the current study excluded central geographic atrophy, primarily because this is a relatively rare feature of AMD in Japanese patients. Finally, this was a cross-sectional study, so we could not offer any information regarding changes in macular function over time. Further longitudinal studies are necessary to fully elucidate the macular function in eyes with AMD of various stages and to study the treatment effects and the natural course of eyes with AMD, especially those with AMD in the early stage. Multimodal evaluations of the entire macular function should be of great help in these endeavors.

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Comparison of Exudative Age-related Macular Degeneration Subtypes in Japanese and French Patients: Multicenter Diagnosis With Multimodal Imaging

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• PURPOSE: To compare and analyze differences and similarities between Japanese and French patients in subtype diagnosis of exudative age-related macular degeneration (AMD) as determined by fundus photography (FP) and fluorescein angiography (FA), and a multimodal imaging involving FP, FA, indocyanine green angiography (ICGA), and optical coherence tomography (OCT).

• DESIGN: Retrospective chart review.

• METHODS: We determined the subtype diagnosis for 99 consecutive Japanese eyes and 94 consecutive French eyes with exudative AMD. The first-step diagnosis was made using FP and FA, while the second-step diagnosis was made using FP, FA/ICGA, and OCT. The diagnoses made by Japanese and French physicians were compared, and when the diagnoses differed, a third institute was consulted to arrive at a final consensus and diagnosis.

• RESULTS: The first-step diagnosis showed 20%-30% disagreement against the final diagnosis, but the second-step diagnosis showed only 10% disagreement. Polypoidal choroidal vasculopathy (PCV) was observed more in Japanese patients (48%) than in French (9%), and the rate of PCV with type 1 or 2 choroidal neovascularization (CNV) was extremely low: 3% in Japanese and 0% in French. Type 1 CNV was found significantly more in French cases (53.3% vs 35.1%, $P = .018$), while the rate of eyes with type 2 CNV only or chorioretinal anastomosis was similar between populations.

• CONCLUSIONS: Multimodality imaging significantly improved the sub-classification of AMD. There were significant differences between the 2 series in the proportions of type 1 CNV and PCV, while the proportions of type 2 CNV only and chorioretinal anastomosis were similar between groups. (Am J Ophthalmol 2014;158:309–318.

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EXUDATIVE AGE-RELATED MACULAR DEGENERATION (AMD), one of the leading causes of visual impairment in developed countries, can be generally categorized clinically into 4 subtypes: (1) AMD with type 1 choroidal neovascularization (CNV), (2) AMD with type 1+2 CNV, (3) AMD with type 2 CNV only, and (4) chorioretinal anastomosis. An additional subtype (5) related to polypoidal choroidal vasculopathy (PCV), either (5a) without CNV or (5b) associated with type 1 or 2 CNV, has been suggested. Previous reports show ethnically different distributions for these subtypes. Differentiating these subtypes is important because the clinical course and response to treatments may differ depending on the subtype. Photodynamic therapy seems to be efficient for achieving the regression of polypoidal lesions in PCV,[1–3] while anti–vascular endothelial growth factor (VEGF) treatment is more useful for regression of subretinal or intaretinal fluid.[4] Photodynamic therapy combined with anti-VEGF treatment is recommended for chorioretinal anastomosis because chorioretinal anastomosis may be more resistant to anti-VEGF monotherapy.[5] Interestingly, some reports have shown that anti-VEGF monotherapy works equally as well for PCV and chorioretinal anastomosis as for typical exudative AMD,[6,7] although the classification of exudative AMD in these reports is not consistent or comparable.

A combined approach using fundus photography and fluorescein angiography (FA) has been the gold standard in the diagnosis and treatment of exudative AMD. However, in some cases, fundus photography and FA alone are insufficient for the final diagnosis of various subtypes of exudative AMD. For example, some PCV lesions can appear similar to "classic" type 2 CNV in FA images.[8] Considering that the pathophysiology of PCV and chorioretinal anastomosis has been established, in large part owing to recent advances in indocyanine green angiography (ICGA) and optical coherence tomography (OCT) devices, the use of multimodality imaging diagnostic devices should be helpful in making more accurate subtype diagnoses of exudative AMD. However, there are sometimes cases in which a clear distinction among the 4 subtypes of exudative AMD and/or the 2 subtypes of PCV cannot be made, even with modern multimodality diagnostic devices. Moreover, polypoidal lesions can develop in eyes diagnosed with

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typical exudative AMD, mainly those with late type 1 or occult CNV.

Besides differences in imaging, the training and experience of physicians in different countries can also affect the subtype diagnosis of exudative AMD. Asian physicians may be more familiar with PCV but less so with type 1 or occult CNV and chorioretinal anastomosis, while Western physicians may be more familiar with type 1 or occult CNV and chorioretinal anastomosis, but less so with typical PCV. However, most previous studies on the subtype distribution of exudative AMD have been conducted in individual facilities, and owing to lack of the standard protocol, this may have introduced biases.

To address these gaps in the literature, we conducted a comparative study in Japan and France to determine (1) whether subtype diagnosis of exudative AMD can be made by fundus photography and FA alone, (2) whether the current multimodality diagnostic devices (fundus photography, FA, ICGA, and OCT) can further help to enhance the accuracy of subtype diagnosis, (3) whether all exudative AMD can be categorized into 4 subtypes of typical exudative AMD and 2 subtypes of PCV, and finally, (4) what is the distribution of AMD subtypes diagnosed with multimodal imaging according to ethnic differences at a French center as compared to that at a Japanese center.

METHODS

WE RETROSPECTIVELY REVIEWED THE MEDICAL RECORDS OF 99 eyes of 99 consecutive patients who visited the Department of Ophthalmology, Kyoto University Hospital with a tentative diagnosis of neovascular AMD (Kyoto cases) and 95 eyes of 85 patients with presumed neovascular AMD at Centre d'Ophtalmologie de Paris (Paris cases). All patients underwent comprehensive ophthalmic examinations, including the measurement of best-corrected visual acuity, intraocular pressure testing, indirect ophthalmoscopy, slit-lamp biomicroscopy with a contact lens, spectral-domain OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany), and FA/ICGA (HRA-2; Heidelberg Engineering). All procedures in this study adhered to the tenets of the Declaration of Helsinki. The Kyoto University ethics committee and Paris University ethics committee approved the retrospective research on their examinations for this study.

At Kyoto University, 2 retina specialists (K.Y. and A.T.) evaluated fundus photography and FA and made the "first-step diagnosis" for both Kyoto cases and Paris cases. If the specialists disagreed regarding the diagnosis, a third retina specialist (N.Y.) was consulted for the final determination. Multimodal images of fundus photography, FA, ICGA, and OCT results were used to make a "second-step diagnosis."

At Centre d'Ophtalmologie de Paris, 2 retina specialists (B.D.U. and F.C.) evaluated fundus photography and FA for the "first-step diagnosis" and multimodal images of fundus photography, FA, ICGA, and OCT assessments were used to make a "second-step diagnosis." In the case of disagreement, a third retina specialist (G.C.) determined the diagnosis.

When the "second-step diagnosis" made by the 2 institutes agreed, the diagnosis was regarded as the "final diagnosis." When the diagnosis by the 2 institutes failed to reach a consensus, retina specialists (C.M.G.C. and T.Y.W.) at Singapore Eye Research Institute were consulted for a diagnosis. In such cases, the diagnosis by Singapore Eye Research Institute was regarded as the "final diagnosis." Singapore Eye Research Institute investigators were asked to use all the data and also to choose 1 from the 2 "second-step diagnoses" suggested previously. In each case, Singapore Eye Research Institute investigators agreed on 1 of the "second-step diagnoses" provided by the Kyoto and Paris investigators. Both Kyoto and Paris cases were subgrouped into: (1) AMD with type 1 CNV; (2) AMD with type 1+2 CNV; (3) AMD with type 2 CNV only; and (4) chorioretinal anastomosis. An additional type (5) is caused by PCV, either (5a) without CNV or (5b) associated with type 1 or 2 CNV. Eyes with PCV with branching vascular network without CNV were categorized to (5a) PCV without CNV.

A diagnosis of PCV was made based on fundus photography, FA/ICGA, and OCT: elevated orange-red lesions, characteristic polypoidal lesions at the edge of a branching vascular network on angiography, and prominent anterior protrusion of the retinal pigment epithelium line in OCT images. A diagnosis of chorioretinal anastomosis was also made based on fundus photography, FA/ICGA, and OCT: subretinal, intraretinal, or preretinal juxtafoveal hemorrhages; dilated retinal vessels; lipid exudates; and retinal–choroidal anastomosis. For the analysis of AMD subtypes, AMD with type 1 CNV, AMD with type 2 CNV, and AMD with type 1+2 CNV were regarded as typical exudative AMD, and PCV associated with type 1 or 2 CNV and PCV without type 1 or 2 CNV were regarded as PCV. Fisher exact test was used to compare groups. *P* values of less than .05 were considered to be statistically significant.

RESULTS

THIS STUDY CONSISTED OF 99 CONSECUTIVE EYES OF 99 JAPANESE patients and 95 consecutive eyes of 85 French patients with presumed neovascular AMD. One eye from among the French was excluded from the study because of angiographic images of low quality. The mean age of the 99 Japanese patients (70 men and 29 women) was 74.0 ± 8.9 years, and all patients were ethnically Japanese. The mean age of the 85 French patients (45 men and 40 women) was 73.5 ± 7.9 years, and 98% were white.

