due to insufficient statistical power or racial/ethnic differences. Further study that includes a larger number of participants is needed to clarify the association between genetic variants of HDL cholesterol-associated genes and the development of PCV.

In the present study, there was a large sex difference between the PCV cases and the general population controls. It remains unknown why there is such a high prevalence of PCV among men. In a previous meta-analysis by Kawasaki et al.,[47] the prevalence of late AMD among Asian women was reported to be much lower than that among Asian men. In contrast, a male predominance was reported in PCV.[4] Considering the high prevalence of PCV among Asian populations, these results suggest that men are more likely to develop PCV. In our study, genetic factors had an enormous influence on whether participants developed PCV (Table 3). However, sex had the largest effect among all covariates on the development of PCV (OR, 3.16). A previous genetic study[23] among Japanese may provide insight into this question because the results suggested that differences in sex would affect phenotypic differences in AMD. Another limitation of the present study was the age difference between cases and controls. Although we enrolled only controls who were 60 years or older, the average age of the control cohort was still younger than that of the case cohort, which means that some of the young controls may develop PCV in the future. To exclude a potential confounder of genetic background with age, a logistic regression analysis adjusting for age and sex was performed in the present study. However, given that the prevalence of late AMD among the Japanese population is reported to be 0.5%,[48] the magnitude of statistical bias of the association analysis is negligible. In addition, considering that case-control association analyses among such subjects are less likely to be statistically significant, our positive results should be acceptable.

Overall, this study provides the first evidence to date that *CETP* variants have a significant role in the risk of developing PCV among the Japanese population. Our study also indicates the same role of HDL cholesterol in both PCV and Caucasian AMD, although the role of fatty acids in Japanese AMD is reported to be different from that in Caucasian AMD.[49] Further studies are needed to increase the understanding of the genetic backgrounds of PCV, as well as the molecular pathogenesis, particularly the role of lipids.

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Acknowledgments

The authors thank the participants of the Nagahama Study, the Nagahama City Office, and the nonprofit organization Zero-ji Club for Health Promotion.

Supported by grants-in-aid from the following organizations: the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2006–2012); Grants 19390442, 22791706, and 22791653 from the Japan Society for the Promotion of Science; the Japanese National Society for the Prevention of Blindness; and Takeda Science Foundation (2008–2012).

Disclosure: **I. Nakata**, None; **K. Yamashiro**, None; **T. Kawaguchi**, None; **N. Gotoh**, None; **H. Nakanishi**, None; **Y. Akagi-Kurashige**, None; **M. Miyake**, None; **A. Tsujikawa**, None; **A. Oishi**, None; **M. Saito**, None; **T. Iida**, None; **R. Yamada**, None; **F. Matsuda**, None; **N. Yoshimura**, None

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Appendix

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Graefes Arch Clin Exp Ophthalmol (2013) 251:1073–1080
DOI 10.1007/s00417-012-2137-y

RETINAL DISORDERS

Two-year outcome of photodynamic therapy combined with intravitreal injection of bevacizumab and triamcinolone acetonide for polypoidal choroidal vasculopathy

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Received: 16 November 2011 / Revised: 6 June 2012 / Accepted: 6 August 2012 / Published online: 25 August 2012

Abstract
Purpose To compare the 2-year results after photodynamic therapy (PDT) alone and PDT combined with intravitreal injections of bevacizumab and triamcinolone acetonide (triple therapy) for polypoidal choroidal vasculopathy (PCV).
Methods We retrospectively reviewed the medical records of 40 consecutive patients (40 eyes) with subfoveal PCV. Of these 40 eyes, 16 were treated with PDT alone and 24 were treated with triple therapy.
Results The change in visual acuity in the triple therapy group was significantly better than that in the PDT group ($P<0.001$). At 24 months, improvement in visual acuity was seen in only two eyes (12.5 %) of the PDT group, while it was seen in ten eyes (41.7 %) of the triple therapy group. Retreatment was given to 12 eyes (75.0 %) in the PDT group and to nine eyes (37.5 %) in the triple therapy group, although the retreatment-free period was significantly longer in the triple therapy group than in the PDT group ($P<0.001$). Post-treatment vitreous hemorrhage was seen in only two eyes (12.5 %), all of which were in the PDT group.
Conclusion Compared with PDT alone, triple therapy appears to reduce the postoperative hemorrhagic complications and recurrences of PCV and to improve the 2-year visual outcomes of PCV.

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Keywords Age-related macular degeneration · Bevacizumab · Photodynamic therapy · Polypoidal choroidal vasculopathy · Triamcinolone acetonide

Introduction

Previously, photodynamic therapy (PDT) with verteporfin was used primarily for the treatment of classic choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD) [1], while today, intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents (bevacizumab or ranibizumab) have become the principal treatment for exudative AMD [2–4]. Three monthly injections of anti-VEGF agents often lead to visual acuity (VA) improvement, but after these initial injections, repeated injections are usually required in order to maintain initial visual recovery. However, repeated injections increase the risk of ophthalmic and systemic adverse events, such as endophthalmitis and stroke [5]. To achieve better visual outcomes with fewer treatments, several reports have shown promising short-term effects of PDT combined with intravitreal injection of bevacizumab and a steroid (triamcinolone acetonide [TA] or dexamethasone) for exudative AMD [6, 7].

