



Age-Related Maculopathy Susceptibility 2 and Complement Factor H Polymorphism and Intraocular Complement Activation in Neovascular Age-Related Macular Degeneration

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Purpose: To investigate the association of risk alleles in complement factor H (CFH) and age-related maculopathy susceptibility 2 (ARMS2) with complement activation products in the aqueous humor in eyes with neovascular age-related macular degeneration (nAMD) including polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP), and pachychoroid neovascularopathy (PNV).

Design: Prospective, comparative, observational study.

Participants: Treatment-naïve patients with nAMD and cataract patients as controls.

Methods: The study included 236 eyes of 236 patients with nAMD and 49 control eyes of 49 patients. Aqueous humor samples were collected from 67 eyes with drusen-associated nAMD, 72 eyes with PCV, 26 eyes with RAP, and 71 eyes with PNV before intravitreal anti-VEGF injection and cataract surgery in the 49 control eyes. Clinical samples were measured for complement component 3a (C3a), C4a, and C5a using a bead-based immunoassay. Genotyping of the ARMS2 A69S (rs10490924), CFH I62V (rs800292), and CFH Y402H (rs1061170) was performed using TaqMan genotyping.

Main Outcome Measures: The levels of complement activation products (C3a, C4a, and C5a) in the aqueous humor in each genotype of ARMS2 and CFH.

Results: The C3a level in the aqueous humor was significantly elevated ($P = 0.006$) in patients with nAMD and the ARMS2 A69S risk allele, whereas the levels of the complement activation products were not associated with CFH I62V and Y402H genotypes. Among the control eyes, no significant differences were seen in any complement activation products for all genetic polymorphisms. The levels of the complement activation products in the aqueous humor of eyes with the nAMD subtypes for each genetic polymorphism did not show significant differences.

Conclusions: The C3a concentration in the aqueous humor was significantly higher in Japanese nAMD patients with the ARMS2 A69S risk allele, whereas it was not elevated in the patients with CFH I62V. Age-related maculopathy susceptibility 2 A69S polymorphism is strongly associated with local complement activation in nAMD patients. *Ophthalmology Science* 2022;2:100167 © 2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

See Editorial (XOPS 100174) in this issue

Age-related macular degeneration (AMD) is a leading cause of irreversible and severe visual impairment in aged persons.¹ The development of AMD, a multifactorial disease, is substantially affected by environmental and genetic factors.^{2,3} Previous genome-wide association studies have reported that some complement proteins, such as complement factor H (CFH),⁴ complement component 3 (C3),⁵ CFI,⁶ CFB,⁷ and CFD,⁸ are associated with the development of AMD. The complement system is responsible for innate immunity, in which reactions occur in 3 pathways, that is, the classic pathway, alternative pathway, and lectin pathway, eventually advancing to the final pathway. Pathologically, complement components 1q, C3a, and C5a were deposited around the drusen and the

choroidal vessels and on Bruch's membrane of AMD patients.^{9,10} On this basis, it is important to clarify the factors involved in the activation of complement in eyes with AMD.

The major genetic risk factors for AMD were identified at chromosome 1q32 in the CFH gene and at chromosome 10q26 in the age-related maculopathy susceptibility 2 (ARMS2) gene.^{3,4} The CFH protein is a regulator of the alternative pathway. The CFH gene mutation alters factor H binding to Bruch's membrane, which is hypothesized to lead to poorly controlled complement activation.¹¹ The ARMS2 protein is associated with extracellular matrix function under oxidative stress or inflammation.¹²⁻¹⁴ Previous reports have suggested that ARMS2 protein also

contributes to complement system activation,¹⁵ although the detailed mechanisms are unknown.

Smailhodzic et al¹⁵ reported activation of the systemic complement system in patients with AMD with CFH and ARMS2 risk alleles, suggesting that these risk alleles are involved in the pathogenesis of AMD due to complement activation. However, Schick et al¹⁶ suggested that the concentration of complement activation products in the eye was not associated with that in the serum. Because the intraocular immune system is independent of the systemic immune system, the intraocular complement responses can differ from those in the blood. Recent studies have suggested that activation product levels of complement system in the aqueous humor may reflect local complement activation in the eye.¹⁶⁻²⁰ To our knowledge, the effect of genetic predisposition on local complement activation has not been reported.

