

# Choroidal Neovascularization in Eyes With Choroidal Vascular Hyperpermeability

Masahiro Miyake,<sup>1,2</sup> Akitaka Tsujikawa,<sup>1</sup> Kenji Yamashiro,<sup>1</sup> Sotaro Ooto,<sup>1</sup> Akio Oishi,<sup>1</sup> Hiroshi Tamura,<sup>1</sup> Isao Nakata,<sup>1,2</sup> Fumihiko Matsuda,<sup>2</sup> and Nagahisa Yoshimura<sup>1</sup>

<sup>1</sup>Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan

<sup>2</sup>Center for Genomic Medicine/Inserm U.852, Kyoto University Graduate School of Medicine, Kyoto, Japan

Correspondence: Akitaka Tsujikawa, Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto 606-8507, Japan; tsujikawa@kuhp.kyoto-u.ac.jp.

Submitted: January 29, 2014

Accepted: April 17, 2014

Citation: Miyake M, Tsujikawa A, Yamashiro K, et al. Choroidal neovascularization in eyes with choroidal vascular hyperpermeability. *Invest Ophthalmol Vis Sci.* 2014;55:3223-3230. DOI:10.1167/iovs.14-14059

**PURPOSE.** We describe the clinical and genetic characteristics of choroidal neovascularization (CNV) in eyes with choroidal vascular hyperpermeability (CVH).

**METHODS.** This cross-sectional study consisted of 438 consecutive patients who underwent fluorescein and indocyanine green angiography for macular disease. We used the genotypes of 1576 age-related macular degeneration (AMD) cases and 3248 general population controls as reference groups for genetic association analyses.

**RESULTS.** Of 871 eyes (438 patients) examined, CVH was found in 227 eyes (26.1%). Of these 227 eyes, 52 (22.6%) had CNV in the macular area. The proportion of patients with drusen and the choroidal thickness were not different between eyes with and without CNV, after adjusting for age ( $P = 0.21$  and  $0.95$ ). Of the 52 eyes with CNV, 51 had type 1 CNV and only one eye had pure type 2 CNV. Of the 51 eyes with type 1 CNV, polypoidal lesions were observed in 17 eyes (33.3%). Genotype distributions of *ARMS2* (A69S) and *CFH* (I62V) in patients with CVH and type 1 CNV significantly differed from those of AMD cases ( $P = 0.0014$  and  $0.0098$ , respectively), but not from general population controls ( $P = 0.33$  and  $0.82$ , statistical power of 88.5% and 72.9%, respectively).

**CONCLUSIONS.** In patients with CVH, type 1 CNV may occur frequently and sometimes accompanies type 2 CNV or polypoidal lesions. In terms of *ARMS2* and *CFH*, genetic background of patients with CVH and type 1 CNV was different from those with AMD, but rather similar to the general Japanese population.

**Keywords:** CSC, type 1 CNV, choroidal vascular hyperpermeability, AMD, genetics

Central serous chorioretinopathy (CSC) is characterized by a serous retinal detachment in the macular area, as confirmed by leakage on fluorescein angiogram (FA). In most eyes with CSC, indocyanine green angiography (ICGA) shows choroidal vascular abnormalities, including choroidal filling delays, dilated vasculature, punctate hyperfluorescent spots, and/or choroidal vascular hyperpermeability (CVH).<sup>1-6</sup> It has been reported that CVH occurs in 90% to 100% of eyes with CSC,<sup>5,7-11</sup> persisting even after serous retinal detachment resolution.<sup>8,11</sup> Interestingly, disease recurrence with new leakage often is seen in the area of CVH.<sup>8,11</sup> In addition, it generally is believed that CVH underlies pathophysiologic abnormalities associated with CSC.

It was noted recently that CVH sometimes is observed in eyes with neovascular age-related macular degeneration (AMD)<sup>12</sup> and polypoidal choroidal vasculopathy (PCV).<sup>12-14</sup> Several studies also have associated CVH with phenotypic variability and AMD treatment efficacy. Jirattanasopa et al.<sup>12</sup> reported that eyes with AMD or PCV that also had CVH had a thicker choroid. Koizumi et al.<sup>14</sup> reported that anti-VEGF treatments were less effective in eyes with PCV accompanied by CVH. However, Maruko et al.<sup>15</sup> found that photodynamic therapy was more effective in eyes with PCV and CVH. These features are consistent with typical CSC characteristics.<sup>2,4,10,16</sup>

It is thought classically that CSC is not accompanied by choroidal neovascularization (CNV) and that patients have a good visual prognosis. In a retrospective study by Mudvari et al.,<sup>17</sup> none of the 340 consecutive CSC patients developed CNV during an approximately 4-year follow-up period (mean of 49 months). However, Spaide et al.<sup>4</sup> reported that older patients with CSC had a lower visual acuity (VA), and were more likely to have diffuse retinal pigment epitheliopathy and secondary CNV than their younger counterparts. Subsequent reports have suggested that classic CNV (mainly type 2) and polypoidal lesions are possible complications of CSC and may contribute to visual loss in these eyes.<sup>5,18-21</sup> Fung et al.<sup>22</sup> recently described 9 eyes with longstanding CSC that went on to develop type 1 CNV. They concluded that a portion of these eyes were given a diagnosis of neovascular AMD, but should have been given a diagnosis of CNV secondary to CSC (i.e., CNV masquerading as AMD).

It still is controversial whether CNV that shares features with CSC originally is AMD or CSC. However, based on the above reports, we hypothesized that most of "AMD with CVH" might truly be "CNV secondary to CSC" masquerading as AMD. Here, to recruit "AMD with CVH" and "CNV secondary to CSC" as one cluster of CNV, we studied consecutive eyes with "CNV with CVH." Although most of these CNV are diagnosed currently as AMD, we demonstrated this cluster of CNV has different characteristics from AMD.