TABLE 1. Two-Step Diagnosis of Neovascular Age-related Macular Degeneration Subtypes in Kyoto Cases (N = 99)

	By Kyoto Investigators		By Paris Investigators		
	First-Step Diagnosis	Second-Step Diagnosis	First-Step Diagnosis	Second-Step Diagnosis	Final Diagnosis
AMD with type 1 CNV	46 (46.5%)	34 (34.3%)	34 (34.3%)	28 (28.3%)	33 (33.3%)
AMD with type 1+2 CNV	17 (17.2%)	8 (8.1%)	1 (1.0%)	3 (3.0%)	6 (6.1%)
AMD with type 2 CNV	2 (2.0%)	3 (3.0%)	6 (6.1%)	7 (7.1%)	5 (5.1%)
Chorioretinal anastomosis	4 (4.0%)	5 (5.1%)	4 (4.0%)	4 (4.0%)	5 (5.1%)
PCV with type 1 or 2 CNV	1 (1.0%)	3 (3.0%)	2 (2.0%)	5 (5.1%)	3 (3.0%)
PCV without type 1 or 2 CNV	24 (24.2%)	41 (41.0%)	48 (48.5%)	47 (47.5%)	42 (42.4%)
Other	5 (5.1%)	5 (5.1%)	4 (4.0%)	5 (5.1%)	5 (5.1%)

AMD = age-related macular degeneration; CNV = choroidal neovascularization; PCV = polypoidal choroidal vasculopathy.
First-step diagnosis was determined using fundus photography and fluorescein angiography, while second-step diagnosis was determined using fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography.

The "first-step diagnosis" for each of the 99 Kyoto cases, made by Kyoto investigators using fundus photography and FA, is shown in Table 1. Among these "first-step diagnoses," 34.3% (34/99) differed from the "final diagnosis" as determined by the 3 facilities together. The number of eyes for which the diagnosis involved disagreement decreased to 10 (10.1%) when considering the "second-step diagnosis," which was based on the additional information provided by ICGA and OCT. Figure 1 shows the breakdown of eyes for which diagnosis differed from the "final diagnosis."

Most eyes for which diagnosis involved disagreement were diagnosed as having type 1 CNV, AMD with type 1 CNV, or AMD with type 1+2 CNV. Notably, despite the higher prevalence of PCV in Japan, Kyoto investigators are rather reluctant to make a diagnosis of PCV with fundus photography and FA alone, and prefer to diagnose eyes with PCV as having AMD with type 1 CNV; 44.9% (22/49) of eyes with PCV with/without CNV were initially diagnosed with AMD with type 1 CNV rather than PCV. However, in the "second-step diagnosis" with fundus photography, FA/ICGA, and OCT, only 3 eyes were judged as AMD; the other 93.9% (46/49) of cases were diagnosed as PCV. Figure 2 shows an example of eyes diagnosed as having AMD with type 1 CNV in the "first-step diagnosis" but as PCV in the "second-step diagnosis."

The "first- and second-step diagnoses" of the Kyoto cases made by the Paris investigators showed different trends. The Paris physicians tended to make a diagnosis of more AMD with type 2 CNV, less AMD with type 1+2 CNV, and more PCV without CNV (Table 1). The "first-step diagnosis" showed disagreement compared to the "final diagnosis" in 18.2% (18/99) of cases, while the "second-step diagnosis" showed disagreement compared to the "final diagnosis" in 13.1% (13/99) of cases. Although most eyes with PCV were correctly diagnosed using fundus photography and FA alone, approximately 20% (8/46 and 10/46, respectively) of eyes with type 1 CNV or chorioretinal anastomosis were diagnosed as having PCV at the time of both "first- and second-step diagnoses" by the Paris investigators (Figure 1).

The "first-step diagnoses" for the 94 Paris cases made by the Paris investigators using fundus photography and FA are shown in Table 2. Among these "first-step diagnoses," 24.5% (23/94) differed from the "final diagnosis" as determined by the 3 facilities together. The number of eyes with any disagreement related to diagnosis decreased to 9 (9.6%) for the "second-step diagnosis" based on the additional information provided by ICGA and OCT. The rate of any disagreement related to diagnosis by Kyoto investigators similarly decreased from 20.2% (19/94) for the "first-step diagnosis" to 9.6% (9/94) for the "second-step diagnosis."

Figure 3 shows the breakdown of eyes for which there was any disagreement related to the "final diagnosis." For the Paris investigators, most disagreements were associated with eyes with a "final diagnosis" of AMD with type 1+2 CNV, while disagreements related to diagnosis among Kyoto investigators were often observed in eyes with type 2 CNV. The diagnoses made by Paris investigators showed more AMD with type 2 CNV and less AMD with type 1+2 CNV in comparison to the diagnoses made by Kyoto investigators. Figure 4 shows an example of eyes diagnosed as having type 1 CNV in the "first-step diagnosis" but as having type 2 CNV in the "second-step diagnosis."

Table 3 shows the subtype distribution for neovascular AMD diagnosed by Kyoto and Paris physicians, after all eyes with a diagnosis other than AMD had been excluded. The rate of AMD with type 1 CNV was significantly higher in Paris cases (35.1% vs 53.3%, $P = .018$), and the rate of AMD with type 1+2 CNV was also significantly higher among Paris cases (21.1% vs 6.4%, $P = .0046$).

When AMD eyes with type 1, type 1+2, and type 2 CNV were evaluated together as typical AMD, the rate of typical AMD was significantly higher among Paris cases (85.6 % vs 46.8%, $P = 2.92 \times 10^{-8}$). In contrast, PCV was significantly more common among Kyoto cases (47.9% vs 8.9%, $P = 3.04 \times 10^{-8}$). PCV with type 1 or 2 CNV was very rare: 3 eyes among the Kyoto cases and 0 eyes among the Paris cases.

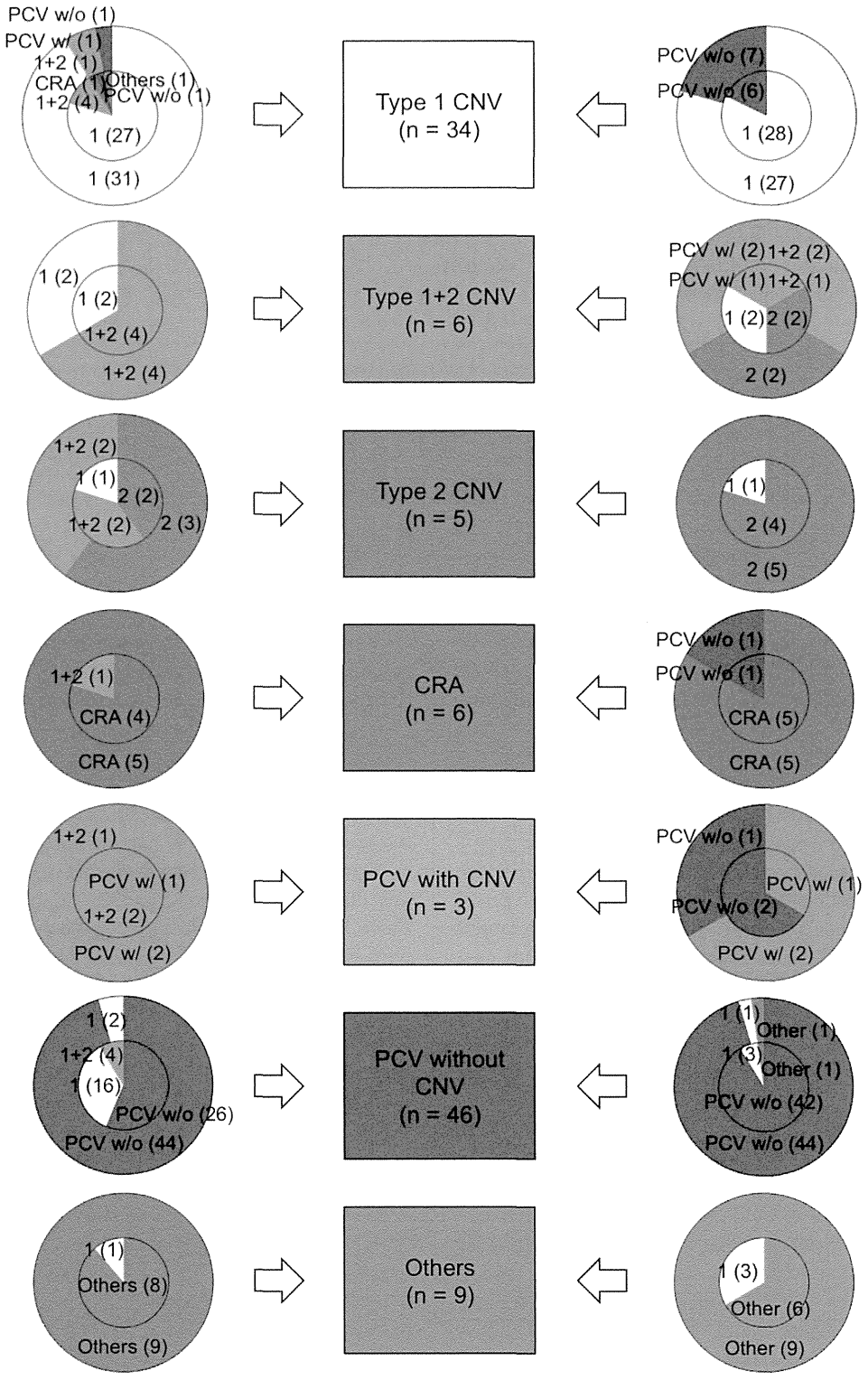

FIGURE 1. Breakdown of eyes with disagreements when "first-step" and "final diagnosis" were compared in Kyoto cases of neovascular age-related macular degeneration. First-step diagnosis was determined using fundus photography and fluorescein angiography, while second-step diagnosis was determined using fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Circles in the left column show the frequency of each diagnosis among Kyoto investigators, while circles in the right column show the frequency of each diagnosis among Paris investigators. The inner circle shows the "first-step diagnosis," and the outer circle shows the "second-step diagnosis." The squares in the middle column show the "final diagnosis" as determined by 3 facilities: Kyoto University, University Paris, and Singapore Eye Research Institute.