In the current study, we measured the complement activation products (C3a, C4a, C5a) in the aqueous humor of patients with neovascular AMD (nAMD), including its 4 subtypes, and compared them by the CFH and ARMS2 genotypes.

Methods

Patients

This prospective, comparative, observational study adhered to the tenets of the Declaration of Helsinki; the research ethics board approved the study before patient enrollment started at the Department of Ophthalmology of Fukushima Medical University Hospital, Fukushima City, Japan. All patients provided written informed consent before enrollment.

All patients were Japanese aged > 50 years. A total of 236 eyes of 236 patients with nAMD were included. Forty-nine eyes of 49 patients who underwent cataract surgery served as controls. A total of 105 eyes of 105 patients were overlapped with our previous report investigating complement activation products in the aqueous humor.¹⁸ All patients underwent comprehensive ophthalmic examinations and slit-lamp biomicroscopy with a noncontact fundus lens. Color fundus photographs and fluorescein angiography (FA) images were obtained using a fundus camera TRC-DX (Topcon). Fundus autofluorescence and indocyanine green angiography (ICGA) images were obtained using HRA2 (Heidelberg Engineering). OCT (Spectralis, Heidelberg Engineering) and OCT angiography (PLEX Elite 9000, Carl Zeiss Meditec, Inc) were also performed. All control patients underwent examinations other than FA and ICGA.

Macular Neovascularization Classifications

All eyes with macular neovascularization (MNV) were classified into 4 subtypes: drusen-associated nAMD, polypoidal choroidal vasculopathy (PCV), retinal angiomatous proliferation (RAP), and polypoidal choroidal vasculopathy (PNV). Drusen-associated nAMD had soft drusen in either eye and no pachychoroid features. We defined pachychoroid features based on the most recent reports as satisfying all of the following criteria:^{18,21-23} (1) type 1 MNV detected in 1 or both eyes; (2) no or only nonextensive drusen (total area $\leq 125 \mu\text{m}^2$ circle) or hard drusen ($\leq 63 \mu\text{m}$) in both eyes; (3) dilated choroidal vessels below type 1 MNV detected by ICGA and OCT; (4) CVH detected on the late-phase ICGA images; and (5) the presence of central serous chorioretinopathy or pachychoroid pigment epitheliopathy-related retinal pigment epithelium (RPE)

abnormalities independent of CNV lesions detected by FA or a history of central serous chorioretinopathy. Polypoidal choroidal vasculopathy is characterized by pachychoroid features with polypoidal lesions and no soft drusen. Polypoidal lesions were diagnosed in the presence of terminal aneurysmal dilatations with a branching vascular network seen on ICGA images that corresponded to steep elevations of the RPE seen on the OCT images. Pachychoroid neovascularopathy is characterized by pachychoroid features and the absence of soft drusen or polypoidal lesions. Retinal angiomatous proliferation was characterized by retinal-retinal anastomosis or retinal-choroidal anastomosis and no pachychoroid features. Retinal-retinal anastomosis and retinal-choroidal anastomosis were diagnosed on ICGA images.²⁴

The exclusion criteria were eyes with a history of any other retinal diseases; uveitis; glaucoma, including ocular hypertension with use of any antiglaucoma eye drops; any intraocular surgery, including cataract surgery; high myopia (spherical equivalent < -6 diopters or axial length > 26.5 mm); or eyes with a treatment history of intravitreal anti-VEGF injections. Patients who had any systemic diseases potentially involved in complement system activation, such as diabetes, autoimmune diseases, cancer, cardiovascular disease, cerebrovascular disease, or systemic corticosteroid medications, were excluded. Control eyes with soft drusen or pachychoroid features were excluded.

Aqueous Humor Collection

Aqueous humor samples were aspirated from the eyes with nAMD before intravitreal injection of aflibercept (Eylea, Regeneron Pharmaceuticals) or ranibizumab (Lucentis, Genentech Inc) under topical anesthesia using a syringe with a 30-gauge needle (Nipro). The procedure and timing of the sample collection were standardized to minimize variations in the sampling procedure. The aqueous humor of controls was aspirated before cataract surgery in the same manner. The aqueous humor samples were immediately mixed with 2 μl of protease inhibitor cocktail (Sigma) to prevent complement activation. Aliquots of the obtained aqueous humor samples were stored at -80°C until analysis.