## METHODS

The current study was approved by the Institutional Review Board (IRB) at Kyoto University Graduate School of Medicine and all study conduct adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each patient who was genotyped. According to our IRB guidelines, it was not mandatory to obtain informed consent from patients before retrospectively reviewing their medical records.

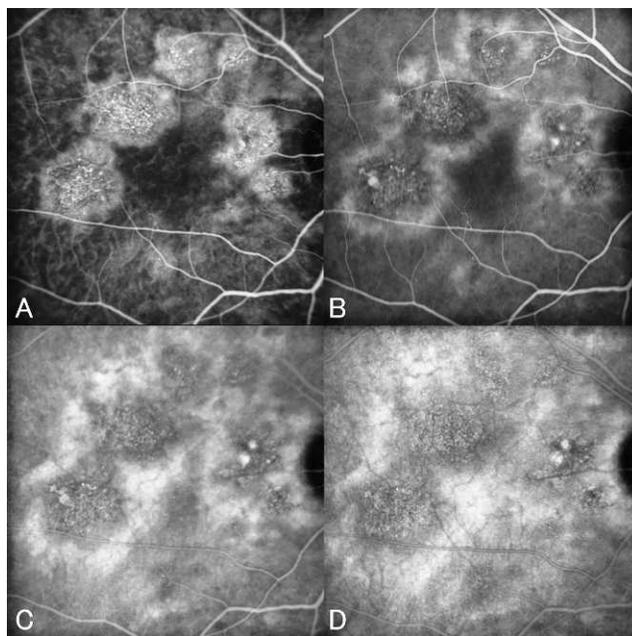
## Subjects

We retrospectively reviewed the medical records of 438 consecutive patients who visited the macular service at Kyoto University Hospital (Kyoto, Japan) between June 2011 and December 2012, and who underwent FA and ICGA to confirm or rule out macular diseases (e.g., AMD, CSC, other CNV, or other diseases requiring angiography for diagnosis). Comprehensive ophthalmic examinations were conducted on all patients, which included best-corrected VA, dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, color fundus photography, FA, and ICGA using a confocal laser scanning ophthalmoscope (HRA2; Heidelberg Engineering, Dossenheim, Germany). Spectral-domain optical coherence tomography (SD-OCT, Spectralis; Heidelberg Engineering) also was performed on all patients. All images were obtained using an eye-tracking system, and 100 scans were averaged automatically to improve the signal-to-noise ratio. Inverted images were obtained routinely in all patients using an enhanced-depth imaging (EDI) technique introduced by Spaide et al.<sup>25</sup>

## CVH and Other Findings

The CVH was evaluated in the late phase of ICGA, approximately 10 to 15 minutes after dye injection. With reference to a report by Guyer et al.,<sup>1</sup> CVH was defined as multifocal areas of hyperfluorescence with blurred margins within the choroid, followed by minimal extension of focal hyperfluorescent area (Fig. 1). This status was evaluated by two retina specialists (MM, AT) who were masked to all other medical information. Both evaluators diagnosed CVH as a binary trait (i.e., present or not), and only the eyes that both evaluators diagnosed with CVH were considered CVH-positive in further analyses. After recruitment, complications were evaluated in all eyes with CVH. Complications, such as type 1 CNV, type 2 CNV, and polypoidal lesion, were determined based on the results of fundus examination, FA, ICGA, and OCT by two retina specialists (MM and AT).

For patients who were judged to have CVH in at least one eye, we evaluated EDI-OCT images in both eyes. Central subfoveal choroidal thickness, defined as the vertical distance between Bruch's membrane and the choriocleral interface, was measured manually by a retinal specialist blinded to study parameters using the built-in caliber. We used data from



**FIGURE 1.** Typical CVH observed in a 43-year-old man. Four images from an ICGA obtained at 1:35:50 (A), 5:37:51 (B), 10:43:80 (C), and 16:17:16 (D) after the intravenous injection of indocyanine green. (A) In the early phase, clusters of small hyperfluorescent spots can be seen in focal areas of hyperfluorescence. (B–D) Focal areas of hyperfluorescence expand over time, eventually forming a ring shape. The center of the initially hyperfluorescent area gradually becomes hypofluorescent.

horizontal line scans. If it was difficult to identify the outer choroid in its entirety, we chose 10 points at which the choriocleral interface could be identified easily and created a segmentation line, based on which subfoveal choroidal thickness was measured.

## Genotyping

Genomic DNAs were prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). We selected two major AMD-associated single nucleotide polymorphisms (SNP), *complement factor H (CFH)* I62V (rs800292),<sup>24–26</sup> and *age-related maculopathy susceptibility 2 (ARMS2)* A69S (rs10490924).<sup>26–28</sup> Samples from patients with CVH and CNV in at least one eye were genotyped using a commercially available assay (Taqman SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA, USA).

For the reference group, we used two cohorts. One was the Kyoto AMD cohort, which consisted of 1576 unrelated AMD patients recruited from the Departments of Ophthalmology at Kyoto University Hospital, Fukushima Medical University

**TABLE 1.** Patient and Ocular Characteristics of Subjects With CVH

	No CNV	Any CNV	P Value	P Value Adjusted for Age
n	175	52	-	-
Age, y	59.6 ± 13.0	68.0 ± 11.2	<0.001	-
Sex, male:female	140:35	44:8	0.55	-
Serous PED (%)	49 (28.0)	7 (13.5)	0.037	0.21
Drusen (%)	43 (24.5)	21 (40.4)	0.028	0.25
Visual acuity, logMAR	-0.03 ± 0.21	0.28 ± 0.34	<0.001	<0.001
Choroidal thickness, μm	362.9 ± 120.1	323.6 ± 102.2	0.035	0.76

All data are presented as mean ± SD, where applicable. PED, pigment epithelial detachment, logMAR, logarithm of minimum angle resolution.