Polypoidal choroidal vasculopathy (PCV) is now recognized as a distinct clinical entity, differing in many ways from exudative AMD [8]. PCV is characterized by a branching vascular network that terminates in polypoidal lesions seen on indocyanine green angiography (IA), and is more common in Asians than in Caucasians [9, 10]. It has been reported that the treatment effects of anti-VEGF agents on the vascular lesions of PCV are limited in short-term follow-up [11, 12]. In contrast, a number of studies have shown encouraging results of PDT for the treatment of PCV. A

small number of sessions of PDT causes regression of the polypoidal lesions, and often results in stable, or even improved VA [13–15]. Subsequent reports, however, have shown extensive hemorrhagic complications and recurrences of the polypoidal lesions after the initially successful treatment with PDT [16, 17].

Recently, PDT combined with anti-VEGF agents has been reported to improve the short-term visual outcome in PCV, compared with PDT alone [18–20]. In addition, it has been suggested that this combination therapy may reduce the risk of postoperative hemorrhagic complications [18]. Furthermore, it has been reported that TA suppresses the early proangiogenic response of retinal pigment epithelium (RPE) cells after PDT treatment [21], and that the intravitreal injection of TA per se has a suppressive effect on CNV [22]. For the treatment of PCV, when PDT is combined with an anti-VEGF agent and also with TA, this triple therapy might reduce postoperative complications and the recurrence rate, and lead to a better visual prognosis. To date, however, no information is available on the effects of this triple therapy for PCV. Accordingly, the study described herein aimed to evaluate the long-term results of this triple therapy on symptomatic subfoveal PCV by comparing it with PDT alone.

Patients and methods

For this retrospective study, we reviewed the medical records of 40 consecutive eyes (40 patients) with treatment-naïve subfoveal PCV, who were treated with PDT alone or with PDT combined with an intravitreal injection of bevacizumab and TA (triple therapy) at Kyoto University Hospital between September 2004 and December 2008. PDT alone was performed on 16 patients (PDT group) between September 2004 and December 2007 and PDT combined with bevacizumab and TA was performed on 24 patients (triple therapy group) between January 2008 and December 2008. Inclusion criteria of the study were: (1) symptomatic PCV in patients older than 50 years, (2) the presence of subfoveal vascular lesions, (3) best-corrected VA of 20/25 or worse, and (4) a minimum follow-up of 24 months after initial treatment. Exclusion criteria were: (1) eyes with other macular abnormalities (i.e., AMD, pathologic myopia, idiopathic CNV, presumed ocular histoplasmosis, angioid streaks, and other secondary CNV), (2) any contraindications for fluorescein angiography (FA), IA, or verteporfin, (3) the presence of an RPE tear, (4) any previous treatment for subfoveal PCV, (5) a history of previous vitrectomy, or (6) any other additional therapy during the study period (i.e., anti-VEGF therapy). This study was approved by the Institutional Review Board at Kyoto University Graduate School of Medicine, and adhered to the tenets of the Declaration of Helsinki.

The diagnosis of PCV was based on IA, which shows a branching vascular network terminating in polypoidal dilation. In the present study, pseudophakic eyes were included. When both eyes with PCV that were treated with PDT or triple therapy met the inclusion criteria, only the eye which was treated initially was included in the current study. Some patients in the PDT group were included in a previous study [17].

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected VA with a Landolt chart, determination of intraocular pressure, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, optical coherence tomography examinations, and FA and IA (HRA-2, Heidelberg Engineering, Dossenheim, Germany).

In eyes of the PDT group, standard-fluence PDT was performed using a 689 nm diode laser unit (Visulas PDT system 690S; Carl Zeiss, Dublin, CA, USA) after an injection of verteporfin (Visudyne; Novartis Pharma AG, Basel, Switzerland), according to PDT guidelines for AMD [23]. In eyes that received triple therapy, injection of bevacizumab (1.25 mg) and TA (2 mg) was performed in a sterile manner, and prophylactic topical antibiotics were applied for 1 week after the injection. At 3–4 days after the intravitreal injection, standard-fluence PDT was performed according to PDT guidelines for AMD. The greatest linear dimension was calculated based on FA and IA, as described in detail previously [24]. All polypoidal lesions, the entire branching vascular network, and type 2 CNV detected by FA and IA were included. Serous pigment epithelial detachment was not included in the lesion area when the absence of underlying CNV was confirmed by IA.

After the initial treatment, each patient was scheduled to be seen at 3 months, at which time they again underwent a comprehensive ophthalmologic examination. When IA showed recurrent or residual polypoidal lesions and exudative change was seen on ocular coherence tomography (OCT), retreatment with PDT or the triple therapy was given according to the initial treatment. When residual polypoidal lesions were detected on IA but no exudative change was seen on OCT, no retreatment was given and the patient was reevaluated at the next visit.