Measurement of Complement Activation Product and Cytokine Levels

Levels of complement activation products C3a, C4a, and C5a in the aqueous humor were measured using a Human Anaphylatoxin Kit (BD Biosciences). The bead-based immunoassay was performed according to the manufacturer's instructions.

Genotyping

Blood samples were obtained from all patients. Genotyping of ARMS2 A69S (rs10490924), CFH I62V (rs800292), and CFH Y402H (rs1061170) was performed using TaqMan genotyping.

Image Analysis

Soft drusen, central retinal thickness, subfoveal choroidal thickness, choroidal vascular hyperpermeability, greatest linear dimension, and MNV lesion size were evaluated as previously reported.¹⁸ The diagnoses of retinal-retinal anastomosis and retinal-choroidal anastomosis were confirmed on ICGA images.

Statistical Analysis

The Kruskal–Wallis test was used to compare the levels of complement activation products in the aqueous humor among the genotype. The Steel–Dwass method was used to analyze the complement activation product levels between the nAMD

subtypes. Statistical analyses were performed using JMP 14 software (SAS Institute Inc). $P < 0.05$ was considered statistically significant.

Results

Clinical Features

A total of 285 patients (236 patients with nAMD, 49 controls) were enrolled. In the 236 eyes with nAMD, the numbers of eyes with drusen-associated nAMD, PCV, RAP, and PNV were 67 (28.5%), 72 (30.5%), 26 (11.0%), and 71 (30.0%), respectively (Table 1).

The mean age was 74.9 ± 8.3 years (mean \pm standard deviation) for patients with nAMD and 69.9 ± 8.1 years for the control patients ($P = 0.001$). The mean ages of the patients with drusen-associated nAMD and RAP were higher than those of patients with PNV (vs. drusen-associated nAMD and vs. RAP, $P < 0.001$ for both comparisons) and PCV ($P < 0.001$ for both comparisons) by pairwise analysis. There were more women in the control group than in the nAMD group (44.8% vs. 30.0%, respectively; $P = 0.042$). No significant differences were seen in the equivalent spherical power or incidence of posterior vitreous detachment among the nAMD subtypes. The central retinal thickness ($P < 0.001$), subfoveal choroidal thickness ($P < 0.001$), greatest linear dimension ($P = 0.001$), and MNV size ($P = 0.026$) differed among the nAMD subtypes.

The frequency of the ARMS2 A69S genotype was significantly different between all eyes with nAMD and controls ($P = 0.001$). The frequency of TT, a homozygous risk allele, was higher in all eyes with nAMD than in controls (nAMD, 41.9%; controls, 10.4%). Among the nAMD subtypes, the genotypic frequencies for the ARMS2 A69S genotype in PNV and PCV significantly differed from those in drusen-associated nAMD ($P < 0.001$, vs. PNV; $P = 0.003$, vs. PCV) and RAP ($P = 0.003$ vs. PNV; $P = 0.018$, vs. PCV). For the CFH I62V and CFH Y402H genotypes, no significant differences were seen between all eyes with nAMD and controls (CFH I62V, $P = 0.89$; CFH Y402H, $P = 0.40$) and among the nAMD subtypes ($P = 0.40$ and $P = 0.61$, respectively).

Levels of Complement Activation Products

In patients with nAMD, a significant ($P = 0.006$) difference in the C3a levels was seen among the ARMS2 A69S genotypes (Table 2). No significant differences in the CFH I62V and CFH Y402H genotypes were seen with any of the complement activation products. Among the controls, there were no significant differences in any complement activation products in any of the genetic polymorphisms. In the ARMS2 A69S genotype, the C3a levels were significantly higher in the nAMD patients with the TT or GT genotype than with the GG genotype ($P = 0.007$, TT vs. GG; $P = 0.007$, GT vs. GG; by the Steel-Dwass test). The C5a levels did not significantly differ among the 3 ARMS2 genotypes. When the C5a levels in the nAMD patients were compared on the basis of the presence or

absence of the risk allele (T), the C5a levels with the risk allele were high (0.020 ng/ml, TT and GT; 0.015 ng/ml, GG) but did not reach significance ($P = 0.051$).

Table 3 shows the C3a levels in the aqueous humor of patients with the nAMD subtypes for each genetic polymorphism. Although the tendency for the C3a level to increase in eyes with the ARMS2 risk alleles was similar to that for all eyes with nAMD, no significant differences in the C3a levels were seen among the genotypes in any genetic polymorphism. There was no difference in the C3a level in the eyes with ARMS2 risk allele between each subtype (Table 4).