TABLE 2. Patient and Ocular Characteristics of Subjects With CVH and CNV

	Type 1 CNV						Pure Type 2 CNV
	Pure Type 1 CNV	P Value* (Adjusted for Age)	With Type 2 CNV	P Value* (Adjusted for Age)	With Polypoidal Lesion	P Value* (Adjusted for Age)	
<i>n</i>	28	-	6	-	17	-	1
Age, y	68.4 ± 9.2	<0.001	63.3 ± 15.5	0.34	70.1 ± 9.9	<0.001	33
Sex, male:female,	26:2	0.12	3:3	0.11	15:2	0.53	0:1
serous PED	4 (14.3%)	0.13 (0.48)	1 (16.7%)	0.55 (0.75)	2 (11.8%)	0.17 (0.57)	0
Drusen	11 (39.3%)	0.11 (0.36)	1 (16.7%)	0.66 (0.54)	9 (52.9%)	0.016 (0.072)	0
Visual acuity, logMAR	0.22 ± 0.26	<0.001 (<0.001)	0.45 ± 0.31	<0.001 (<0.001)	0.31 ± 0.46	<0.001 (<0.001)	0.22
Choroidal thickness, μm	353.3 ± 110.7	0.69 (0.15)	331.7 ± 62.5	0.53 (0.98)	268.5 ± 81.1	0.0039 (0.077)	327.0

Data presented as mean ± SD.

\* Compared to eyes without CNV.

Hospital, and Kobe City Medical Center General Hospital. The AMD diagnosis was confirmed by 3 retinal specialists. A fourth specialist (NY) was consulted when the initial three reviewers could not reach a consensus. These patients were genotyped using Illumina OmniExpress or HumanOmni2.5M Arrays (Illumina, Inc., San Diego, CA, USA). Another cohort of the general population made up the control group and consisted of 3248 unrelated individuals, recruited from the Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience (The Nagahama Study).<sup>29-31</sup> These patients were genotyped using HumanHap610K Quad Arrays, HumanOmni2.5M Arrays, and/or HumanExome Arrays (Illumina, Inc.),

and the two SNPs genotypes were extracted from the cohort's fixed dataset.

### Statistical Analysis

Every 2 × 2 table was compared using a Fisher's exact test, while continuous variables were compared using unpaired *t*-tests. Linear or logistic regression analyses were performed and adjusted for age. Genotypes were compared using  $\chi^2$  tests for trend. Statistical power of the genetic association test also was calculated. These statistical analyses were conducted using Software R (R Foundation for Statistical Computing, Vienna, Austria). A *P* value <0.05 was considered statistically significant.

### RESULTS

Of the 871 eyes (438 patients) examined, 120 eyes (61 patients) had high myopia (axial length ≥ 26 mm). No eyes with high myopia had signs of CVH on ICGA. In the current study, CVH was seen in 227 eyes (26.1%) from 141 patients (112 men, 29 women), ranging in age from 33 to 89 years (62.0 ± 13.1 years). All patients were Japanese and of Asian ancestry. Further analysis was performed on data from these 227 eyes with CVH.

Of the 227 eyes with CVH, 52 (22.6%) had CNV in the macular area. Table 1 summarizes the demographics of these eyes. The mean age of patients with CNV was significantly higher than that of subjects without CNV. The proportion of eyes with a serous pigment epithelium detachment (PED) or any drusen was higher in eyes with CNV. Measurements of choroidal thickness also seemed to be larger in eyes with CNV, but none of these differences was statistically significant after adjusting for age (PED *P* = 0.34, drusen *P* = 0.21, and choroidal thickness *P* = 0.95, respectively).

Table 2 shows subclassification of the 52 eyes with CVH and CNV. Of all eyes, 51 (22.6%) had type 1 CNV in the macular area (Fig. 2). Pure type 2 CNV, without any type 1 CNV, was seen in only one eye (0.4%) and data from this eye were excluded from all further analyses. Of the 51 eyes with type 1 CNV, polypoidal lesions were observed in 17 eyes (33.3%, Fig. 3), and type 2 CNV was observed in 6 eyes (11.8%, Fig. 4). The remaining 28 eyes (54.9%) had only type 1 CNV. The mean age of patients with either type 1 CNV or with polypoidal lesions was significantly higher than that of patients with no CNV, and their VA was significantly lower than that of eyes with no CNV. Additionally, more eyes with polypoidal lesions had concomitant drusen and thinner choroids than eyes without these