All values are presented as mean ± standard deviation. For statistical analysis, best-corrected VA as measured with a Landolt chart was converted to a logarithm of the minimum angle of resolution (logMAR). VA was considered to be improved or deteriorated when the logMAR change was greater than 0.2. On OCT scans, foveal thickness was defined as the distance between the inner surface of the neurosensory retina and the RPE beneath the fovea. In each group, VA or foveal thickness after treatment was studied by one-way repeated measures analysis of variance with the

Table 1 Baseline characteristics of study population

	Photodynamic therapy group	Triple therapy group	*P* value
Number of patients	16	24	
Age (years)	73.3±9.9	73.7±5.8	0.887*
Gender (female/male)	5/11	6/18	0.665†
Initial visual acuity (logMAR)	0.36±0.13	0.50±0.31	0.119*
Initial foveal thickness (μm)	264.9±87.4	392.1±129.5	0.002*
Cystoid macular edema	4 (25.0 %)	11 (45.8 %)	0.182†
Serous retinal detachment	11 (68.8 %)	24 (100 %)	0.003†
Subretinal hemorrhage	8 (50.0 %)	7 (29.2 %)	0.182†
Pigment epithelial detachment	13 (81.3 %)	23 (95.8 %)	0.132†
Greatest linear dimension (μm)	2584±998	3193±1194	0.101*

logMAR, logarithm of the minimum angle of resolution

*Unpaired *t*-test

†Chi-squared test

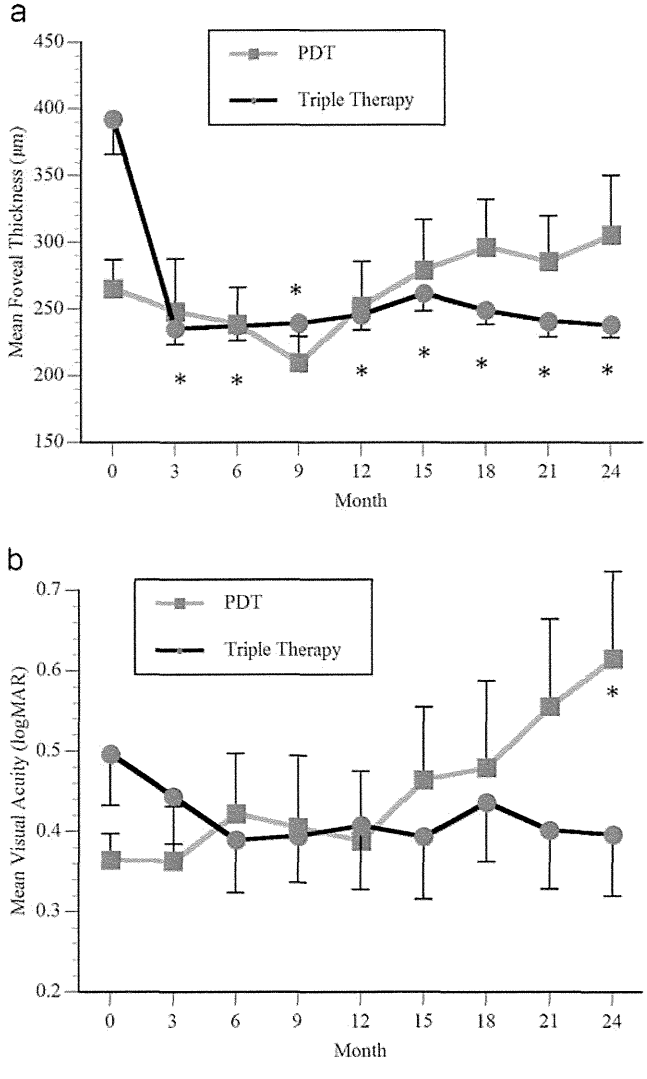

Fig. 1 Mean foveal thickness (**a**) and mean visual acuity (**b**) in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT group) or with PDT combined with intravitreal injections of bevacizumab and triamcinolone acetonide (triple therapy group). Visual acuity is shown in logMAR fashion. *$P<0.05$, compared with pretreatment values. *Error bars* represent the standard error

Dunnett test. To compare VA and foveal thickness between the PDT group and the triple therapy group, two-factor repeated measures analysis of variance was used. The retreatment-free period was calculated from the date of the initial therapy to the date when the treating physician determined the necessity of retreatment by PDT or by triple therapy. Survival analysis using Kaplan–Meier methods was used to compare the difference in the retreatment-free period after initial treatment between the PDT and the triple therapy groups. In patients who underwent no retreatment by either PDT or triple therapy, the retreatment-free period was established at 2 years of follow-up. Descriptive statistics for all demographic and clinical variables were calculated, and comparisons made using the unpaired *t*-test for means with continuous data (e.g., age) and the Chi-squared test for categorical data (e.g., gender). Statview version 5.0

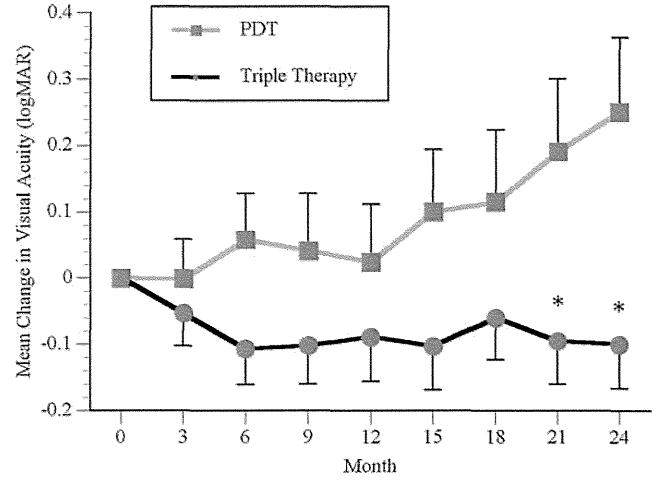

Fig. 2 Change in visual acuity in eyes with polypoidal choroidal vasculopathy treated with photodynamic therapy (PDT group) or with a combination of PDT and intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). Change in visual acuity in eyes treated with triple therapy was significantly better than that in eyes treated with PDT alone ($P<0.001$). Visual acuity is shown in logMAR fashion. *$P<0.05$, compared with values in the PDT group. *Error bars* represent the standard error

