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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.

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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-

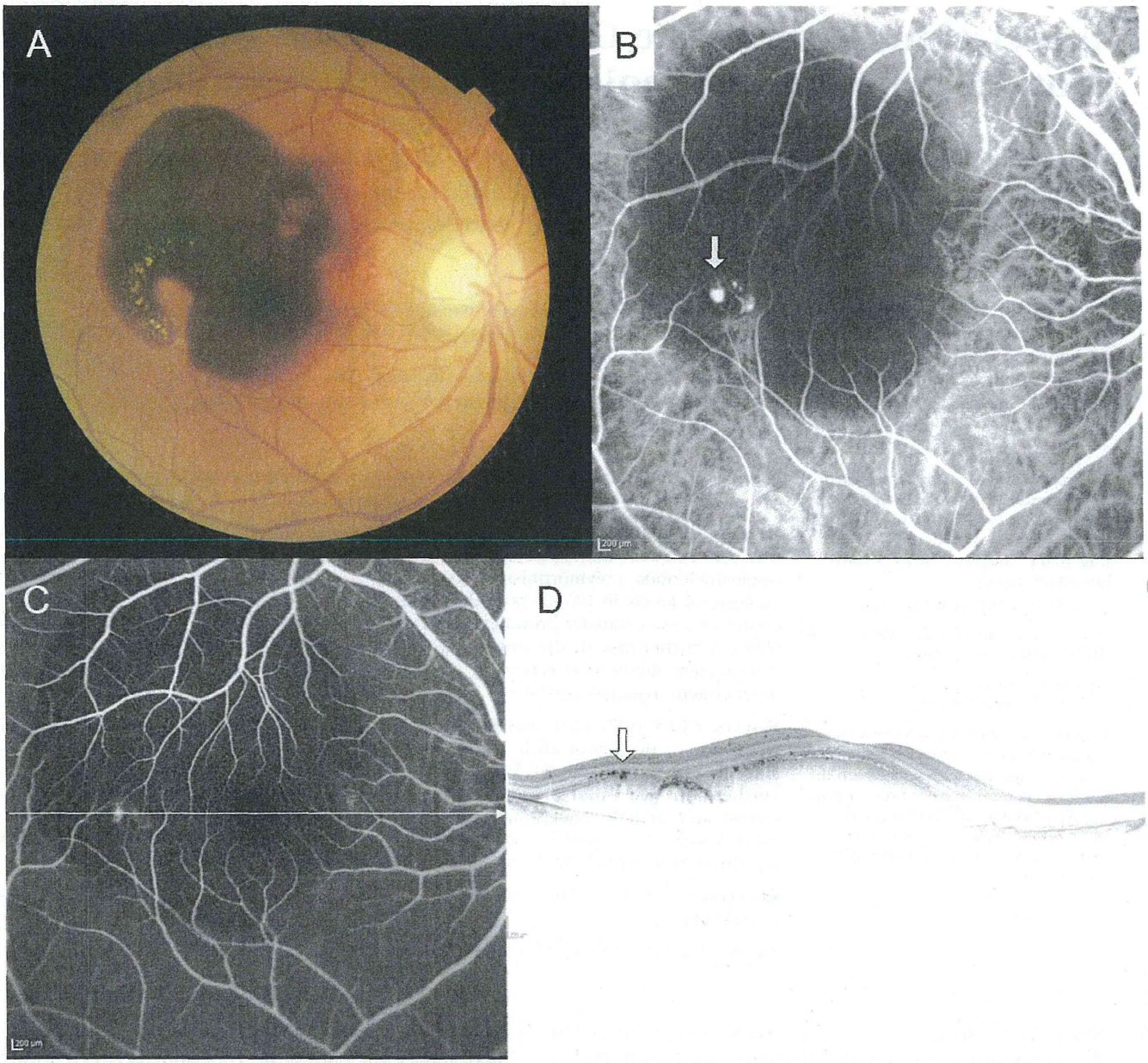

FIGURE. A 64-year-old woman with a typical case of PCV in the right eye. (**A**) Fundoscopic examination shows massive subretinal hemorrhage, lipid deposits, and reddish orange nodules. (**B**) Indocyanine green angiography demonstrates a small branching vascular network terminating in polypoidal lesions (*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.