The associations between the ARMS2 A69S and CFH I62V risk alleles and the levels of C3a in the aqueous humor are shown in Figure 1. The levels of C3a in all eyes with nAMD with both risk alleles were higher than in all eyes with only the CFH risk allele ($P = 0.028$) and in controls with both risk alleles ($P = 0.002$). No significant difference was found in C3a levels except between the groups mentioned.

Discussion

The current results on the frequency of the risk alleles in the ARMS2 and CFH genes were mostly consistent with previous reports on Japanese patients with AMD.^{25,26} In the current study, the ARMS2 A69S polymorphism was strongly associated with C3a elevation in the nAMD eyes. The C4a level did not significantly differ between genotypes. The C5a level tended to be high but did not significantly differ between genotypes. No association was seen between the CFH I62V and Y402H genotypes and the local complement activation products. The current results suggest that ARMS2 is involved in local complement activation in the eyes of patients with nAMD, whereas CFH is unlikely in Japanese patients.

A few previous reports have suggested a relationship between the ARMS2 A69S risk allele and the systemic complement concentration.^{15,27} One study reported a high plasma C3d:C3 ratio, meaning activation of complement, in patients with AMD with the ARMS2 A69S risk allele,¹⁵ whereas another study reported no relationship between the ARMS2 risk allele and serum complement levels.²⁸ In the current study, the intraocular C3a levels were significantly higher in patients with the ARMS2 A69S risk alleles than those without the risk alleles. No elevation of the C4a level suggested less of a relationship between ARMS2 A69S and the classic pathway and lectin pathway. An elevated C5a level was seen in the eyes with ARMS2 risk alleles but did not reach significance, and it seems that the final pathway was activated. Age-related maculopathy susceptibility 2 A69S may be involved in local complement activation, especially in the alternative pathway, in patients with nAMD. Local complement inhibition might be another target of anti-complement therapy in patients with the ARMS2 risk allele.

Although the presence of the ARMS2 risk allele may be involved in intraocular complement activation of patients with AMD, the precise role of ARMS2 in the nAMD

Table 1. Baseline Demographics and Genotypic Characteristics of the Study Population

	Eyes with nAMD					P Value*	All Eyes with nAMD (n = 236)	Controls (n = 49)	P Value†
	Drusen-Associated nAMD (n = 67)	PCV (n = 72)	RAP (n = 26)	PNV (n = 71)					
Age, yrs mean (SD)	78.1 (7.0)	72.9 (7.9)	80.6 (6.5)	72.0 (8.7)	0.001	74.9 (8.3)	69.9 (8.1)	0.001	
Female gender, No. (%)	28 (41.8)	13 (18.0)	14 (53.8)	15 (21.1)	0.001	70 (30.0)	22 (44.8)	0.042	
Complete PVD, No. (%)	39 (58.2)	43 (59.7)	17 (65.4)	36 (50.7)	0.54	135 (57.2)	33 (67.4)	0.18	
Spherical equivalent, Diopters median (IQR)	0 (−0.87 to 1.37)	0 (−1.59 to 1.12)	0.12 (−0.78 to 0.90)	0.12 (−1.00 to 1.25)	0.98	0.12 (−1.00 to 1.25)	−0.25 (−2.68 to 0.62)	.044	
CRT, μm , median (IQR)	428 (341–555)	319 (254–466)	461 (339–655)	325 (244–444)	<0.001	-	-	-	
SFCT, μm , median (IQR)	188 (110–274)	259 (180–345)	151 (105–173)	319 (232–395)	<0.001	-	-	-	
GLD, μm , median (IQR)	4.67 (3.24–6.38)	3.86 (2.89–5.23)	2.39 (1.93–4.14)	3.08 (2.27–4.54)	0.001	-	-	-	
MNV size, μm^2 , median (IQR)	1.58 (1.03–2.86)	1.42 (0.71–3.07)	0.68 (0.24–1.84)	1.26 (0.63–2.77)	0.026	-	-	-	
ARMS2 A69S, No. (%)									
TT	41 (61.2)	23 (32.0)	16 (61.6)	19 (26.8)	0.001	99 (41.9)	5 (10.4)	0.001	
GT	18 (26.8)	35 (48.6)	9 (34.6)	33 (46.5)		95 (40.3)	22 (44.8)		
GG	8 (12.0)	14 (19.4)	1 (3.8)	19 (26.7)		42 (17.8)	22 (44.8)		
CFH I62V, No. (%)									
AA	37 (55.3)	32 (44.4)	12 (46.2)	38 (53.6)	0.40	119 (50.5)	24 (49.0)	0.89	
AG	27 (40.2)	32 (44.4)	9 (34.6)	26 (36.6)		94 (39.8)	21 (42.9)		
GG	3 (4.5)	8 (11.2)	5 (19.2)	7 (9.8)		23 (9.7)	4 (8.1)		
CFH Y402H, No. (%)									
CC	2 (2.9)	2 (2.8)	0 (0)	0 (0)	0.61	4 (1.7)	0 (0)	0.40	
TC	12 (17.9)	12 (16.6)	4 (15.4)	12 (17.0)		40 (17.0)	10 (20.5)		
TT	53 (79.2)	58 (80.6)	22 (84.6)	59 (83.0)		192 (81.3)	39 (79.5)		

ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; CRT = central retinal thickness; GLD = greatest linear dimension; IQR = interquartile range; MNV = macular neovascularization; nAMD = neovascular age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; PNV = pachychoroid neovascularopathy; PVD = posterior vitreous detachment; RAP = retinal angiomatous proliferation; SD = standard deviation; SFCT = subfoveal choroidal thickness; TT, GT, GG, AA, AG, CC, and TC indicate base pairs of genes.

*Kruskal-Wallis test (among MNV subtypes).

†Mann-Whitney U test (between all eyes with MNV and controls).

Table 2. Levels of Complement Activation Products in the Aqueous Humor of Patients with nAMD and Controls for each Genetic Polymorphism

A. Patients with nAMD							
		C3a (ng/ml)		C4a (ng/ml)		C5a (ng/ml)	
		P Value*		P Value*		P Value*	
ARMS2	TT	3.15	.006	1.54	.20	0.020	.17
A69S	GT	3.08		1.63		0.021	
	GG	2.25		1.38		0.016	
CFH	AA	3.00	.25	1.54	.64	0.021	.46
I62V	AG	3.02		1.54		0.017	
	GG	3.11		1.57		0.021	
CFH	CC	4.86	.51	1.40	.79	0.041	.06
Y402H	CT	3.03		1.64		0.022	
	TT	2.98		1.51		0.019	
B. Controls							
		C3a (ng/ml)		C4a (ng/ml)		C5a (ng/ml)	
		P Value*		P Value*		P Value*	
ARMS2	TT	1.43	.56	0.93	.21	0.017	.15
A69S	GT	2.03		1.27		0.041	
	GG	2.12		1.50		0.023	
CFH	AA	1.92	.99	1.35	.32	0.027	.39
I62V	AG	2.00		1.21		0.021	
	GG	2.23		1.69		0.029	
CFH	CC	-	.27	-	.56	-	.81
Y402H	CT	2.24		1.50		0.021	
	TT	1.93		1.25		0.025	

ARMS2 = age-related maculopathy susceptibility 2; C3a = complement component 3a; C4a = complement component 4a; C5a = complement component 5a; CFH = complement factor H; nAMD = neovascular age-related macular degeneration. TT, GT, GG, AA, AG, CC, CT, and TC indicate base pairs of genes.

*Kruskal-Wallis test.

pathogenesis remains unknown. The ARMS2 protein has been identified in the interstitial space of the choriocapillaris, the ellipsoidal region of the photoreceptors,^{12,29} and

intracellularly in the cytosol or mitochondria.^{12,29,30} The functions of ARMS2 may be maintenance of the homeostasis of the interstitial structure and Bruch's membrane,¹² a role in the phagocytosis function of RPE cells,³¹ and resistance to oxidative stress by involvement in the mitochondrial function.²⁹ Age-related maculopathy susceptibility 2 also has been suggested to be involved in the complement system. Experimentally, reductions of C3 and C5 expression in the RPE have been reported in individuals with ARMS2 deficiency.³² The ARMS2 protein is a surface molecule that enhances C3b opsonization via binding of properdin.¹³ Age-related maculopathy susceptibility 2 may be involved in debris removal by activating the complement system. Further studies are needed to elucidate the local complement activation in patients with ARMS2 polymorphism.