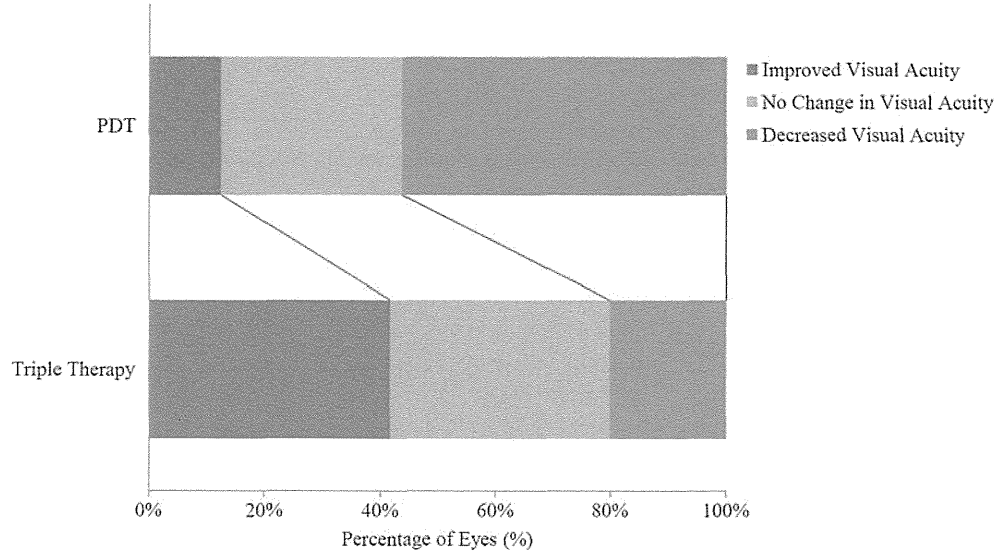

Fig. 3 Percentages of eyes with improved and deteriorated visual acuity at 24 months after treatment. All eyes with polypoidal choroidal vasculopathy were treated with photodynamic therapy (PDT group) or PDT combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). Visual acuity was considered to be improved or deteriorated when the change in logMAR units was greater than 0.2. Improvement of visual acuity was seen more frequently in the triple therapy group (P=0.044)

software (SAS Institute, Inc., Cary, NC, USA) was used for statistical analyses. A P value<0.05 was considered to be statistically significant.

Results

In the study described herein, a total of 40 patients with treatment-naïve PCV were evaluated; 16 eyes which received only treatment with PDT (PDT group) and 24 eyes which received only triple therapy (triple therapy group) during the 24-month study period. Table 1 shows baseline characteristics of each group. There were no significant differences in age, gender, or baseline VA between the two groups. However, baseline foveal thickness of the triple therapy group was significantly larger than that of the PDT group, and the rate of serous retinal detachment was also higher in the triple therapy group.

Figure 1a shows the time-course in mean foveal thickness of each group. In the triple therapy group, the mean foveal thickness decreased immediately after initiation of treatment and remained throughout the 2-year follow-up period. Figure 1b shows the time-course of mean VA in each group. In the PDT group, there was no significant improvement of VA after initiation of treatment; in fact, mean VA was significantly decreased (+0.25±0.45) at 24 months (P=0.041). In contrast, VA somewhat improved after initiation of treatment in the triple therapy group, while the improvement was not statistically significant. Some improvement was maintained throughout the 2-year follow-up period.

Table 2 Final characteristics of study population and complications during study period

	Photodynamic therapy group	Triple therapy group	P value
Number of patients	16	24	
Final visual acuity (logMAR)	0.62±0.43	0.40±0.38	0.099*
Final foveal thickness (μm)	283±204	230±66	0.234*
Final conditions			
Cystoid macular edema	5 (31.3 %)	0 (0 %)	0.003†
Serous retinal detachment	5 (31.3 %)	0 (0 %)	0.003†
Subretinal hemorrhage	2 (12.5 %)	1 (4.2 %)	0.327†
Pigment epithelial detachment	11 (68.8 %)	5 (20.8 %)	0.002†
Polypoidal lesions	3 (18.8 %)	2 (8.3 %)	0.385†
Complications			
Cataract	2 (12.5 %)	1 (4.2 %)	0.327†
Suprachoroidal hemorrhage	1 (6.3 %)	0 (0 %)	0.215†
Vitreous hemorrhage	2 (12.5 %)	0 (0 %)	0.076†
Tear of retinal pigment epithelium	1 (6.3 %)	0 (0 %)	0.215†
Number of photodynamic therapy or triple therapy sessions (range)	2.19±0.91 (1–3)	1.50±0.78 (1–3)	0.015*
Retreatment-free period (months)	11.7±8.6	20.6±6.8	<0.001‡

logMAR, logarithm of the minimum angle of resolution; VEGF, vascular endothelial growth factor