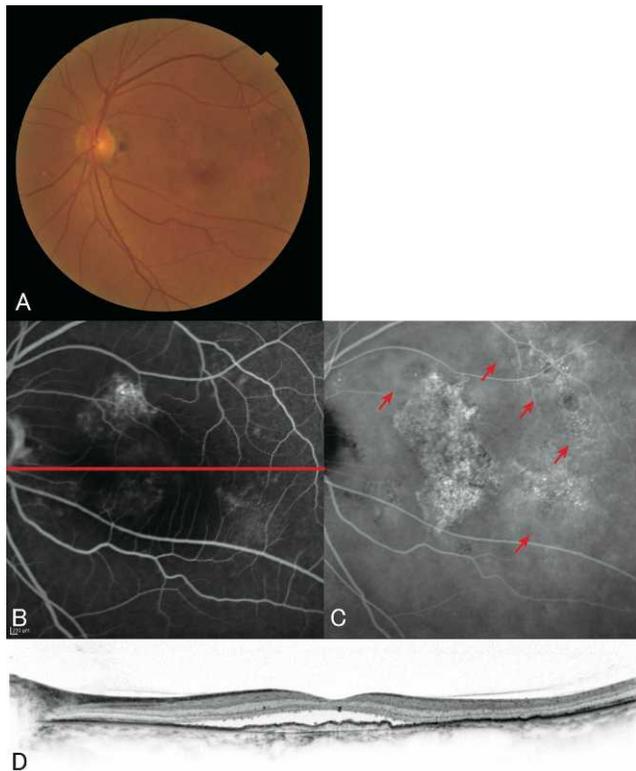
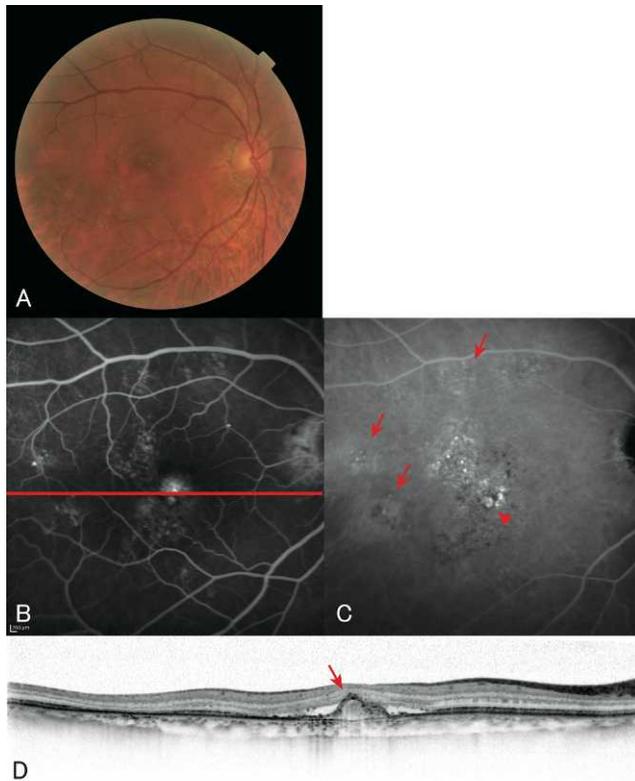


FIGURE 2. CVH with pure type 1 CNV. (A) A 70-year-old male with a serous retinal detachment in the left eye (VA = 20/40). (B) An FA showing occult CNV. (C) Late-phase ICGA showing typical CVH (arrows). (D) A horizontal OCT cross-sectional image reveals a subfoveal fibrovascular pigment epithelium detachment (type 1 CNV) with subretinal fluid. Neither a polypoidal lesion nor classic CNV is apparent in the angiogram.

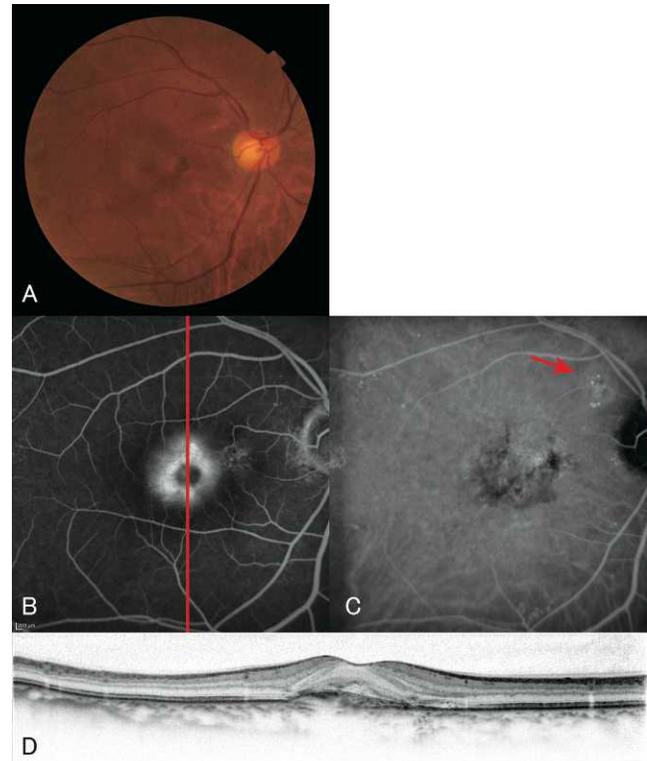


**FIGURE 3.** A CVH with type 1 CNV accompanied by a polypoidal lesion. (A) A 70-year-old man had visual impairment in the right eye (VA = 20/50). (B) An FA showing classic CNV with numerous dot-like hyperfluorescent spots. (C) Late-phase ICGA showing CVH (arrows) with a polypoidal lesion (arrowhead). (D) A horizontal OCT cross-sectional image reveals a sharply protruding retinal pigment epithelium, consistent with the polypoidal lesion seen in ICGA (arrow).

lesions, but these differences were not statistically significant after adjusting for age.

Figure 5 shows the distribution of eyes with each feature in the context of the association between choroidal thickness and age. The solid line indicates the best-fit line (choroidal thickness [ $\mu\text{m}$ ] =  $-4.10 \times \text{age} [\text{years}] + 606$ ) for data on the effect of choroidal thickness on age. Most eyes with only type 1 CNV, types 1 and 2 CNV, and type 1 CNV with polypoidal lesion belonged to patients who were  $\geq 60$  years old. Choroidal thickness in these eyes was distributed almost evenly along the regression line. Eyes with serous PED tended to belong to younger patients, while those with drusen tended to belong to older patients.

Supplementary Table S1 summarizes patient characteristics of the two cohorts used as reference groups for genetic association testing. Tables 3 and 4 show results of the genetic association tests. Genotype distributions of *ARMS2* and *CFH* were significantly different in our subjects with CVH and type 1 CNV, and the Kyoto AMD cohort (*ARMS2*,  $P = 1.4 \times 10^{-3}$ ; *CFH*,  $P = 9.8 \times 10^{-3}$ ), but not with Nagahama control group (*ARMS2*,  $P = 0.33$ ; *CFH*,  $P = 0.82$ ). Power calculations revealed that for associations of the reported effect size (odds ratio [OR] = 2.7 for *ARMS2* and 0.42 for *CFH*),<sup>32</sup> we could have detected an association by 88.5% for *ARMS2* and 72.9% for *CFH*. Furthermore, genotype distribution of individuals with CVH and type 1 CNV was similar to Hapmap Japanese in Tokyo (Hapmap JPT, available in the public domain at <http://hapmap.ncbi.nlm.nih.gov/index.html>).