Table 1. Characteristics of the Study Population

Variable	Cases, n = 581	Controls, n = 793	P Value
Age, y			
Mean 6 SD	72.59 6 8.13	65.99 6 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 6 SD HDL cholesterol level of the control samples was 61.3 6 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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Appendix

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Investigative Ophthalmology & Visual Science

Prevalence and Characteristics of Age-Related Macular Degeneration in the Japanese Population: The Nagahama Study

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• PURPOSE: To estimate the age- and sex-specific prevalence of early age-related macular degeneration (AMD; drusen and retinal pigment abnormalities) and late AMD (exudative AMD and geographic atrophy) in the Japanese population.
• DESIGN: Community-based, cross-sectional study.
• METHODS: The study was held in Nagahama, Japan, and included 6065 Japanese individuals (aged ≥50 years) recruited in 2008-2010. We graded fundus photographs of both eyes for the AMD phenotype based on drusen size, the presence of retinal pigment abnormalities, and late AMD. The associations between smoking and AMD phenotypes were also evaluated.
• RESULTS: We assessed 5595 subjects (women, 65%) with a gradable macular condition. Early and late AMD prevalence increased from 16.1% and 0.27% at 50-59 years to 31.2% and 0.98%, respectively, at 70-74 years and was predominant in male subjects in each age group. Smoking was associated with both early and late AMD stages and retinal pigment abnormalities ($P < .0001$), but not with drusen ($P = .305$). The prevalence of retinal pigment abnormalities was significantly higher in men ($P < .0001$), which was associated with high rates of cigarette smoking. We found no sex difference for the prevalence of large drusen ($P = .264$).
• CONCLUSIONS: The prevalence of early AMD among adult Japanese persons was similar to the rates in white populations. The prevalence of late AMD in Japanese people aged <70 years was similar to that observed in white populations, whereas that in Japanese people aged ≥70 years was relatively lower. (Am J Ophthalmol 2013;156:1002–1009. © 2013 by Elsevier Inc. All rights reserved.)

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AGE-RELATED MACULAR DEGENERATION (AMD) IS the leading cause of visual impairment in the elderly and is the most common cause of blindness in developed countries.[1] The stages of AMD are categorized as early, in which visual symptoms are inconspicuous,[2] and late, in which severe vision loss is typical. Early AMD is characterized by drusen or by pigment abnormalities of the retinal pigment epithelium (RPE) in the macula, without visible choroidal vessels.[1] The presence or absence of these 2 features is characteristic of AMD and is highly associated with the development of late AMD, especially when the status of both eyes is considered.[3]

To date, the introduction of anti–vascular endothelial growth factor (VEGF) intravitreal injections has offered remarkable clinical benefits for patients with late AMD.[4] However, because these benefits are associated with an increased financial burden of providing care for these patients,[5] determining the precise incidence of AMD and identifying its risk factors are still required in order to develop preventive measures for this disease. In fact, an increasing number of studies have reported the epidemiology of AMD in different racial/ethnic groups over the last 10 years.[6–8] However, although the state of health, food intake, nutritional intake, and lifestyle of the Japanese people have been changing,[9] only 2 small cohorts, the Hisayama study[10] (1998), with 1486 participants aged ≥50 years, and the Funagata study[11] (2000-2002), with 1246 participants aged ≥50 years, have evaluated the prevalence of AMD in the Japanese population.