*Unpaired t-test

†Chi-squared test

‡Survival analysis

Figure 2 shows the change in mean VA from baseline in each group. The mean change in VA in the triple therapy group was significantly better than that in the PDT group ($P<0.001$). At 24 months after initial treatment, mean change in VA in the triple therapy group (−0.10±0.32) was significantly better than that in the PDT group (+0.25±0.45, $P=0.007$). Figure 3 shows the percentage of eyes with improved or decreased VA at 24 months; improvement in VA was seen in two eyes (12.5 %) of the PDT group and in ten eyes (41.7 %) of the triple therapy group, and reduction in VA was seen in nine eyes (56.3 %) of the PDT group and in five eyes (20.1 %) of the triple therapy group. Thus, improvement in VA was seen more frequently in the triple therapy group ($P=0.044$).

Table 2 summarizes final characteristics and complications during the study period. In both groups, the polypoidal lesions disappeared after treatment (Fig. 4). At 24 months, complete disappearance of the polypoidal lesions was confirmed in 13 eyes (81.3 %) in the PDT group and in 22 eyes (91.7 %) of the triple therapy group. Fifteen eyes (62.5 %) of the triple therapy group and four eyes (25.0 %) of PDT group underwent a single session of PDT during the 24-month study period ($P=0.020$). Retreatment by PDT was done in 12 eyes (75.0 %) in that group, and nine eyes (37.5 %) in the triple therapy group received retreatment by triple therapy (Fig. 5). The mean numbers of treatment were 2.19±0.91 in the PDT group and 1.50±0.78 in the triple therapy group ($P=0.015$). Figure 6 shows the overall survival analysis curve for the retreatment-free periods in each group, which was significantly longer in the triple therapy group (20.6±6.8 months) than in the PDT group (11.7±8.6 months, $P<0.001$).

In the current study, two eyes (12.5 %) in the PDT group developed cataract and underwent surgery during the study period; additionally, one eye (4.2 %) in the triple therapy group ($P=0.327$) underwent similar surgery. In the PDT group, two eyes (12.5 %) developed a vitreous hemorrhage and one eye developed an RPE tear during the study period, while no eye in the triple therapy group developed a vitreous hemorrhage. No eye underwent glaucoma surgery for ocular hypertension after intravitreal injections of TA.