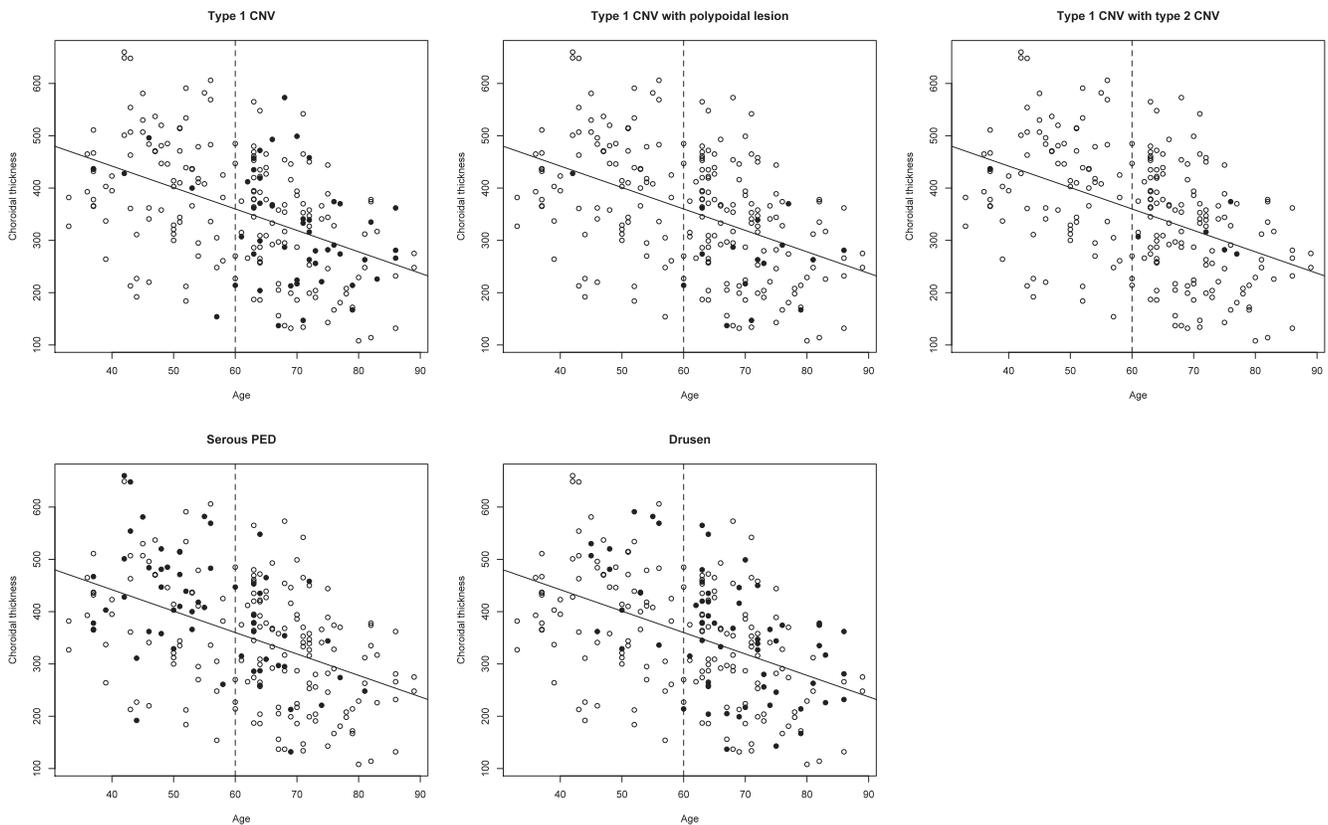
**FIGURE 4.** A CVH with both type 1 and 2 CNV. (A) A 72-year-old woman had visual impairment in the right eye (VA = 20/32). (B) An FA showing classic CNV. (C) Late-phase ICGA showing typical CVH (arrow) and numerous hyperfluorescent spots. (D) A vertical OCT cross-sectional image reveals subfoveal type 1 and 2 CNV and a small amount of subretinal fluid.

We also compared the genotype distributions in subjects with CVH and type 1 CNV to those in typical AMD cases or PCV cases from the Kyoto AMD cohort, summarized in Table 5. The subjects with CVH and type 1 CNV showed significantly different genotype distributions from those with typical AMD or PCV, respectively.

## DISCUSSION

To date and to our knowledge, there is no available information on the prevalence of CVH in the cohort study. In the current study, CVH was seen in 26.1%. However, data were collected through a retrospective review of medical records of consecutive patients examined with ICGA, performed because of macular disease suspicions. Because asymptomatic subjects rarely visit the clinic, we may have overestimated the CVH prevalence. On the other hand, ICGA often reveals CVH in the asymptomatic fellow eye of patients with unilateral CSC. Therefore, the prevalence of asymptomatic CVH may have been higher than we assumed.

In the current study, 175 of 227 eyes (77.4%) with CVH had no evidence of CNV, but type 1 CNV in the macular area was confirmed in 51 eyes (22.5%). Pure type 2 CNV (with no type 1 CNV) was seen in only one eye (0.4%). Therefore, we estimated that 22.6% (95% confidence interval [CI], 17.6%–28.9%) of eyes with CVH also had CNV. The true prevalence of type 1 CNV in eyes with CVH would be lower because of the selection bias our inclusion criteria introduced. Nevertheless, type 1 CNV is not a rare complication in eyes with CVH based on the estimates of the current study.



**FIGURE 5.** Choroidal thickness versus patient age. All eyes with CVH were plotted. Each *solid line* represents the best-fit line of how choroidal thickness is affected by age (choroidal thickness [ $\mu\text{m}$ ] =  $-4.10 \times \text{age}$  [years] + 606). Each *broken line* is a line of a 60-year-old. (A) Eyes with pure type 1 CNV are highlighted in *closed circles*. (B) Eyes with type 1 CNV and polypoidal lesions are highlighted in *closed circles*. (C) Eyes with type 1 and 2 CNV are highlighted in *closed circles*. (D) Eyes with serous pigment epithelial detachment are highlighted in *closed circles*. (E) Eyes with drusen are highlighted in *closed circles*.