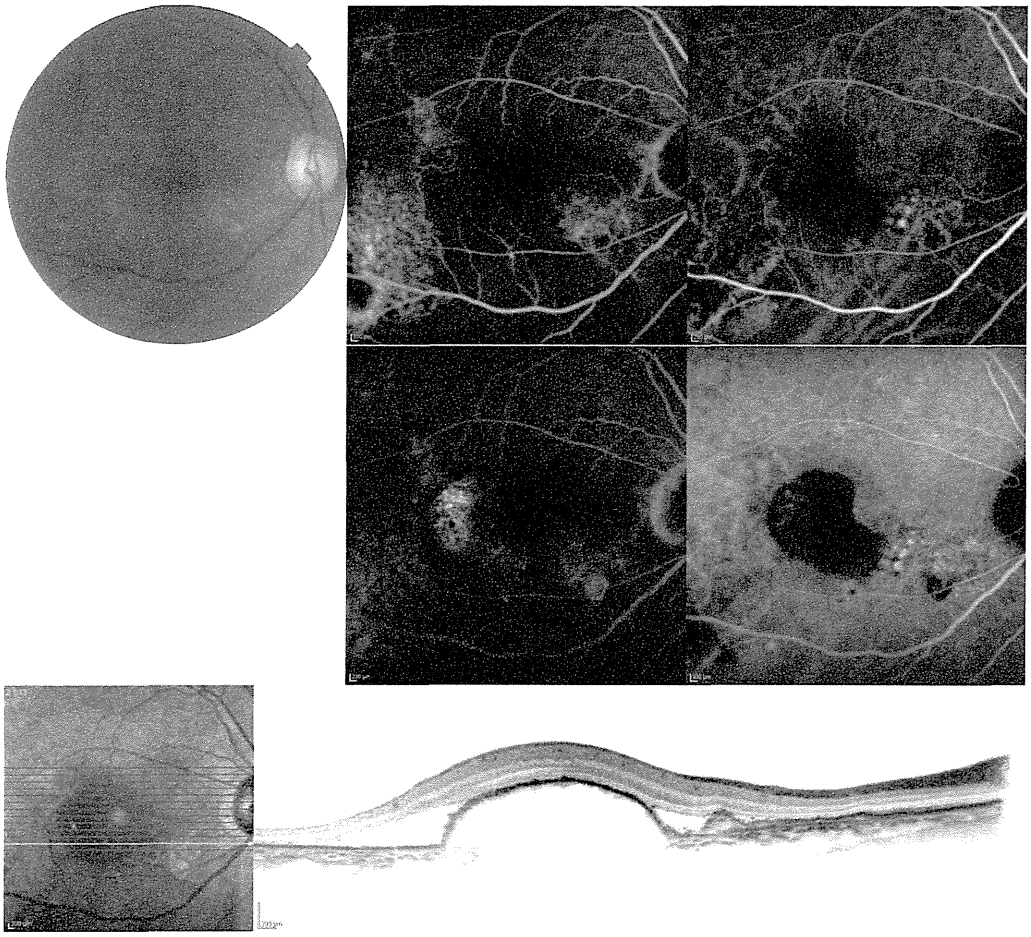

FIGURE 2. Right eye of an 81-year-old man with age-related macular degeneration among the Kyoto cases; an example of eyes with different diagnosis between the "first-step diagnosis" determined by fundus photography and fluorescein angiography and the "second-step diagnosis" determined by fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Fundus photography (Top left) shows depigmentation of the retinal pigment epithelium at the nasal-lower area of the macula, suggesting the existence of choroidal neovascularization. Fluorescein angiography shows the faint leakage of fluorescein at the nasal-lower area of the macula in the early phase (Top middle) and substantial leakage in the late phase (Middle middle). The "first-step diagnosis" was age-related macular degeneration with type 1 CNV. Indocyanine green angiography shows polypoidal lesions at the edge of the subfoveal pigment epithelium detachment in the early phase (Top right). The polypoidal lesions and network vessels between the polypoidal lesions and the disc are clearly visible in the late phase (Middle right). Optical coherence tomography (Bottom) shows protrusions of the retinal pigment epithelium line indicating serous pigment epithelium detachment and a polypoidal lesion at its edge. The flat elevation of the retinal pigment epithelium line next to the polypoidal lesion indicates network vessels. The "second-step diagnosis" was polypoidal choroidal vasculopathy without type 1 or type 2 choroidal neovascularization.

DISCUSSION

IN THE PRESENT STUDY, WE ADDRESSED SEVERAL GAPS IN the literature. First, our initial purpose was to try to determine whether the subtype diagnosis of exudative AMD can be made based on the results of fundus photography and FA only, and whether current multimodality diagnostic devices could help to enhance the accuracy of subtype diagnosis. We compared the results of "first-step diagnosis" with fundus photography and FA only to the results of "second-step diagnosis" with multimodality technology including fundus photography, FA, ICGA, and OCT in Kyoto cases and Paris cases.

The "first-step diagnosis" showed 20%–30% disagreement against the "final diagnosis" in both facilities; the level of disagreement decreased to approximately 10% with use of the multimodality approach. Furthermore, we compared the diagnoses by the Kyoto investigators to those by the Paris investigators. The Kyoto investigators tended to diagnose PCV cases as AMD with type 1 CNV when using fundus photography and FA alone. Of the 53 eyes with PCV, Kyoto investigators diagnosed 22 eyes (41.5%) as AMD with type 1 CNV, while 7 eyes (13.2%) were diagnosed as such by Paris investigators. In contrast, Paris investigators tended to diagnose AMD cases with type 1 CNV as PCV; they diagnosed Japanese AMD cases with

TABLE 2. Two-step Diagnosis of Neovascular Age-related Macular Degeneration Subtypes in Paris Cases (N = 94)

	By Kyoto Investigators		By Paris Investigators		
	First-Step Diagnosis	Second-Step Diagnosis	First-Step Diagnosis	Second-Step Diagnosis	Final Diagnosis
AMD with type 1 CNV	50 (53.2%)	52 (55.3%)	50 (53.2%)	50 (53.2%)	48 (51.1%)
AMD with type 1+2 CNV	25 (26.6%)	20 (21.3%)	12 (12.8%)	14 (14.9%)	19 (20.2%)
AMD with type 2 CNV	6 (6.4%)	6 (6.4%)	16 (17.0%)	13 (13.8%)	10 (10.6%)
Chorioretinal anastomosis	4 (4.3%)	5 (5.3%)	5 (5.3%)	5 (5.3%)	5 (5.3%)
PCV with type 1 or 2 CNV	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PCV without type 1 or 2 CNV	6 (6.4%)	7 (7.4%)	3 (3.2%)	8 (9.5%)	8 (9.5%)
Other	3 (3.2%)	4 (4.3%)	7 (7.4%)	4 (4.3%)	4 (4.3%)

AMD = age-related macular degeneration; CNV = choroidal neovascularization; PCV = polypoidal choroidal vasculopathy.
The first-step diagnosis was determined using fundus photography and fluorescein angiography, while the second-step diagnosis was determined using fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography.

type 1 CNV as PCV in 9 out of 39 eyes (23.1%) that were not diagnosed as such by the Japanese investigators. Although these findings on the part of Kyoto and Paris investigators may not represent tendencies typical of other institutes in Asia and Europe, differences in the AMD subtype distribution between Asia and Europe may have precipitated the differing attitudes in the diagnosis of exudative AMD.

Previous studies have reported that the prevalence of PCV is higher among Asians, particularly East Asians (eg, Japanese, Chinese), while the prevalence of chorioretinal anastomosis is higher among whites. Maruko and associates reported that 54.7% of Japanese exudative AMD is PCV, while 4.5% is chorioretinal anastomosis.[9] This rate of PCV is much higher than that found among whites,[10,11] but this rate of chorioretinal anastomosis is lower than that reported for whites.[12–16] However, most studies used diagnoses determined at the same institution where the study was performed. In this study, we determined the diagnosis with the collaboration of specialists at 3 facilities: Kyoto University, University of Paris, and Singapore Eye Research Institute.

Although the number of eyes with disagreements related to diagnosis decreased substantially from the "first-step diagnosis" to the "second-step diagnosis," this improvement with the use of multimodality technology seemed to be dependent on the backgrounds of investigators, the subtypes of AMD involved, and differing definitions of typical PCV (with or without CNV): most of the "polyps" observed in Europe and Western countries are associated with advanced AMD with type 1 CNV.

The rate of disagreement related to the "first-step diagnosis" was not high in the diagnosis of typical exudative AMD, which suggests that the benefit of multimodality technology was small for the differentiation of typical exudative AMD from chorioretinal anastomosis and PCV for both Kyoto and Paris investigators. When Kyoto cases and Paris cases were evaluated together, 122 eyes were diagnosed with typical exudative AMD: 82 cases of AMD with type 1 CNV, 25 cases of AMD with type 1+2 CNV, and 15 cases of AMD with type 2 CNV. Of these 122 eyes, 4 eyes (3.3%) at the time of the "first-step diagnosis" and 2 eyes (1.7%) at the time of the "second-step diagnosis" were not diagnosed with typical exudative AMD by Kyoto investigators: 1 PCV, 2 chorioretinal anastomosis, and 1 other case in the "first-step" analysis; and 2 cases of PCV in the "second-step" analysis. On the other hand, 10 eyes (8.2%) were not diagnosed with typical exudative AMD during "first- or second-step" diagnosis by Paris investigators: 7 PCV, 1 chorioretinal anastomosis, and 2 others in the "first-step diagnosis"; 9 PCV and 1 chorioretinal anastomosis in the "second-step diagnosis."