These 2 population-based studies (the Hisayama study and Funagata study) arrived at similar conclusions regarding the prevalence of late AMD: late AMD is less common among Japanese people (with a reported overall prevalence of 0.87% and 0.6%, respectively) than among white subjects.[10,11] However, these 2 studies arrived at different conclusions regarding the prevalence of early AMD in Japanese. Although the Hisayama study suggested a lower prevalence of early AMD in the Japanese,[10] the Funagata study indicated that the prevalence of early AMD is similar to that reported in the Blue Mountains Eye Study (BMES).[11] A recent meta-analysis in 4 Asian populations reported that the prevalence of early AMD in Asians is lower than that in white populations.[12] It is well known that polypoidal choroidal vasculopathy (PCV) has a higher

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prevalence as a subtype of AMD in Asians than in whites.[13] Therefore, these results showing a lower prevalence of early AMD in Asians were convincing because previous studies reported a lower prevalence of drusen in PCV.[14–16] However, a subsequent clinical study suggested that drusen is not an uncommon feature of PCV.[17–19] Because the small number of participants in previous Japanese studies limits meaningful comparisons of the prevalence between the Japanese and other populations, a study with a larger number of participants is required to estimate the precise prevalence of AMD in the Japanese.

Nagahama is a regional mid-sized city located in the central region of the main island of Japan. The municipality has a population of approximately 126 000 (2010 Japan census). The aim of the present study was to describe the age- and sex-specific prevalence of early and late AMD in a general adult population of Nagahama, Japan.

METHODS

THE NAGAHAMA PROSPECTIVE GENOME COHORT FOR THE Comprehensive Human Bioscience, hereinafter referred to as the Nagahama Study, is a community-based prospective cohort study that aims to determine the prevalence and risk factors of various diseases in a community. At baseline, all participants underwent automatic refractometry (Autorefractor ARK-530; Nidek, Tokyo, Japan), axial length measurement (IOL Master; Carl Zeiss, Jena, Germany), and fundus photography using a digital retinal camera (CR-DG10; Canon, Tokyo, Japan) in a darkened room. For this study, residents of Nagahama City who satisfied the following criteria were recruited as participants and were examined between November 2008 and November 2010: (1) age ≥30 years and ≤74 years; (2) ability to participate on one's own; (3) no significant problems communicating in Japanese; (4) no current serious diseases/symptoms or health issues; and (5) voluntarily decided to participate in this study. Information regarding recruitment was provided through newsletters/homepages of government and citizen organizations, newspaper flyers, and brochures. The goal for the number of participants was set at 10 000 (approximately 15% of the population; age, 30-74 years). All procedures in this study adhered to the tenets of the Declaration of Helsinki. The Kyoto University Graduate School and Faculty of Medicine Ethics Committee, the Ad Hoc Review Board of the Nagahama Cohort Project, and the Nagahama Municipal Review Board of Personal Information Protection approved all protocols and informed consent procedures.

Overall, 6118 healthy Japanese individuals aged ≥50 years participated in the Nagahama Study. In the present study, we evaluated subjects who had nonmydriatic fundus photographs of both eyes showing sufficient quality for grading lesions (Figure 1). Participants with other retinal diseases that would disturb the precise grading for

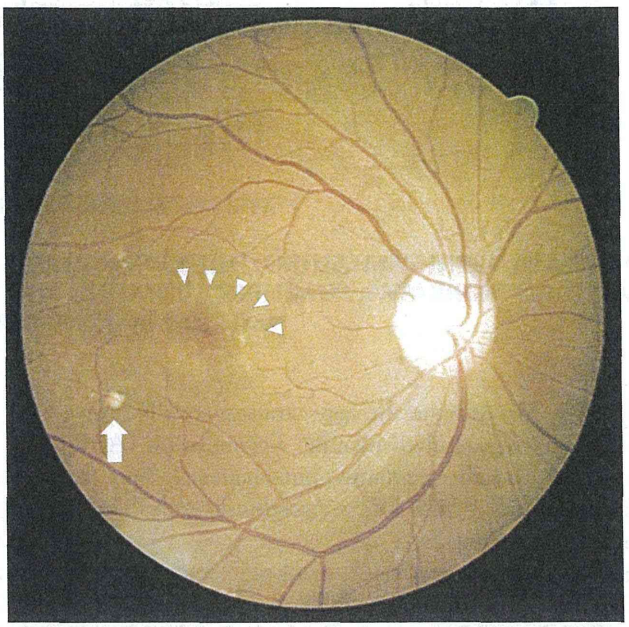

FIGURE 1. Fundus photograph of a 64-year-old Japanese woman with a large drusen (white arrow) and retinal pigment epithelial abnormalities (arrowheads).