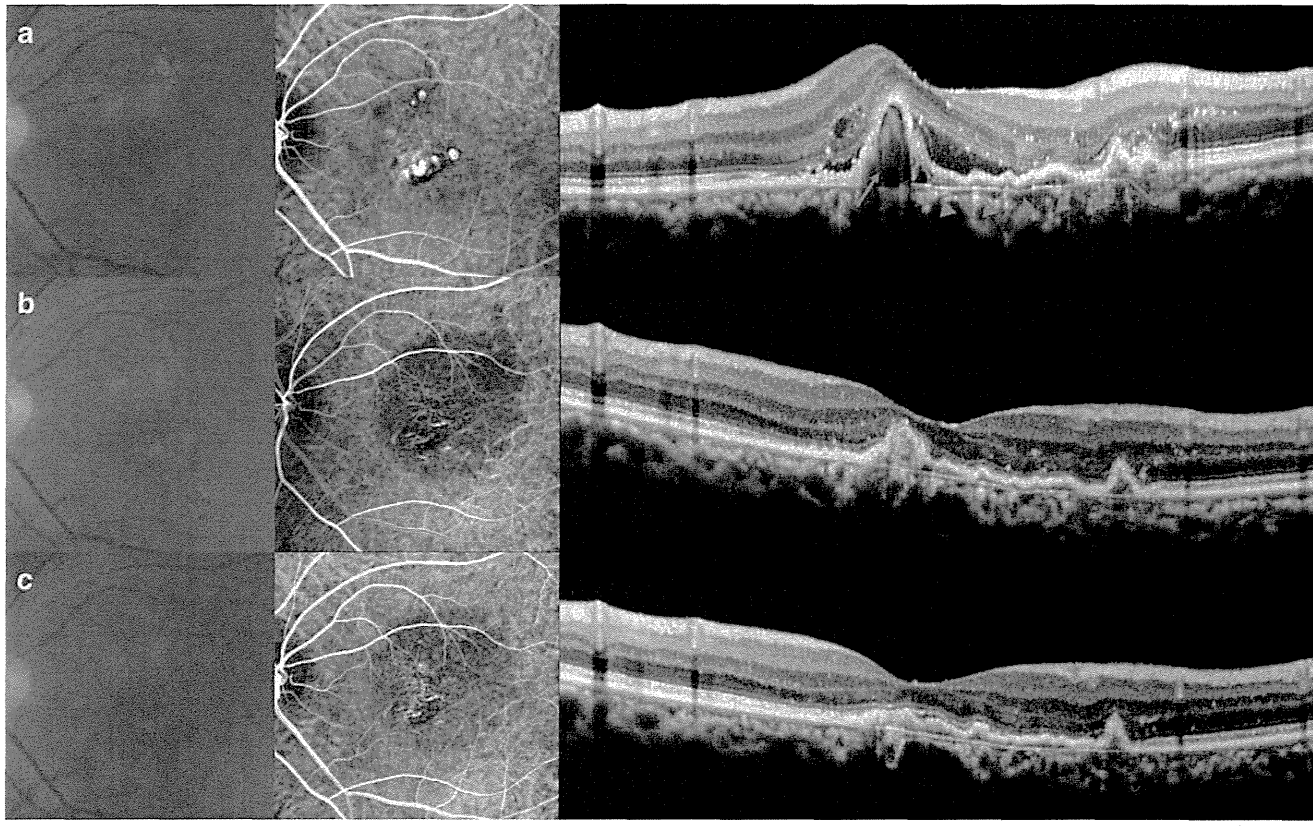

Fig. 4 Polypoidal choroidal vasculopathy successfully treated with photodynamic therapy combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy). **a** Initial fundus photograph (*left*) shows reddish-orange nodules and fibrin exudate (20/50 OS). Indocyanine green angiography (*middle*) shows a branching vascular network that terminates in polypoidal lesions. A vertical optical coherence tomography section through the fovea (*right*) reveals sharp protrusions of the retinal pigment epithelium due to polypoidal lesions (*arrows*). A branching vascular network is seen as flat protrusions (arrowheads). **b** Three months after triple therapy. No polypoidal lesions are seen on indocyanine green angiogram. A branching vascular network is still seen. Protrusions due to polypoidal lesions have become flattened (20/60 OS). **c** 12 months after treatment, no recurrence is seen (20/50 OS)

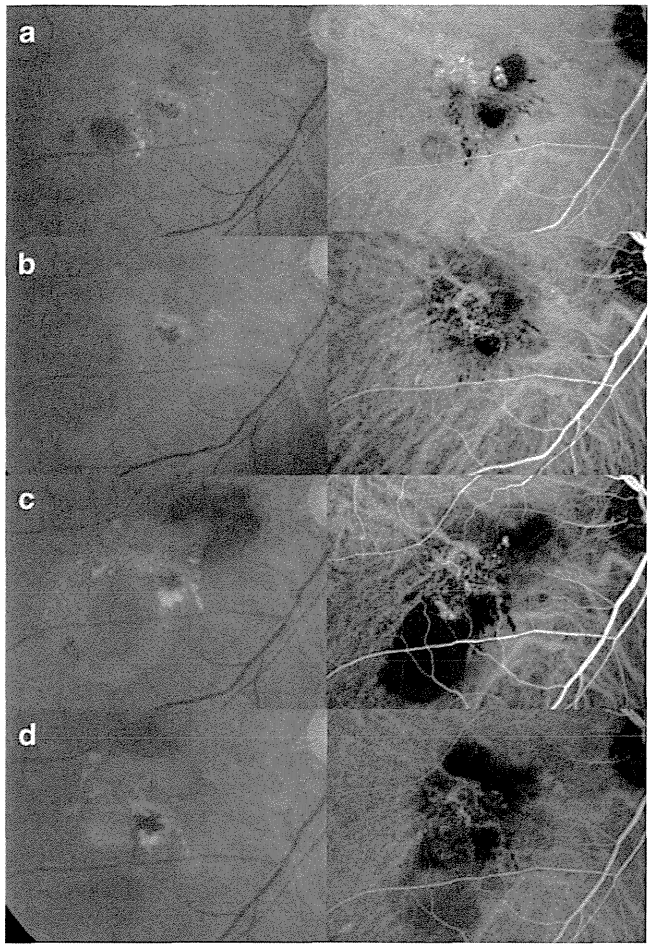

Fig. 5 Recurrence of polypoidal lesions after successful treatment with photodynamic therapy of polypoidal choroidal vasculopathy. **a** Initial fundus photograph (*left*) shows a reddish-orange nodule with a hard exudate and with a subretinal hemorrhage (20/40 OD). Indocyanine green angiography (*right*) shows a branching vascular network that terminates in polypoidal lesions. **b** Three months after photodynamic therapy. Reddish-orange nodules and polypoidal lesions have regressed (20/30 OD). **c** At 12 months after treatment, recurrence has occurred. Fundus photograph shows pigment epithelial detachments with surrounding subretinal hemorrhage and hard exudate (20/100 OD). Polypoidal lesions have recurred at the terminus of the remaining branching vascular network. **d** At 3 months after retreatment with photodynamic therapy, pigment epithelial detachments and polypoidal lesions have completely regressed (20/70 OD)

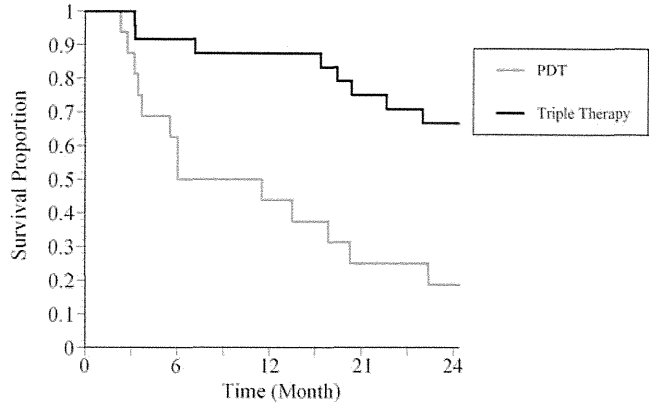

Fig. 6 Overall survival curve for the retreatment-free period in both treatment groups. Eyes with polypoidal choroidal vasculopathy were treated with either photodynamic therapy (PDT group) or PDT combined with intravitreal injection of bevacizumab and triamcinolone acetonide (triple therapy group). The period until the treating physician opted to perform additional treatment was significantly longer in the triple therapy group than in the PDT group ($P<0.001$)