The mean age of patients with type 1 CNV was significantly higher than in patients without CNV. Although the proportion of eyes with a serous PED or drusen, along with choroidal thickness, tended to be higher in eyes with type 1 CNV, these differences were not statistically significant after age adjustments were made. Spaide et al.<sup>4</sup> previously reported that older patients with CSC had a lower VA, and were more likely to have diffuse retinal pigment epitheliopathy, bilateral involvement, and secondary CNV than their younger counterparts. Although most eyes with classic CSC also have CVH in younger patients, they rarely develop CNV. It is possible that some patients with CVH go on to develop CNV at an older age.

Of the 51 eyes with type 1 CNV, polypoidal lesions and type 2 CNV also were present in 33.3% and 11.8% of eyes, respectively. Several previous reports also have indicated that

the choroid is thicker in eyes with PCV than in eyes with AMD.<sup>33-35</sup> On the other hand, Jirattanasopa et al.<sup>12</sup> reported that choroidal thickness was significantly greater in eyes with PCV and no CVH (225.7  $\mu\text{m}$ ) than in eyes with AMD and no CVH (158.9  $\mu\text{m}$ ). They also reported that choroidal thickness is not different in eyes with CVH and AMD (278.2  $\mu\text{m}$ ), and in eyes with CVH and PCV (283.4  $\mu\text{m}$ ). In our patients with CVH, eyes with polypoidal lesions (268.5  $\mu\text{m}$ ) had a thinner choroid than those with pure type 1 CNV (353.0  $\mu\text{m}$ ). However, choroidal thickness still was greater than in healthy eyes, as reported previously in patients averaging 64.6 years of age (203.6  $\mu\text{m}$ )<sup>36</sup> and in AMD (158.9  $\mu\text{m}$ ) or PCV (225.7  $\mu\text{m}$ ) eyes with no CVH.<sup>12</sup> Therefore, CVH is associated primarily with an increase in choroidal thickness.

**TABLE 3.** Comparison of A69S Genotype Frequency

	GG	GT	TT	T Allele Frequency	P Value*
AMD	302	587	620	0.605	$1.4 \times 10^{-3}$
Hyperpermeability + type 1 CNV	15	19	8	0.417	Reference
Nagahama control	1312	1499	435	0.365	0.33†
Hapmap JPT	15	21	9	0.433	0.83

\* Indicates  $\chi^2$  test for trend.

† Indicates statistical power = 88.5% (assuming OR = 2.7, minor allele frequency = 0.40).

**TABLE 4.** Comparison of the I62V Genotype Frequency

	GG	GA	AA	A Allele Frequency	P Value*
AMD	856	578	121	0.264	$9.8 \times 10^{-3}$
Hyperpermeability + type 1 CNV	16	19	7	0.393	Reference
Nagahama control	1162	1538	546	0.405	0.82†
Hapmap JPT	16	22	6	0.386	0.93

\* Indicates  $\chi^2$  test for trend.

† Indicates statistical power = 72.9% (assuming OR = 0.42, minor allele frequency = 0.40).

TABLE 5. Differences of Genotype Distributions Against Typical AMD or PCV

	ARMS2 A69S						CFH I62V					
	T Allele			Frequency	OR (95% CI)	P Value*	A Allele			Frequency	OR (95% CI)	P Value*
	GG	GT	TT				GG	GA	AA			
CVH + type 1 CNV	15	19	8	0.417	–	–	16	19	7	0.393	–	–
PCV	186	310	282	0.562	1.79 (1.15–2.80)	0.017	438	294	67	0.268	0.57 (0.36–0.89)	0.015
Typical AMD	111	272	308	0.645	2.52 (1.61–3.94)	1.0×10 <sup>-4</sup>	402	270	47	0.253	0.52 (0.33–0.82)	4.9×10 <sup>-3</sup>

\*  $\chi^2$  test for trend.

Recently, Fung et al.<sup>22</sup> reported that, compared to patients with type 1 neovascularization secondary to AMD, patients with type 1 CNV associated with CSC are more likely to be younger and male, have thicker choroids, and have polypoidal lesions. Although it is rather difficult to compare our findings to those of this report because of inclusion criteria differences, choroidal thickness in our patients (323.5  $\mu$ m) was compatible with that measured in eyes with “CNV secondary to CSC” (356.5  $\mu$ m). In addition, the rate of concomitant polypoidal lesions was comparable between our study (33.3%) and that of Fung et al.<sup>22</sup> (36%).

Both *ARMS2* and *CFH* generally are thought as the two most important SNPs associated with the development of AMD and PCV in Caucasian and Japanese populations.<sup>12,24–27</sup> In the current study, genetic association analysis showed that the genetic background of patients with type 1 CNV and CVH was different from that of patients with AMD. Statistical testing showed that the probability of such a deviation occurring by chance is 0.14% and 0.98% for the *ARMS2* and *CFH* gene variations, respectively. Their different genetic background from those of typical AMD cases and PCV cases (shown in Table 5) also is important; it raises the possibility that type 1 CNV with CVH is different not only from typical AMD, but also PCV. Furthermore, no significant differences were observed in genotype between patients with type 1 CNV and CVH and Japanese controls. Because the statistical powers of these association tests were adequate (88.5% and 72.9% for *ARMS2* and *CFH*, respectively), it is unlikely that a false-negative occurred, especially for the *ARMS2* gene variation.