Complement factor H is a central regulator in the complement activation, especially in the alternative pathway.³³ Several studies have reported the relationship between the CFH genetic polymorphism and systemic complement activity. Ansari et al³⁴ found that the CFH I62V and Y402H polymorphisms were associated with plasma CFH levels based on logistic analysis. Smailhodzic et al¹⁵ reported an increased plasma C3d:C3 ratio in patients with AMD with the CFH Y402H risk allele. However, Silva et al³⁵ reported no significant difference in the serum CFH and C3 levels when the CFH Y402H genotypes were compared. Kelly et al³⁶ reported no significant difference in the C3b inactivation between CFH V62/H402 (both risk variants) and CFH I62/Y402 (both nonrisk variants). In the current study, no significant increase in the complement levels of the aqueous humor was observed in patients with the CFH Y402H and I62V risk alleles. The CFH Y402H and I62V polymorphisms may not be directly involved in local complement activation.

The CFH molecule has heparan sulfate-binding domains that facilitate binding to the host cell surface, thereby protecting the host tissue from complement damage.³⁷ The CFH H402 variant binds to Bruch's membrane less than the CFH Y402 variant, suggesting that the defense

Table 3. Levels of C3a in the Aqueous Humor of Patient with Drusen-Associated nAMD, PCV, RAP, and PNV for Each Genetic Polymorphism

		Drusen-Associated nAMD		PCV		RAP		PNV	
		P Value*		P Value*		P Value*		P Value*	
ARMS2	TT	3.16	.08	3.19	.14	2.96	.18	3.03	.18
A69S	GT	2.94		3.31		3.11		2.93	
	GG	1.88		2.33		5.48		2.19	
CFH	AA	2.82	.26	3.16	.82	3.01	.75	2.40	.08
I62V	AG	3.11		2.89		3.02		2.89	
	GG	5.83		3.02		3.11		3.59	
CFH	CC	3.00	.86	4.86	.41	-	1.00	-	.59
Y402H	CT	3.21		3.34		3.06		2.98	
	TT	3.74		3.10		3.06		2.58	

ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; C3a = complement component 3a; nAMD = neovascular age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; PNV = pachychoroid neovascularopathy; RAP = retinal angiomatous proliferation. TT, GT, GG, AA, AG, CC, and TC indicate base pairs of genes.

*Kruskal-Wallis test.

Table 4. Comparison between Subtypes of the Levels of C3a in the Aqueous Humor of Patient with ARMS2 A69S Risk Allele

	Drusen-associated nAMD*	PCV*	RAP*	PNV
Drusen-associated nAMD	-	-	-	-
PCV	$P = 0.99$	-	-	-
RAP	$P = 0.95$	$P = 0.81$	-	-
PNV	$P = 0.68$	$P = 0.64$	$P = 0.99$	-

ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; C3a = complement component 3a; nAMD = neovascular age-related macular degeneration; PCV = polypoidal choroidal vasculopathy; PNV = pachychoroid neovascularopathy; RAP = retinal angiomatous proliferation.

*By the Steel-Dwass test.

mechanism of Bruch’s membrane is vulnerable in the CFH H402 variant.¹¹ Besides, CFH is a major malondialdehyde-binding protein and an innate defense protein against malondialdehyde, a marker of oxidative stress.³⁸ The CFH H402 variant binds malondialdehyde less efficiently than the CFH Y402 variant, suggesting that patients with the CFH H402 variant have weak resistance to oxidative

stressors.³⁸ On the basis of these factors, the CFH polymorphism associated with AMD may be related to the defense mechanism of Bruch’s membrane and its resistance to oxidative stress.

In the current study, we examined whether there is an interaction between ARMS2 A69S and CFH I62V. The C3a levels in the aqueous humor of the patients with both ARMS2 A69S and CFH I62V risk alleles were higher than those of the patients with only the CFH I62V risk alleles, whereas there was no difference between the group with both ARMS2 A69S and CFH I62V risk alleles and only ARMS2. This may suggest that the presence of ARMS2 risk alleles has a more significant effect on complement activation. We also showed that the patients with nAMD with both ARMS2 A69S and CFH I62V risk alleles had higher C3a levels in the aqueous humor compared with controls with both risk alleles. A previous report on systemic complement activation found that the C3d:C3 ratio in plasma was higher in patients with AMD with both ARMS2 A69S and CFH Y402H risk alleles than in controls with both risk alleles.¹⁵ Considering our result and the previous report, a trigger may be needed for complement activation or AMD development.