Discussion

Several investigators have reported the short-term outcomes of anti-VEGF agents for the treatment of PCV, and have shown the limited effect of these agents on the vascular lesions of PCV, even though the anti-VEGF agents reduced the exudative change that was due to PCV. It has been reported that complete resolution of the polypoidal lesions was achieved in only 16.1 % of eyes, with a mean of 3.3 injections of bevacizumab over a 12 month period [25]. In an earlier report, although monthly injections of ranibizumab successfully reduced the exudative manifestations of PCV, a reduction in the polypoidal lesions was seen in only 33 % of eyes [11].

In contrast, a number of studies have shown encouraging results of PDT for the vascular lesions of PCV, with complete regression of the polypoidal lesions achieved in many cases with fewer sessions. In a report by Chan et al. [26], PDT led to complete regression of the polypoidal lesions in 95 % of eyes with PCV, and resulted in either stable or improved VA 1 year after treatment in 95 % of eyes. However, a year or more after successful treatment with PDT, recurrences of the polypoidal lesions sometimes cause a substantial decrease in VA. Using Kaplan–Meier methods, Yamashiro et al. reported that the recurrence of polypoidal lesions after successful PDT treatment was estimated to be 11.5 % at 15 months, 20.4 % at 18 months, and 38.8 % at 21 months [16]. Thus, the recurrence of polypoidal lesions after PDT is a major problem in the treatment of PCV.

In the combination therapy, anti-VEGF agents, which can cause rapid reduction of the exudative change, are thought to contribute to the visual recovery that is associated with regression of the polypoidal lesions induced by PDT. Previous experimental studies have shown increased expression of VEGF shortly after PDT treatment [27, 28], which suggests that an intravitreal injection of bevacizumab before PDT may well exert a protective effect. With regard to the injection of TA, Okubo and colleagues reported a case of PCV successfully treated with trans-Tenons retrobulbar injection of TA [29], and Mukai and colleagues reported the protective effects of TA against occlusion of the choriocapillaris which was induced by PDT [30]. However, Lai et al. reported that the adjunctive use of TA during PDT did not appear to result in additional benefit for the treatment of PCV [31], so the effect of TA on PCV remains controversial.

In the current study, the PDT group showed no significant improvement in VA after initiation of treatment; mean VA was significantly decreased at 24 months. In the triple therapy group, however, VA was improved at 3 months after treatment and mean VA was improved by −0.11 (logMAR) at 6 months and by −0.09 (logMAR) at 12 months. In this triple therapy group, although improvement was not statistically significant, at least some improvement in VA was maintained throughout the 2-year follow-up period. At 24 months, VA improvement was achieved in only 12.5 % of eyes in the PDT group and in 41.7 % in the triple therapy group (P=0.044), so, based on our findings, triple therapy for PCV, compared to PDT alone, results in more rapid visual recovery and improved visual outcome at 24 months.

In our case series, both PDT alone and the triple therapy successfully reduced polypoidal lesions and exudative change, with complete disappearance of the polypoidal lesions confirmed at 24 months in 81.3 % of cases in the PDT group and in 91.7 % of cases in the triple therapy group. There were significant differences in the number of eyes with a recurrence and in the number of PDT treatments between the two groups. Furthermore, the retreatment-free period was significantly longer in the triple therapy group (20.6± 6.8 months) than in the PDT group (11.7±8.6 months).

Following treatment for PCV, one of the most vision-threatening complications of PDT is extensive hemorrhage. A previous report of PCV treated with PDT indicated that postoperative subretinal hemorrhage was seen in 28 of 91 eyes, and that bleeding resulted in a vitreous hemorrhage in six eyes [32]. In the current study, no eye in the triple therapy group developed a vitreous hemorrhage, although two eyes in the PDT group developed a vitreous hemorrhage. Recent reports by Gomi et al. [18] and by Sato et al. [19] suggested a lower incidence of subretinal hemorrhage after PDT when it was combined with bevacizumab, and it has been reported that the vasoconstrictive effect of bevacizumab may contribute to the suppression of postoperative hemorrhages [33].

Major limitations of the current study are its retrospective nature and its relatively small sample size. In addition, there were some statistical differences between the two groups, including baseline foveal thickness and the rate of serous retinal detachment, which may affect the response to treatment. Furthermore, this study was not a randomized, comparative trial. However, selection bias is small as both groups consisted of consecutive eyes that were treated at different time periods. Our findings suggest that intravitreal injection of bevacizumab and TA combined with PDT improves the 2-year visual outcome of PCV and may reduce postoperative hemorrhagic complications and the recurrence rate. However, because our findings are based on an observation period of only 24 months, it remains unclear whether triple therapy has a long-term effect.