The benefit of multimodality technology becomes clear when the subtypes of typical exudative AMD are differentiated into 3 subtypes: AMD with type 1 CNV, AMD with type 1+2 CNV, and AMD with type 2 CNV. Of the 122 typical exudative AMD eyes, the diagnosed subtype of CNV differed from the "final diagnosis" in 22 eyes (18.0%) in the "first-step diagnosis" by Kyoto investigators, which improved to 12 eyes (9.8%) in the "second-step diagnosis." As for the diagnosis by Paris investigators, the type of CNV differed from the "final diagnosis" in 19 eyes (15.6%) at the "first-step diagnosis" and 9 eyes (7.4%) at the "second-step diagnosis." Multimodality improved diagnosis agreement by 50% among CNV subtype, regardless of investigator background.

The benefit of multimodality was most clearly demonstrated in the diagnosis of PCV by Kyoto investigators. In the "final diagnosis," the number of eyes diagnosed as having PCV with or without CNV was 49 among the Kyoto cases and 8 among the Paris cases. Of these 57 eyes, Kyoto investigators reported disagreements related to the diagnosis in 27 eyes (47.4%) at the time of the "first-step diagnosis": 26 eyes were diagnosed with AMD with type 1 CNV or type 1+2 CNV, and 1 eye was diagnosed with a condition other than AMD. This disagreement significantly decreased to 3 eyes (5.3%) with the help of multimodality diagnostic technology. Among Paris investigators, the rate

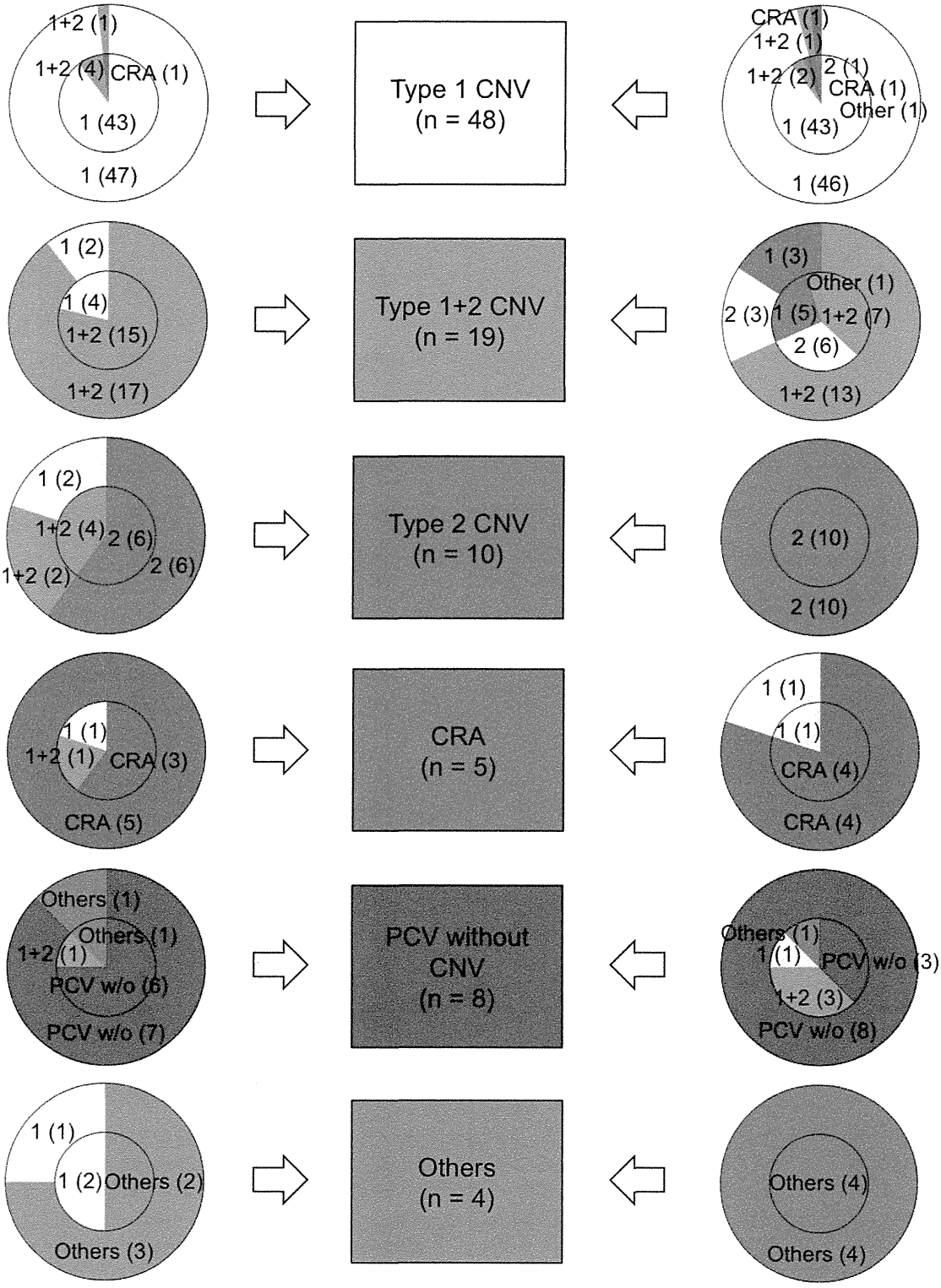

FIGURE 3. Breakdown of eyes with disagreement between "first-step" and "final diagnosis" among the Paris cases of neovascular age-related macular degeneration. First-step diagnosis was determined using fundus photography and fluorescein angiography, while second-step diagnosis was determined using fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Circles in the left column show the frequency of each diagnosis determined by Kyoto investigators, while circles in the right column show the frequency of each diagnosis determined by Paris investigators. The inner circle shows the diagnosis in the "first-step diagnosis," and the outer circle shows the diagnosis in the "second step diagnosis." The squares in the middle column show the "final diagnosis" determined by 3 facilities: Kyoto University, University Paris, and Singapore Eye Research Institute.

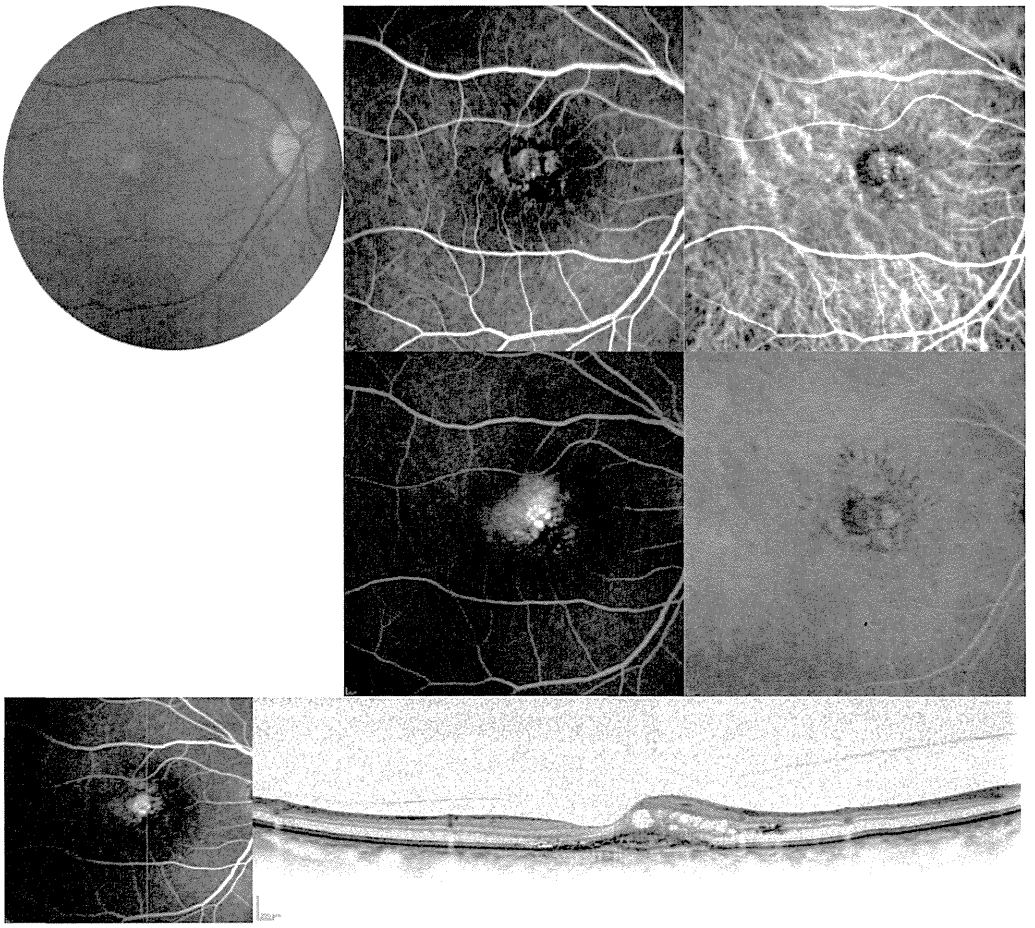

FIGURE 4. Right eye of a 78-year-old man with age-related macular degeneration from among the Paris cases; an example of eyes with different diagnosis between the "first-step diagnosis" determined by fundus photography and fluorescein angiography and the "second-step diagnosis" determined by fundus photography, fluorescein angiography, indocyanine green angiography, and optical coherence tomography. Fundus photograph (Top left) shows a white-gray lesion at the fovea, suggesting type 2 choroidal neovascularization. Fluorescein angiography shows staining of the subfoveal choroidal neovascularization in the early phase (Top middle) and substantial leakage from subfoveal choroidal neovascularization in the late phase (Middle middle). The "first-step diagnosis" was age-related macular degeneration with type 2 choroidal neovascularization. Indocyanine green angiography clearly shows choroidal neovascularization in both the early phase (Top right) and the late phase (Middle right). Optical coherence tomography (Bottom) shows protrusion of the retinal pigment epithelium line indicating type 1 choroidal neovascularization and a high-density mass on the type 1 choroidal neovascularization, suggesting type 2 choroidal neovascularization. The "second-step diagnosis" was age-related macular degeneration with type 1+2 choroidal neovascularization.

of disagreement also improved, from 8 eyes (14.0%) in the "first-step diagnosis" to 4 eyes (7.0%) in the "second-step diagnosis." It could be suggested that multimodality is very important to differentiate PCV from AMD with type 1 CNV.