macular lesions (such as diabetic retinopathy, retinal vein occlusion, and epiretinal membrane) were excluded from the analysis. Two independent ophthalmologists (I.N. and Y.A. or M.M.) graded each image twice for drusen, RPE abnormalities (hyperpigmentation or hypopigmentation), and late AMD (exudative AMD and geographic atrophy) according to the simplified severity scale for age-related macular degeneration in the Age-Related Eye Disease Study (AREDS).[3] We used the maximum drusen size within the grid (a 3000-μm radius centered on the fovea) at baseline to assess drusen phenotypes. Drusen size was determined using standard circles with diameters corresponding to 63, 125, and 250 μm. Reticular drusen, which were enhanced with the blue channel of the color photograph,[20–22] were considered as soft drusen for the purpose of the analysis.[23] Before grading was initiated for all subjects, intergrader and intragrader agreements were assessed on a random subset of images of 80 eyes of 40 participants. In this initial assessment, the level of agreement between the graders was 1.0 for the presence of late AMD and the agreements between the presence of retinal pigment changes and of drusen size were 0.75 and 0.85-0.90, respectively (crude agreement ratios). The senior reviewers (K.Y. and N.Y.) discussed the cases in which the 2 independent ophthalmologists disagreed and made the final diagnosis. After an agreement had been reached regarding the diagnosis, each photograph was graded twice for all subjects. The level of overall agreement between the grading ophthalmologists was more than 0.94 for most features.

Early AMD was defined by the presence of large drusen (soft distinct and soft indistinct drusen of ≥125 μm in

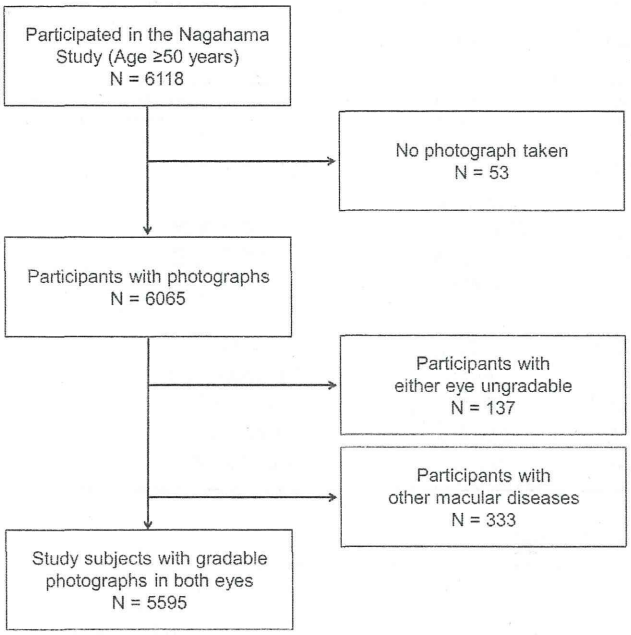

FIGURE 2. Flowchart describing participants from the Nagahama Study who were included and excluded from the analysis for age-related macular degeneration in the Japanese population. Of the 6065 subjects aged ≥50 years, 5595 (92.3%) had gradable fundus photographs in both eyes.