Another limitation is that the safety and efficacy of the triple therapy were not compared with PDT combined with anti-VEGF therapy. Recently, the EVEREST study has shown the 6-month effects of PDT in combination with ranibizumab for PCV [34], in which the eyes treated with PDT combined with ranibizumab achieved the highest gains at 6 months. However, it remains unclear whether this combination therapy reduces the recurrence of polypoidal lesions after successful initial treatment. Further prospective, randomized, long-term studies are necessary to determine the efficacy and safety of triple therapy for PCV.

Acknowledgments This study was supported in part by the Japan Society for the Promotion of Science (JSPS), Tokyo, Japan (Grant-in-Aid for Scientific Research, no. 21592256), and by the Japan National Society for the Prevention of Blindness, Tokyo, Japan. We thank the following clinicians at Kyoto University Hospital for their assistance in gathering the treatment histories for our study: Hiroshi Tamura, MD, Hideo Nakanishi, MD, Hisako Hayashi, MD, Satoko Nakagawa, MD, Kohei Takayama, MD, Yumiko Ojima, MD, and Takahiro Horii, MD.

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PLOS ONE

Mdm20 Stimulates PolyQ Aggregation via Inhibiting Autophagy Through Akt-Ser473 Phosphorylation

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Abstract

Mdm20 is an auxiliary subunit of the NatB complex, which includes Nat5, the catalytic subunit for protein N-terminal acetylation. The NatB complex catalyzes N-acetylation during *de novo* protein synthesis initiation; however, recent evidence from yeast suggests that NatB also affects post-translational modification of tropomyosin, which is involved in intracellular sorting of aggregated proteins. We hypothesized that an acetylation complex such as NatB may contribute to protein clearance and/or proteostasis in mammalian cells. Using a poly glutamine (polyQ) aggregation system, we examined whether the NatB complex or its components affect protein aggregation in rat primary cultured hippocampal neurons and HEK293 cells. The number of polyQ aggregates increased in Mdm20 over-expressing (OE) cells, but not in Nat5-OE cells. Conversely, in Mdm20 knockdown (KD) cells, but not in Nat5-KD cells, polyQ aggregation was significantly reduced. Although Mdm20 directly associates with Nat5, the overall cellular localization of the two proteins was slightly distinct, and Mdm20 apparently co-localized with the polyQ aggregates. Furthermore, in Mdm20-KD cells, a punctate appearance of LC3 was evident, suggesting the induction of autophagy. Consistent with this notion, phosphorylation of Akt, most notably at Ser473, was greatly reduced in Mdm20-KD cells. These results demonstrate that Mdm20, the so-called auxiliary subunit of the translation-coupled protein N-acetylation complex, contributes to protein clearance and/or aggregate formation by affecting the phosphorylation level of Akt indepenently from the function of Nat5.

Citation: Yasuda K, Ohyama K, Onga K, Kakizuka A, Mori N (2013) Mdm20 Stimulates PolyQ Aggregation via Inhibiting Autophagy Through Akt-Ser473 Phosphorylation. PLoS ONE 8(12): e82523. doi:10.1371/journal.pone.0082523

Editor: Maria Gasset, Consejo Superior de Investigaciones Cientificas, Spain

Received August 6, 2013; **Accepted** October 24, 2013; **Published** December 16, 2013

Funding: This work was supported by Grants-in-Aid for Scientific Research from MEXT Japan (Kiban B) and also, in part, by a fund from Smoking Research Foundation (http://www.srf.or.jp/) and the Asian CORE program of JSPS (to N.M.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Protein modifications, including phosphorylation, acetylation, methylation, ubiquitination, and SUMOylation, are important for maintaining cellular homeostasis, and disruption of protein modifications can lead to cellular dysfunction during aging and the resulting age-related disorders [1-3]. Among the post-translational modifications, protein acetylation controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) participates in transcriptional regulation through controlling the acetylation levels of histone or various transcriptional factors in the nucleus. However, in the cytoplasm, the functional significance of protein acetylation are not well understood except for α-tubulin, which is well known to stabilize microtubule networks [4]. HDAC6 and Sirt2, class II and III HDACs, respectively, deacetylate α-tubulin in the cytoplasm [5,6]. Interestingly, HDAC6 interacts with ubiquitinated proteins and regulates protein degradation using either the ubiquitin-proteasome system (UPS) [7-9] or ubiquitin-selective quality control-autophagy [10]. HDAC6 rescues neurodegeneration and closely links between autophagy and the UPS [11]. SIRT2 is also well studied in the relationship with both brain physiology (physiological brain aging) and aging-related neurodegenerative diseases (Alzheimer disease, poly glutamine (polyQ) disease, Parkinson disease) [12,13]. These findings clearly indicate that acetylation regulates, at least in part, protein clearance to maintain cellular homeostasis during the brain ageing. While recent studies showed that HDAC6 and Sirt2 have no effects on the progression of Huntington's disease using the gene dificient mice [14,15], the relationship of the deacetylases, i.e. HDAC6 and Sirt2 with both acetylation and protein aggreagtes clearance is not brought out and the full repertoire of acetylated proteins and acetylase enzymes is mostly unknown.

Recently, proteomic analyses of acetylated proteins have identified a number of cytoplasmic proteins that include cytoskeletal proteins, molecular chaperones, and ribosomal proteins [16-18]. These data suggest that acetylation occurs in