For the diagnosis of chorioretinal anastomosis, however, multimodality only improved the diagnosis among Kyoto investigators. Of the 11 eyes diagnosed with chorioretinal anastomosis in the "final diagnosis," disagreements existed for 3 eyes (27.3%) during the "first-step diagnosis" by Kyoto investigators and only 0 eyes (0%) in the "second-step diagnosis" by Kyoto investigators. Among Paris investigators, disagreements were observed in the "first- and second-step diagnoses" of 2 eyes (18.2%). Considering the low number of evaluated eyes with chorioretinal anastomosis, however, the benefit of multimodality for chorioretinal anastomosis diagnosis should be further investigated.

Although multimodality improved the unanimity of diagnosis, we failed to reach a consensus for some cases—even with multimodal diagnostic technology. The diagnoses by Kyoto investigators and Paris investigators showed disagreement in the "second-step diagnosis" for 22 eyes (20.2%) among the Kyoto cases and 16 eyes (17.0%) among the Paris cases. For subsequent analyses, we decided to employ the Singapore Eye Research Institute diagnosis as the "final diagnosis." In clinical settings, it is important to differentiate AMD subtypes because the response to treatments may differ among subtypes. Aside from the

TABLE 3. Prevalence of the Neovascular Age-related Macular Degeneration Subtypes in Kyoto Cases (N = 94) and Paris Cases (N = 90)

	Kyoto Cases	Paris Cases		Kyoto Cases	Paris Cases
AMD with type 1 CNV	33 (35.1%)	48 (53.3%)	tAMD	44 (46.8%)	77 (85.6%)
AMD with type 1+2 CNV	6 (6.4%)	19 (21.1%)			
AMD with type 2 CNV	5 (5.3%)	10 (11.1%)			
Chorioretinal anastomosis	5 (5.3%)	5 (5.6%)	Chorioretinal anastomosis	5 (5.3%)	5 (5.6%)
PCV with type 1 or 2 CNV	3 (3.2%)	0 (0%)	PCV	45 (47.9%)	8 (8.9%)
PCV without type 1 or 2 CNV	42 (44.7%)	8 (8.9%)			
Total	94	90		94	90

AMD = age-related macular degeneration; CNV = choroidal neovascularization; PCV = polypoidal choroidal vasculopathy; tAMD = typical age-related macular degeneration.

neovascular lesion itself, other features captured by fundus photography, FA/ICGA, and OCT might be helpful for the diagnosis of exudative AMD subtypes. For example, PCV is thought to result in fewer drusen than typical neovascular AMD or chorioretinal anastomosis. However, we have shown that the presence of drusen in PCV is not rare.[17] Although differences in choroidal thickness or choroidal vascular hyperpermeability could be used to distinguish PCV from typical neovascular AMD,[18,19] we have also demonstrated the development of polypoidal lesions in typical AMD.[20] Moreover, typical neovascular AMD is usually restricted to the macula, while PCV is very frequently multifocal and located in the peripapillary region. It is therefore necessary to carefully differentiate AMD cases into 4 subtypes (type 1, type 1+2, type 2 only, and chorioretinal anastomosis) or PCV (either without CNV, or with type 1 or 2 CNV) using multimodal diagnostic technology. It might be suggested that "PCV" (without CNV) should not be considered altogether as a subtype of type 1 AMD.

Even when considering the disagreements discussed above, our findings would support that the prevalence of PCV is significantly higher among East Asians, which would affect ophthalmologists' examinations of patients with AMD. The prevalence of PCV in the Kyoto cases (49.0%) is compatible with that in previous reports,[9,21] and the prevalence of PCV among the Paris cases (8.9%) is similar to that in previous reports in whites.[10,11,22,23] Asian ophthalmologists should recognize the importance of multimodality for the diagnosis of PCV, which they often encounter in the clinic. Asian ophthalmologists may become rather reluctant to make a diagnosis of PCV without ICGA and OCT, whereas European ophthalmologists may become too cautious of overlooking PCV when diagnosing Asian cases.

The prevalence of chorioretinal anastomosis found among the Kyoto cases (6.0%) is also compatible with previously reported rates: 4.5% in Japanese,[9] 4.5% in Chinese,[24] and 11.1% in Korean subjects.[25] However, previous reports on the prevalence of chorioretinal anastomosis in whites have not been consistent: 5.0%,[12] 9.1%,[13] 10%–15%,[15] 15.1%,[14] and 21.7%.[16] Our multimodal findings showed the rate of chorioretinal anastomosis was 5.6% among the Paris cases. Given that previous studies did not necessarily include ICGA, further study using recently developed FA/ICGA and OCT devices is required.

The current study has various limitations, including its retrospective nature and the relatively small sample size of each subtype. Because this is a retrospective study, we did not create a detailed protocol for image acquisition with each modality. Each examination was performed in the clinical setting, with examination and diagnosis performed at the level of common clinical practice.

In summary, our study showed the importance of multimodal diagnosis for neovascular AMD and the prevalence of AMD subtypes in Japanese vs French patients. Further prospective study with a large sample size would be necessary to confirm our current findings.

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Biosketch

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Biosketch

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New loci and coding variants confer risk for age-related macular degeneration in East Asians

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Age-related macular degeneration (AMD) is a major cause of blindness, but presents differently in Europeans and Asians. Here, we perform a genome-wide and exome-wide association study on 2,119 patients with exudative AMD and 5,691 controls, with independent replication in 4,226 patients and 10,289 controls, all of East Asian descent, as part of The Genetics of AMD in Asians (GAMA) Consortium. We find a strong association between *CETP* Asp442Gly (rs2303790), an East Asian-specific mutation, and increased risk of AMD (odds ratio (OR) = 1.70, $P = 5.60 \times 10^{-22}$). The AMD risk allele (442Gly), known to protect from coronary heart disease, increases HDL cholesterol levels by 0.17 mmol l^{-1} ($P = 5.82 \times 10^{-21}$) in East Asians ($n = 7{,}102$). We also identify three novel AMD loci: *C6orf223* Ala231Ala (OR = 0.78, $P = 6.19 \times 10^{-18}$), *SLC44A4* Asp47Val (OR = 1.27, $P = 1.08 \times 10^{-11}$) and *FGD6* Gln257Arg (OR = 0.87, $P = 2.85 \times 10^{-8}$). Our findings suggest that some of the genetic loci conferring AMD susceptibility in East Asians are shared with Europeans, yet AMD in East Asians may also have a distinct genetic signature.

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Age-related macular degeneration (AMD) is a progressive, blinding disease affecting millions of elderly individuals worldwide[1,2]. Several genome-wide association studies (GWAS) have identified common variants associated with AMD in European-ancestry populations[3–6], and recently, rare genetic variation at *CFH*, *CFI*, *C3* and *C9* were also shown to strongly associate with AMD in Europeans[7–10]. However, there are few such studies in Asians[11]. Importantly, Asians appear to have a distinct clinical presentation of the disease (for example, absence of drusen and minimal fibrous scarring in polypoidal choroidal vasculopathy, a variant of AMD accounting for 20–55% of Asian patients with exudative AMD) and different responses to treatment (for example, poorer response to inhibitors of vascular endothelial growth factor (VEGF) compared with patients of European ancestry)[12,13]. It remains unclear whether there are differences in underlying genetic characteristics of AMD between patients of Asian versus European ancestry.

Concurrently, previous GWAS studies provide limited coverage of low-frequency coding variants, which may result in the loss of function and are often ethnic-specific. There is thus interest in genetic studies of AMD and other diseases beyond standard-content GWAS to discover potentially causative coding variants in different ethnic groups.

To address these questions, the Genetics of AMD in Asians Consortium perform a genome-wide (GWAS) and exome-wide association study (EWAS) of advanced AMD solely on the exudative (neovascular) disease subtype in East Asians. Compared with standard-content GWAS arrays, the exome array has significantly increased marker density across the coding human exome, thus increasing power to detect disease associations located within the coding frame. EWAS of AMD have not been previously conducted in either Europeans or Asians. In this paper, we present data from eight independent AMD case–control collections enrolled across multiple sites in East Asia, totalling 6,345 exudative AMD cases and 15,980 controls. This is the largest sample, to our knowledge, of East Asians ever assembled for genetic studies of AMD.