The current result of the *CFH* association test is supported by a previous report<sup>12</sup> that compared the *CFH* I62V genotype between patients with AMD and CVH, and patients with AMD and no CVH. This report showed that patients with AMD and CVH had a more similar genotype distribution (A allele frequency of 34%) to Japanese controls than did patients with AMD and no CVH (A allele frequency of 24%). Because *ARMS2* or *CFH* genotyping is not the gold standard in diagnosing AMD, we cannot clearly distinguish AMD from CSC by simply examining the *ARMS2* or *CFH* genotypes. However, judging from the two major genes (*ARMS2* and *CFH*), CNV with CVH has a different genetic background than CNV associated with AMD, and has similar genetic background as control subjects.

Yannuzzi et al.<sup>37</sup> previously published a report on 13 patients initially suspected of having CSC, but ultimately were diagnosed with PCV. Because none of these cases also had CVH, we believe that these eyes were likely to be PCV from the start. Based on accumulating ICGA and OCT evidence, it generally is believed that CVH is a principal pathophysiologic abnormality underlying CSC. Since Sasahara et al.<sup>15</sup> reported an association between CVH and PCV, various investigators have examined the clinical features of these eyes and thought that these eyes had AMD or PCV that was accompanied by CVH.<sup>12,14,15,33,38</sup> However, in a recent report by Fung et al.,<sup>22</sup> CVH was attributed to CNV or polypoidal lesions secondary to CSC. Unfortunately, whether CNV with CVH originally began

as AMD or as CSC remains controversial. However, our study results added further insight to this argument, from the genetic point of view.

This study has various limitations. First, this study is a retrospective, hospital-based study. Ideally, we would have enrolled consecutive subjects from a long-term, prospective, population-based cohort. However, because it is ethically questionable to perform ICGA on healthy subjects, accurately estimating CVH prevalence in the general population would have been difficult. Second, eyes were determined to have CVH only if independent diagnoses of 2 retinal specialists agreed. This increased diagnostic specificity for CVH, but decreased the sensitivity, and marginal cases had to be ignored. Additional objective criteria or diagnostic methods are needed to eliminate subjective interpretation of CVH in the further studies. Third, we only examined the two most important SNPs associated with the development of AMD and PCV (*ARMS2* and *CFH*). Genotypes of many other diseases susceptible to SNPs might provide further understanding of the current issues. Fourth is the quality of controls in the genetic association tests. If the prevalence of CVH with type 1 CNV was high in the general population, then the statistical power of the genetic association test is lower than we estimated. Although we can assume that the CVH with type 1 CNV prevalence in the general population is not high based on the low prevalence of CNV<sup>30</sup> and CSC,<sup>39,40</sup> we must interpret the negative associations with caution. Finally, this is a cross-sectional study, and lacks an investigation of treatment efficacy and long-term visual prognosis. These should be explored in future prospective, long-term studies.

In summary, the current study describes the clinical characteristics of CNV that is seen in eyes with CVH. Type 1 CNV was seen more frequently than it was thought, and they sometimes accompanied type 2 CNV or polypoidal lesions. Additionally, the genetic background of these patients was different from AMD patients, but rather similar to the general Japanese population, suggesting that “CNV with CVH” might be one cluster of CNV distinguished from AMD.

### Acknowledgments

The authors thank Takeo Nakayama (Department of Health Informatics, Kyoto University School of Public Health, Kyoto, Japan), Akihiro Sekine (Department of Genome Informatics, Kyoto University School of Public Health, Kyoto, Japan), Shinji Kosugi (Department of Medical Ethics, Kyoto University School of Public Health, Kyoto, Japan), Yasuharu Tabara (Center for Genomic Medicine, Graduate School of Medicine, Kyoto University), and Ryo Yamada (Center for Genomic Medicine, Graduate School of Medicine, Kyoto University) for their efforts with the Nagahama study.

The authors alone are responsible for the content and writing of the paper.

Disclosure: **M. Miyake**, None; **A. Tsujikawa**, Pfizer (F); **K. Yamashiro**, None; **S. Ooto**, None; **A. Oishi**, None; **H. Tamura**,

None; I. Nakata, None; F. Matsuda, None; N. Yoshimura, Topcon Corporation (F), Nidek (F), Canon (F)