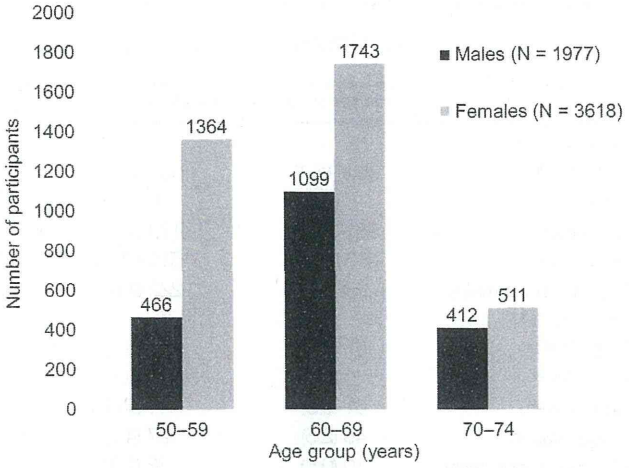

FIGURE 3. Age and sex distribution of the study subjects for age-related macular degeneration in the Japanese population (n = 5595).

diameter) or RPE pigment abnormalities within the grid in the absence of late AMD in either eye.[10,24] Late AMD was defined as the presence of exudative AMD or geographic atrophy (GA). Signs of exudative AMD were retinal pigment epithelial detachment or serous detachment of the sensory retina, subretinal or sub-RPE hemorrhages, and subretinal fibrous scars. GA was defined as a circular discrete area (of at least 175 μm in diameter) of retinal depigmentation with visible choroidal vessels in the absence of exudative AMD.

Information on smoking status was obtained via a self-reported questionnaire. To assess the association between the effect of cigarette smoking and the development of AMD in detail, we used 2 methods of analysis: (1) total cigarette amount using the Brinkman index, which was calculated by the daily number of cigarettes 3 years of smoking[25]; and (2) smoking status, in which the subjects were categorized as never smokers (had smoked less than 100 cigarettes in the past) and ever smokers (had smoked more than 100 cigarettes in the past).

We assessed the age- and sex-specific prevalence of early AMD and late AMD, including the phenotypes of AMD lesions. The age- and sex-adjusted standardized incidences of AMD were calculated using the direct method with reference to the World Health Organization standard population in 2010. We used analysis of variance or the χ^2 test to compare demographic characteristics. P values less than .05 were considered statistically significant.

RESULTS

FUNDUS PHOTOGRAPHS WERE AVAILABLE FOR 6065 subjects aged ≥50 years, and 5595 of these subjects (92.3%) had photographs that were gradable for AMD lesions in both eyes (Figures 2 and 3). Photographs were not taken for 53 participants because of significant media opacities, poor fixation, and/or poor participant cooperation/refusal. Photographs were ungradable in either eye (n = 137) because of media opacities (such as asteroid hyalosis of the vitreous) or poor camera focus. We excluded 333 participants with other macular disease, such as diabetic retinopathy, from the analysis. Thus, a total of 523 participants who had missing or ungradable photographs or who had macular conditions that were inadequate were excluded from this analysis. The participants with gradable photographs (n = 5595) who were included in the analyses were younger (mean age, 62.5 6 6.5 years) than those excluded from the analysis (65.9 6 5.9 years; P < .0001). However, no differences were found in sex between those with gradable and ungradable photographs (P = .588). Thus, the following prevalence data are from 5595 participants with gradable photographs in both eyes.

The summary of the prevalence of phenotypes of AMD in the Nagahama cohort is shown in Table 1. In the study cohort of participants aged ≥50 years, the prevalence of soft drusen (defined as drusen of > 63 μm) was 39.4% (39.2%, standardized) and that of large drusen (defined as drusen of ≥125 μm) was 17.4% (17.5%, standardized). Overall, 22.3% of all subjects had early AMD in at least 1 eye, and the prevalence increased from 16.1% in subjects aged 50-59 years to 31.2% in subjects aged ≥70 years. The overall prevalence of late AMD was 0.52% (0.58%, standardized), which increased from 0.27% in subjects aged 50-59 years to 0.98% in subjects aged 70-74 years.