Results

Association with previously identified AMD variants. After genotype imputation, synchronization and stringent quality filters were performed, a total of 4,471,719 SNPs were assessed for the GWAS and 120,027 autosomal coding-frame SNPs for EWAS from 2,119 AMD cases and 5,691 controls (Table 1). Overall genomic inflation was very low ($\lambda_{gc} = 1.031$; Supplementary Fig. 1), suggesting minimal confounding of the disease association analysis by population stratification or other systematic study design biases. Data from the discovery stage analysis confirmed previously identified AMD variants in *ARMS2-HTRA1* rs10490924 ($P = 1.20 \times 10^{-103}$), *CFH* rs10737680 ($P = 7.54 \times 10^{-38}$), *CETP* rs3764261 ($P = 1.66 \times 10^{-12}$), *ADAMTS9* rs6795735 ($P = 1.13 \times 10^{-5}$), *C2-CFB* rs429608 ($P = 1.06 \times 10^{-4}$), as well as *CFI* rs4698775 ($P = 7.5 \times 10^{-4}$; Supplementary Table 1 and Supplementary Fig. 2). Our data also showed nominal evidence of replication in the same direction as the initial study for a further three previously reported variants (*TGFBR1* rs334353, *APOE* rs4420638, and *VEGFA* rs943080; $P < 0.05$ for each). The remaining 8 out of 17 previously described SNPs that were non-monomorphic in our East Asian collections did not show evidence of replication in our study (Supplementary Table 1). A recently described rare, functional and highly penetrant genetic mutation within *CFI* (G119R, rs141853578) shown to confer markedly elevated risk of AMD in Europeans[14] was observed to be non-polymorphic in our East Asian samples (Supplementary Table 1). Similarly, recently described rare mutations in *C3* (K155Q, rs147859257) and *C9* (P167S, rs34882957) were also shown to be non-polymorphic in our East Asian samples (Supplementary Table 1).

Discovery of new SNP variants associated with AMD. Apart from verifying previous observations, our discovery analysis also revealed genome-wide significant association at *C6orf223* (rs2295334 encoding for A231A, $P = 1.41 \times 10^{-8}$; Table 2, Supplementary Table 2 and Supplementary Fig. 2), a novel SNP marker not previously reported to associate with AMD risk. We observed a further 21 independent SNPs from distinct loci not previously implicated with susceptibility to AMD showing evidence of association surpassing $P < 1 \times 10^{-4}$. We then brought forward all the 22 markers (Table 2) for replication genotyping in independent sample collections comprising 4,226 exudative AMD cases and 10,289 controls (Table 1). Replication evidence was compelling for *CETP* rs2303790 (encoding D442G; odds ratio (OR) = 1.73, $P = 2.95 \times 10^{-16}$), as well as for *C6orf223* rs2295334 (A231A; OR = 0.80, $P = 5.25 \times 10^{-11}$), *SLC44A4* rs12661281 (D47V; OR = 1.22, $P = 5.13 \times 10^{-6}$) and *FGD6* rs10507047 (Q257R; OR = 0.88, $P = 7.69 \times 10^{-5}$), leading to genome-wide significant findings in the meta-analysis of all 6,345

Table 1 | Baseline characteristics of exudative age-related macular degeneration cases and controls in the discovery and replication sample collections.

Sample collection	Ethnicity	No. of cases*	No. of controls*	AMD phenotyping	Genotyping platform
Discovery					
Singapore	Chinese	631	1,967	Dilated fundoscopy, FA, ICG & OCT	Illumina OmniExpress, 610 K & Exome Chips
Hong Kong	Chinese	507	2,967	Dilated fundoscopy, FA & ICG	Illumina OmniExpress & Exome Chips
Japan	Japanese	981	757	Dilated fundoscopy, FA, ICG & OCT	Illumina OmniExpress & Exome Chips
Subtotal		2,119	5,691		
Replication					
Korea	Korean	757	1,829	Dilated fundoscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Japan	Japanese	1,213	4,035	Dilated fundoscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Guangdong, China	Chinese	398	2,478	Dilated fundoscopy, FA & ICG	Sequenom MassArray & Taqman
Sichuan, China	Chinese	1,055	1,089	Dilated fundoscopy, FA & ICG	SNAPSHOT
Beijing, China	Chinese	803	858	Dilated fundoscopy, FA, ICG & OCT	Sequenom MassArray & Taqman
Subtotal		4,226	10,289		
All samples		6,345	15,980		

AMD, age-related macular degeneration; FA, fluorescein angiography; ICG, indocyanine green angiography; OCT, optical coherence tomography.
*No. of samples reflect those passing quality checks.

Table 2 | Summary of results of the genome-wide and exome-wide association study on exudative age-related macular degeneration.

SNP	Chr	Position	Nearest gene	Minor allele	Discovery (2,119/5,691)*		Replication (4,226/10,289)*		Combined (6,345/15,980)*	
					OR	$P_{discovery}$	OR	$P_{replication}$	OR	$P_{combined}$
Loci reaching $P_{discovery} < 1 \times 10^{-4}$ *and* $P_{combined} < 5 \times 10^{-8}$										
rs2303790	16	57,017,292	*CETP*	G	1.69	3.36×10^{-7}	1.73	2.95×10^{-16}	1.70	5.60×10^{-22}†
rs2295334	6	43,970,827	*C6orf223*	A	0.75	1.41×10^{-8}	0.80	5.25×10^{-11}	0.78	6.19×10^{-18}‡
rs12661281	6	31,842,598	*SLC44A4*	T	1.38	1.23×10^{-7}	1.22	5.13×10^{-6}	1.27	1.08×10^{-11}§
rs10507047	12	95,604,290	*FGD6*	G	0.83	4.75×10^{-5}	0.88	7.69×10^{-5}	0.87	2.85×10^{-8}†
Loci reaching $P_{discovery} < 1 \times 10^{-4}$ *but* $P_{combined} \geq 5 \times 10^{-8}$										
rs62191056	2	227,779,676	*RHBDD1*	A	1.33	2.27×10^{-5}	1.14	0.046	1.23	1.10×10^{-5}
rs7274811	20	32,333,181	*ZNF341*	T	0.83	5.72×10^{-5}	0.92	0.011	0.89	1.26×10^{-5}
rs3894326	19	5,843,784	*FUT3*	T	0.75	6.22×10^{-6}	0.91	0.27	0.81	1.85×10^{-5}
rs117581914	19	9,236,724	*OR7G3*	G	2.02	3.27×10^{-5}	1.36	0.19	1.78	3.05×10^{-5}
rs2287921	19	49,228,272	*RASIP1*	C	1.74	7.70×10^{-6}	1.20	0.13	1.43	3.56×10^{-5}
rs17143419	7	70,829,578	*WBSCR17*	T	1.49	2.28×10^{-5}	1.12	0.34	1.34	8.66×10^{-5}
rs4280803	4	57,760,424	*REST*‖	T	0.79	4.87×10^{-5}	0.65	0.76	0.79	4.68×10^{-5}
rs202018816	19	10,445,066	*ICAM3*	A	5.13	1.39×10^{-5}	1.42	0.28	2.47	2.46×10^{-4}
rs7165901	15	102,021,219	*PCSK6*	C	0.80	1.46×10^{-5}	0.96	0.44	0.88	3.21×10^{-4}
rs1891359	10	127,495,153	*UROS*	G	1.77	5.42×10^{-5}	0.95	0.74	1.36	0.0044
rs215736	7	32,443,119	*PDE1C-LSM5*‖	T	3.28	3.82×10^{-5}	0.71	0.38	1.93	0.0049
rs2221338	2	68,224,745	*C1D*‖	A	1.22	2.60×10^{-5}	1.02	0.65	1.13	5.83×10^{-4}
rs1538240	13	101,968,310	*NALCN*	T	1.24	2.92×10^{-5}	1.03	0.59	1.14	6.54×10^{-4}
rs7560053	2	228,220,769	*MFF*	T	0.83	7.00×10^{-5}	0.98	0.76	0.90	0.0023
rs73509026	19	12,059,467	*ZNF700*	G	1.65	1.25×10^{-5}	0.85	0.34	1.32	0.0039
rs1241050	4	141,410,099	*LOC152586*	T	0.72	3.35×10^{-5}	1.02	0.79	0.86	0.0068
rs7612209	3	177,596,989	*AK056252*	A	1.21	6.45×10^{-5}	0.93	0.11	1.06	0.087

Summary of SNPs that exceeded the threshold of $P < 1 \times 10^{-4}$ on the basis of score-based tests using logistic regression in the discovery stage and were brought forward to the replication stage. In the combined meta-analysis of the discovery and replication samples, four new variants (the first four SNPs in the table) reached the threshold of genome-wide significance ($P < 5 \times 10^{-8}$). All of the four new variants are coding variants (*CETP* rs2303790 encoding D442G; *C6orf223* rs2295334 encoding A231A; *SLC44A4* rs12661281 encoding D47V; and *FGD6* rs10507047 encoding Q257R). Physical positions and nearest genes are based on NCBI build 37 of the human genome.
SNP, single-nucleotide polymorphism; Chr, chromosome; OR, odds ratio for per copy of the minor allele.
*Number in parentheses presents the no. of cases and no. of controls, respectively.
†Heterogeneity $I^2 = 0\%$ for *CETP* rs2303790 and *FGD6* rs10507047.
‡Heterogeneity $I^2 = 41.5\%$, random-effects P in the combined analysis $= 1.61 \times 10^{-13}$.
§Heterogeneity $I^2 = 23.1\%$, random-effects P in the combined analysis $= 6.91 \times 10^{-9}$.
‖These index SNPs are located in intergenic regions. Other index SNPs are located within the genes.

AMD cases and 15,980 controls ($P < 5.0 \times 10^{-8}$ for each of the four loci; Table 2 and Supplementary Table 2). Genotyping clusters were directly visualized for the top SNPs and confirmed to be of good quality (Supplementary Fig. 3).

Of note, we did not observe any substantial difference in the association signals of the most significant SNPs in the subgroup analysis of our AMD cases by typical neovascular AMD ($n = 1{,}083$ cases) and polypoidal choroidal vasculopathy ($n = 1{,}015$ cases; Supplementary Table 3). The effect size for each of the top SNPs was similar between the two AMD subgroups.