## References

- Guyer DR, Puliafito CA, Mones JM, et al. Digital indocyanine-green angiography in chorioretinal disorders. *Ophthalmology*. 1992;99:287-291.
- Piccolino FC, Borgia L. Central serous chorioretinopathy and indocyanine green angiography. *Retina*. 1994;14:231-242.
- Spaide RF, Yannuzzi LA, Slakter JS, et al. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. *Retina*. 1995;15:100-210.
- Spaide RF, Campeas L, Haas A, et al. Central serous chorioretinopathy in younger and older adults. *Ophthalmology*. 1996;103:2070-2079. discussion 9-80.
- Lafaut BA, Salati C, Priem H, De Laey JJ. Indocyanine green angiography is of value for the diagnosis of chronic central serous chorioretinopathy in elderly patients. *Graefes Arch Clin Exp Ophthalmol*. 1998;236:513-521.
- Tsujikawa A, Ojima Y, Yamashiro K, et al. Punctate hyperfluorescent spots associated with central serous chorioretinopathy as seen on indocyanine green angiography. *Retina*. 2010;30:801-809.
- Guyer DR, Yannuzzi LA, Slakter JS, et al. Digital indocyanine green videoangiography of central serous chorioretinopathy. *Arch Ophthalmol*. 1994;112:1057-1062.
- Piccolino FC, Borgia L, Zinicola E, Zingirian M. Indocyanine green angiographic findings in central serous chorioretinopathy. *Eye*. 1995;9:324-332.
- Spaide RF, Hall L, Haas A, et al. Indocyanine green videoangiography of older patients with central serous chorioretinopathy. *Retina*. 1996;16:203-213.
- Menchini U, Virgili G, Lanzetta P, Ferrari E. Indocyanine green angiography in central serous chorioretinopathy. ICG angiography in CSC. *Int Ophthalmol*. 1997;21:57-69.
- Iida T, Kishi S, Hagimura N, Shimizu K. Persistent and bilateral choroidal vascular abnormalities in central serous chorioretinopathy. *Retina*. 1999;19:508-512.
- Jirarattanasopa P, Ooto S, Nakata I, et al. Choroidal thickness, vascular hyperpermeability, and complement factor H in age-related macular degeneration and polypoidal choroidal vasculopathy. *Invest Ophthalmol Vis Sci*. 2012;53:3663-3672.
- Sasahara M, Tsujikawa A, Musashi K, et al. Polypoidal choroidal vasculopathy with choroidal vascular hyperpermeability. *Am J Ophthalmol*. 2006;142:601-607.
- Koizumi H, Yamagishi T, Yamazaki T, Kinoshita S. Relationship between clinical characteristics of polypoidal choroidal vasculopathy and choroidal vascular hyperpermeability. *Am J Ophthalmol*. 2013;155:305-313.
- Maruko I, Iida T, Sugano Y, et al. Subfoveal retinal and choroidal thickness after verteporfin photodynamic therapy for polypoidal choroidal vasculopathy. *Am J Ophthalmol*. 2011;151:594-603.
- Quin G, Liew G, Ho IV, et al. Diagnosis and interventions for central serous chorioretinopathy: review and update. *Clin Exp Ophthalmol*. 2013;41:187-200.
- Mudvari SS, Goff MJ, Fu AD, et al. The natural history of pigment epithelial detachment associated with central serous chorioretinopathy. *Retina*. 2007;27:1168-1173.
- Cooper BA, Thomas MA. Submacular surgery to remove choroidal neovascularization associated with central serous chorioretinopathy. *Am J Ophthalmol*. 2000;130:187-191.
- Chan WM, Lam DS, Lai TY, et al. Treatment of choroidal neovascularization in central serous chorioretinopathy by photodynamic therapy with verteporfin. *Am J Ophthalmol*. 2003;136:836-845.
- Yang CS, Chen KC, Lee SM, Lee FL. Photodynamic therapy in the treatment of choroidal neovascularization complicating central serous chorioretinopathy. *J Chin Med Assoc*. 2009;72:501-505.
- Ahuja RM, Downes SM, Stanga PE, et al. Polypoidal choroidal vasculopathy and central serous chorioretinopathy. *Ophthalmology*. 2001;108:1009-1010.
- Fung AT, Yannuzzi LA, Freund KB. Type 1 (sub-retinal pigment epithelial) neovascularization in central serous chorioretinopathy masquerading as neovascular age-related macular degeneration. *Retina*. 2012;32:1829-1837.
- Spaide RF, Koizumi H, Pozzoni MC. Enhanced depth imaging spectral-domain optical coherence tomography. *Am J Ophthalmol*. 2008;146:496-500.
- Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2005;102:7227-7232.
- Mori K, Gehlbach PL, Kabasawa S, et al. Coding and noncoding variants in the CFH gene and cigarette smoking influence the risk of age-related macular degeneration in a Japanese population. *Invest Ophthalmol Vis Sci*. 2007;48:5315-5319.
- Hayashi H, Yamashiro K, Gotoh N, et al. CFH and ARMS2 variations in age-related macular degeneration, polypoidal choroidal vasculopathy, and retinal angiomatous proliferation. *Invest Ophthalmol Vis Sci*. 2010;51:5914-5919.
- Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14:3227-3236.
- Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am J Hum Genet*. 2006;78:852-864.
- Yoshimura K, Nakayama T, Sekine A, et al. B-type natriuretic peptide as an independent correlate of nocturnal voiding in Japanese women. *NeuroUrol Urodyn*. 2012;31:1266-1271.
- Nakata I, Yamashiro K, Nakanishi H, et al. Prevalence and characteristics of age-related macular degeneration in the Japanese population: the Nagahama study. *Am J Ophthalmol*. 2013;156:1002-1009.
- Nakata I, Yamashiro K, Kawaguchi T, et al. Association between the cholesterol ester transfer protein gene and polypoidal choroidal vasculopathy. *Invest Ophthalmol Vis Sci*. 2013;54:6068-6073.
- Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. *Nat Genet*. 2013;45:433-439.
- Koizumi H, Yamagishi T, Yamazaki T, et al. Subfoveal choroidal thickness in typical age-related macular degeneration and polypoidal choroidal vasculopathy. *Graefes Arch Clin Exp Ophthalmol*. 2011;249:1123-1128.
- Baja ES, Schwartz JD, Wellenius GA, et al. Traffic-related air pollution and QT interval: modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study. *Environ Health Perspect*. 2010;118:840-846.
- Kim SW, Oh J, Kwon SS, et al. Comparison of choroidal thickness among patients with healthy eyes, early age-related maculopathy, neovascular age-related macular degeneration, central serous chorioretinopathy, and polypoidal choroidal vasculopathy. *Retina*. 2011;31:1904-1911.
- Hirata M, Tsujikawa A, Matsumoto A, et al. Macular choroidal thickness and volume in normal subjects measured by swept-source optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:4971-4978.

37. Yannuzzi LA, Freund KB, Goldbaum M, et al. Polypoidal choroidal vasculopathy masquerading as central serous chorioretinopathy. *Ophthalmology*. 2000;107:767-777.
38. Chung SE, Kang SW, Lee JH, Kim YT. Choroidal thickness in polypoidal choroidal vasculopathy and exudative age-related macular degeneration. *Ophthalmology*. 2011;118:840-845.
39. Tsai DC, Chen SJ, Huang CC, et al. Epidemiology of idiopathic central serous chorioretinopathy in Taiwan, 2001-2006: a population-based study. *PLoS One*. 2013;8:e66858.
40. Kitzmann AS, Pulido JS, Diehl NN, et al. The incidence of central serous chorioretinopathy in Olmsted County, Minnesota, 1980-2002. *Ophthalmology*. 2008;115:169-173.