Conditional analysis. The presence of the mutant *CETP* 442G (rs2303790) allele is seen only in East Asians (for example, Chinese, Japanese and Koreans; minor allele frequency (MAF) <5% in our controls) and not in South Asians, Europeans or Africans. This mutation is independent from all previously described common, non-coding polymorphisms near the *CETP* locus ($r^2 < 0.1$; Supplementary Fig. 4). Regional association analysis conditioning on other known common AMD variants in *CETP* confirmed the independence of D442G from other nearby common variants (Supplementary Tables 4 and 5). We also genotyped and assessed multiple, rare, protein-changing mutations at *CETP*, including Y74Stop, G331S, N358S and A390P (Fig. 1). None of them showed association with AMD (Table 3). Mutational load and haplotypic analysis considering all amino-acid changes within *CETP* confirmed that the D442G mutation drove all signals of association between *CETP* and AMD (Table 4).

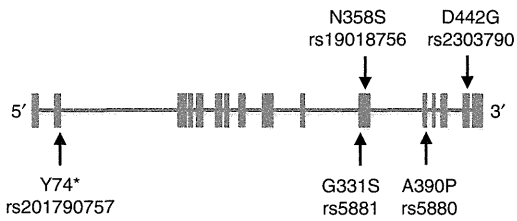

Figure 1 | Genomic organization of the *CETP* gene. The position of five rare (minor allele frequency <0.05) amino-acid changes observed in the discovery samples are shown as indicated by the arrows. Horizontal bars represent the position of exons in the *CETP* gene.

C6orf223 is located ~220,000 base pairs downstream of *VEGFA* and ~150,000 base pairs from rs943080, a marker previously shown to strongly associate with AMD in Europeans[5,6]. In this study of AMD in East Asians, the evidence of association for *VEGFA* rs943080 was only nominally significant ($P = 0.041$ in the discovery stage; Supplementary Table 1). Linkage disequilibrium analysis revealed no correlation between *C6orf223* rs2295334 and *VEGFA* rs943080 ($r^2 = 0.0$), with both markers separated by significant recombination events (Fig. 2a). Logistic regression adjusting for the allele dosage at *VEGFA* rs943080 did not reveal any attenuation of the association signal for *C6orf223* rs2295334 ($P = 1.66 \times 10^{-8}$; Supplementary Table 6). Regional association

Table 3 | Single variant analysis (score-based tests using logistic regression) of the five observed *CETP* rare variants with exudative age-related macular degeneration in the discovery stage.

Sample collections	SNP	Amino-acid change	A1	A2	Genotype*		MAF		OR	P value
					Cases	Controls	Cases	Controls		
Hong Kong	rs201790757	Y74*	C	A	0/0/507	0/3/2,962	0	0.0005	N/A	1
Hong Kong	rs5881	G331S	A	G	0/5/502	12/1/52	0.0049	0.0024	1.94	0.18
Japan	rs5881	G331S	A	G	0/3/970	0/4/759	0.0015	0.0026	0.59	0.49
Singapore	rs5881	G331S	A	G	0/5/635	0/21/1,935	0.0039	0.0054	0.73	0.52
Hong Kong	rs190187567	N358S	G	A	0/0/507	0/1/2,964	0	0.0002	N/A	1
Japan	rs190187567	N358S	G	A	0/1/972	0/0/763	0.0005	0	N/A	1
Hong Kong	rs5880	A390P	C	G	0/5/502	0/36/2,926	0.0049	0.0061	0.81	0.66
Singapore	rs5880	A390P	C	G	0/13/627	0/28/1,925	0.0102	0.0072	1.43	0.30
Hong Kong	rs2303790	D442G	G	A	4/45/458	2/172/2,791	0.0523	0.0297	1.80	2.8×10^{-4}
Japan	rs2303790	D442G	G	A	4/84/885	0/41/722	0.0473	0.0269	1.79	0.0025
Singapore	rs2303790	D442G	G	A	1/44/595	0/98/1,858	0.0359	0.0251	1.48	0.034

SNP, single-nucleotide polymorphism; A1, minor allele; A2, major allele; MAF, minor allele frequency; OR, odds ratio for per copy of the minor allele.
*Data are number of genotypes A1A1/A1A2/A2A2.

Table 4 | Results of association of the five observed *CETP* rare variants with exudative age-related macular degeneration using gene-based tests in 2,119 cases and 5,691 controls in the discovery stage.

Gene	No. of variants	Variants (minor allele counts)	Unconditional analysis*	Conditional analysis*
CETP	5	Tyr74*(3), Gly331Ser (56), Asn358Ser (2), Ala390Pro (81), Asp442Gly (536)	$P = 5.38 \times 10^{-6}$	$P = 0.96$

*Gene-based tests on mutational load (additive allele based using Burden tests) at *CETP*, unconditioned and conditioned for *CETP* Asp442Gly.

analysis including all markers from the genome and exome array data conditioning on *C6orf223* rs2295334 also did not reveal any secondary signal of association within its 1 Mb flanking region (Supplementary Table 7), thus pointing to *C6orf223* rs2295334 as a novel and uncharacterized genetic risk factor for exudative AMD in East Asians.

SLC44A4 is located ~116,000 base pairs away from a previously reported AMD locus, *C2-CFB* (rs429608)[6]. Nevertheless, *SLC44A4* rs12661281 has no correlation with *C2-CFB* rs429608 ($r^2 = 0.01$) and showed the strongest evidence of association with AMD within the genomic region (Fig. 2b), suggesting it to be also a new and uncharacterized risk factor for AMD. Logistic regression analysis adjusting for allele dosage at *C2-CFB rs429608* did not result in any significant change in magnitude of the association either at *SLC44A4* rs12661281, testifying to their mutual independence ($OR_{unconditioned} = 1.38$, $P_{unconditioned} = 1.10 \times 10^{-7}$; $OR_{conditioned\ for\ rs429608} = 1.35$, $P_{conditioned\ for\ rs429608} = 1.49 \times 10^{-6}$; Supplementary Table 8).

Gene-based tests on mutational load. We next proceeded to conduct gene-based tests on mutational load to further investigate the role of low-frequency variants in exudative AMD for all the patient collections in the discovery stage. Gene-based tests are an alternative to single-marker tests for association, which are often underpowered to detect association with rare variants. We performed our tests as previously described[15]. To more directly address the impact of low-frequency, non-synonymous genetic variants, we considered only 109,296 such variants with MAF <5%. As a result, we were able to assess a total of 10,736 genes having at least two such variants using the sequence kernel association optimal (SKAT-O) test[16]. We did not detect significant evidence of association ($P < 5 \times 10^{-8}$) between mutational load and AMD at any of the 10,736 genes tested, which are consistent across all three discovery sample collections. Nonetheless, while looking up on previously reported 22 genes within 17 distinct loci (Supplementary Table 1) associated with AMD in European populations, we note nominal evidence of association between genetic load at *CETP* ($P_{unconditioned} = 5.38 \times 10^{-6}$), whereby the association was almost entirely driven by D442G ($P_{conditioned\ for\ D442G} = 0.96$; Table 4) as well as *C2* and AMD ($P = 1.83 \times 10^{-6}$, Supplementary Table 9). All the observations exceeding $P < 1 \times 10^{-4}$ for gene-based tests on mutational load summarized across the three discovery collections are appended as Supplementary Table 9.

CETP 442G, HDL and coronary heart disease. The mutant *CETP* 442G allele was shown to result in an abnormally functioning CETP protein[17]. As CETP is a critical component of the pathways that regulate high-density lipoprotein cholesterol (HDL-c)[18], we assessed this mutation for associations with serum HDL-c levels using linear regression, with adjustment for age, gender and body mass index, in three population-based cohorts of Singaporean Chinese[19–21] and Japanese[22] ($n = 7,102$, see details on the study cohorts in Supplementary Methods) where GWAS data were available. We noted a strong association between 442G allele and increased HDL-c levels ($\beta = 0.174\ mmol\ l^{-1}$ per copy of 442G allele; reflecting an ~10% shift within the normal HDL range, $P = 5.82 \times 10^{-21}$; Table 5). This effect size is at least twice that observed for other *CETP* variants reported in European populations (Supplementary Table 10)[23,24].

Due to its strong effect on serum HDL-c, we assessed whether the mutant *CETP* 442G allele conferred any effect on individual susceptibility to coronary heart disease (CHD) in East Asians. Using 683 CHD cases and 1,281 controls from the Singapore Chinese Health Study (Supplementary Methods)[25], we noted some degree of enrichment of the HDL-increasing, mutant 442G allele in the controls (2.89%) compared with the cases (2.42%,

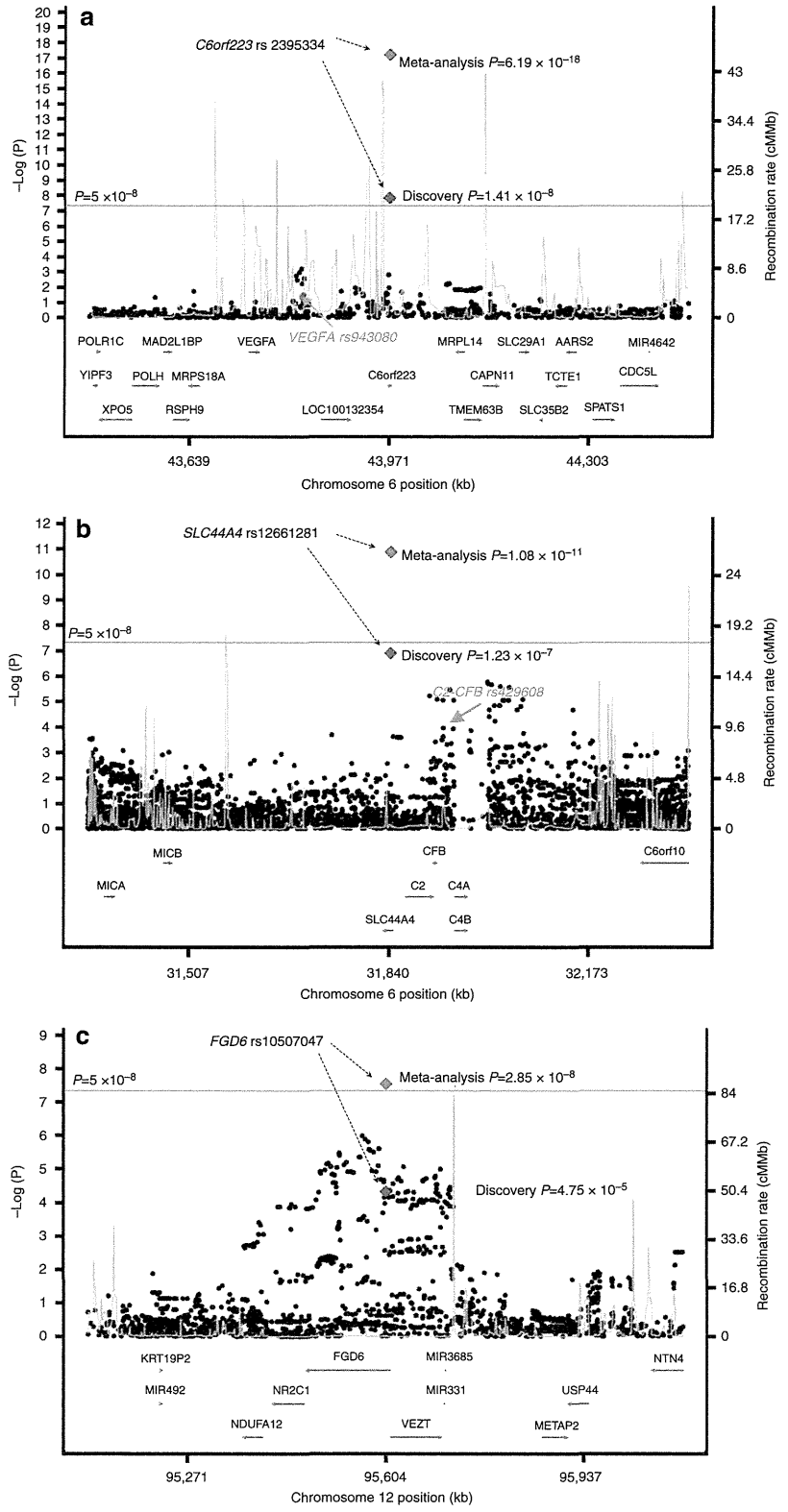

Figure 2 | Regional association plots at the three risk loci for exudative age-related macular degeneration. (**a**) The *C6orf223* locus (rs2295334): *VEGFA* rs943080, which is located in the intergenic, non-coding region, was very strongly associated with AMD in Europeans, but this study of exudative AMD in East Asians could not reveal significant association with it ($P = 0.041$ by score-based tests using logistic regression in 2,119 cases and 5,691 controls). The two markers are independent from each other ($r^2 = 0.0$), as well as separated by strong recombination events in Asians. (**b**) The *SLC44A4* locus (rs12661281): *C2-CFB* rs429608 was very strongly associated with AMD in Europeans, but revealed modest evidence of association in our discovery stage ($P = 1.06 \times 10^{-4}$). The two markers are independent from one another ($r^2 = 0.01$). (**c**) The *FGD6* locus (rs10507047).

Table 5 | Association of serum HDL cholesterol level and *CETP* D442G in East Asian population-based cohorts.

Cohort	No. of samples	D442G genotype	Serum HDL cholesterol mean (s.d.), mmol l^{-1}	Association of HDL with D442G				
				Effect allele	Effect allele frequency	Effect,* mmol l^{-1}	s.e.m.	*P* value
SCES	1,922	Gly/Gly	1.46†	442Gly	0.02	0.17	0.04	1.46×10^{-6}
		Asp/Gly	1.49 (0.010)					
		Asp/Asp	1.32 (0.009)					
SP2-set1‡	1,044	Gly/Gly	—†	442Gly	0.03	0.17	0.04	5.38×10^{-5}
		Asp/Gly	1.70 (0.014)					
		Asp/Asp	1.52 (0.011)					
SP2-set2§	888	Gly/Gly	—†	442Gly	0.03	0.17	0.05	3.46×10^{-4}
		Asp/Gly	1.59 (0.012)					
		Asp/Asp	1.43 (0.012)					
Nagahama	3,248	Gly/Gly	2.32 (0.344)	442Gly	0.04	0.18	0.03	1.85×10^{-9}
		Asp/Gly	1.83 (0.030)					
		Asp/Asp	1.66 (0.008)					
Meta-analysis	7,102			442Gly	0.03	0.174	0.018	5.82×10^{-21}

SCES, Singapore Chinese Eye Study; SP2, Singapore Prospective Study Program; Nagahama, Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience.
*Effect per copy of the 442Gly allele on serum HDL-c in mmol l^{-1}.
†Only one individual in SCES was homozygous (Gly/Gly) for the *CETP* 442G mutant allele. No Gly/Gly homozygotes were observed in both SP2 collections.
‡Genotyped on Illumina 610 K chip.
§Genotyped on Illumina 1 M chip.

OR = 0.83), although this did not reach statistical significance ($P = 0.39$). However, our observations were consistent with a recent study from Japan which analysed *CETP* D442G in 4,399 CHD cases and 7,672 controls whereby enrichment of the 442G allele were also found in the controls (3.4%) compared with the CHD cases (2.8%; OR = 0.83, $P = 0.02$)[26]. We thus performed a meta-analysis of our study and the Japanese study, resulting in a consistent protective effect of this mutation with CHD (OR = 0.83, $P = 0.011$, $I^2 = 0.0\%$; Supplementary Fig. 5).

Discussion

Our studies of exudative AMD in East Asians identified three novel loci (*C6orf233*, *SLC44A4* and *FGD6*), two of which (*SLC44A4* and *FGD6*) harbour coding, non-synonymous variants. These have not been identified in large samples of AMD patients of European descent[6], thus validating the role of searching for coding variations in diverse ethnic groups to better understand mechanistic basis of complex diseases such as AMD.

Our most interesting findings were the identification of an uncommon East Asian-specific mutation at *CETP* (D442G) associated with exudative AMD. The association is the strongest (per-allele OR = 1.70) observed outside of the classical *CFH* and *ARMS2-HTRA1* loci in East Asians. A common variant (rs3764261) mapping to the intergenic region between *HERPUD1* and *CETP* was previously linked to AMD in Europeans, yet its effect size was modest (OR = 1.15)[5] and independent from the D442G association. The mutant 442G allele is known to impair CETP function with reduction in plasma CETP mass and activity[17,27], and is associated with elevated HDL-c in Japanese families[17,27]. This allele is absent in European populations[28] and appears to be present only in East Asians, rendering it independent from all other previously described common, non-coding *CETP* polymorphisms. We showed that each copy of the dysfunctional 442G allele confers, on average, a rise in HDL-c levels of 0.174 mmol l^{-1} and confirmed findings from the earlier Japanese studies. Given the mean serum HDL-c concentration is 1.3 mmol l^{-1}, this is a mutation of considerable effect size even from a population-based perspective. Notably, no other amino-acid substitutions within *CETP* detected by our GWAS and EWAS showed any evidence of association with AMD (Fig. 1 and Table 3), suggesting that the D442G mutation is a possible causative mutation of AMD in East Asians in the *CETP* locus. We are not surprised that *CETP* D442G did not show a clearly significant protective effect against CHD as increasing lines of evidence more directly implicate LDL as the driving force for CHD susceptibility[29,30].

C6orf223 is a newly mapped gene with yet unknown functional role. Its A231A synonymous coding change is fivefold rarer in Europeans (MAF = 0.03) as compared with Asians (MAF > 0.15, Supplementary Table 2). It is thus unsurprising that the European GWAS efforts have yet to detect this locus. *VEGFA* rs943080, a marker strongly associated with AMD in European-ancestry populations[5], is in the vicinity of *C6orf223*, but its association with AMD is much weaker in this study of East Asians (Supplementary Tables 1 and 6), possibly reflecting the differences in therapeutic response to anti-VEGF treatment between Asians and Europeans[13,31].

The D47V mutation within *SLC44A4* is not included in most of the routinely used genotyping arrays, but is now included as part of the exome array used in this study. The genomic region around the *SLC44A4* locus is more complex, being located within the broad MHC region on Chromosome 6 between *HLA* class I and class II genes. As this region is very polymorphic, and allele frequency differences between cases and controls could be confounded by even minor population stratification, we thus reassessed the associations with AMD by adjusting for the first 10 principal components (PCs) of genetic ancestry. We did not observe any change in the association signals observed from our standard analysis, which adjusted for the first five PCs (analysis adjusting for the top 10 PCs; OR = 1.39, $P = 2.33 \times 10^{-7}$ in the discovery phase), consistent with previous observations in Asian studies with well-replicated associations within the MHC region[32,33]. *SLC44A4* encodes for choline transporter protein-4, involved in sodium-dependent choline uptake by cholinergic neurons[34]. Defects in *SLC44A4* have been linked to sialidosis, which presents with a spectrum of symptoms including eye abnormalities[35].

Prior studies on the basis of European patient collections have identified a total of four distinct AMD-associated loci on Chromosome 6 alone[6]. In this light, the burden of proof for the positive identification of *C6orf223* and *SLC44A4* (both are also