In this study, there were no differences of the C3a level in the eyes with ARMS2 A69S risk allele between each

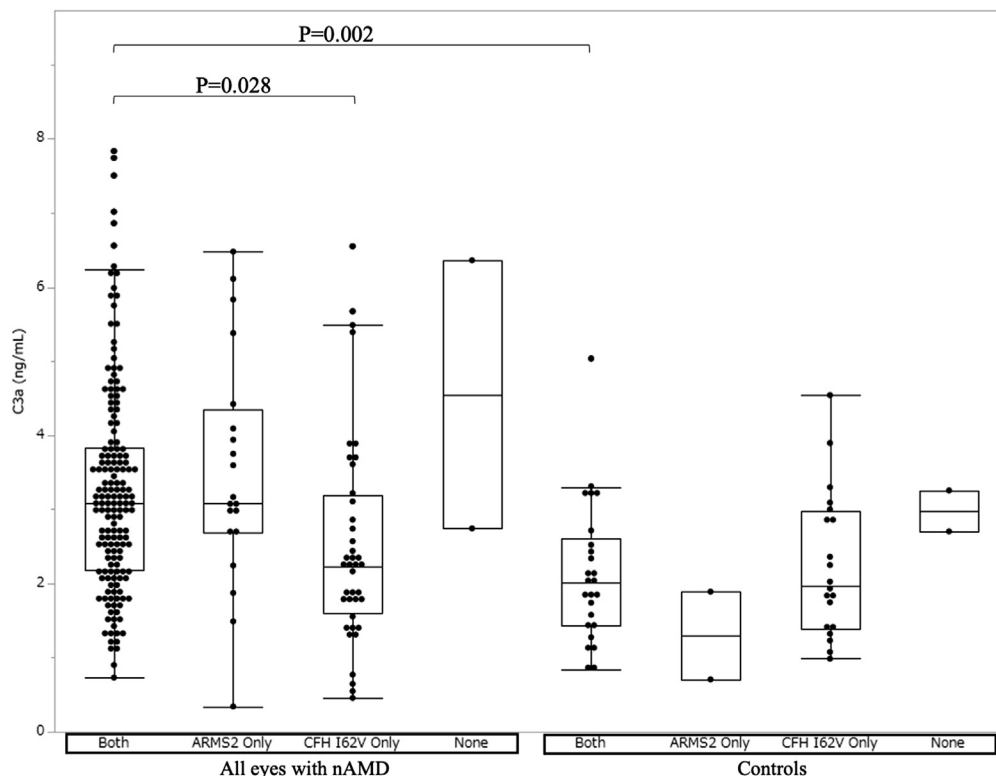


Figure 1. Association between the risk alleles of age-related maculopathy susceptibility 2 (ARMS2) and complement factor H (CFH) I62V and the levels of complement component 3a (C3a) in the aqueous humor. The box plot shows the median levels of C3a. The “both group” is composed of individuals with both the ARMS2 and CFH I62V risk alleles. The “ARMS2 only group” is composed of individuals with the ARMS2 risk allele but not the CFH I62V risk allele. The “CFH I62V only group” is composed of individuals with the CFH I62V risk allele but not the ARMS2 risk allele. The “none group” is composed of individuals without risk alleles in either gene. Compared with controls, the C3a level is significantly elevated ($P = 0.002$) in the “both group.” In all eyes with neovascular age-related macular degeneration (nAMD), the C3a level in the “both group” is significantly ($P = 0.028$) higher than in the “CFH I62V only group.” *Kruskal–Wallis test.

nAMD subtype and in the eyes with each nAMD subtype among the genotypes. Hayashi et al³⁹ reported that ARMS2 A69S has a strong association with all 3 nAMD subtypes, including typical AMD, PCV, and RAP, with the association being strongest for RAP and weakest for PCV. A strong association of ARMS2 variants with RAP may contribute the elevation of C3a in RAP patients. The reduction in numbers of patients by dividing into nAMD subtypes may have affected the results of this study.

The pathophysiology of PNV and PCV remains unknown. Although the frequency of the ARMS2 risk allele in PNV and PCV was lower than in drusen-associated nAMD, some patients with PNV and PCV were homozygous for ARMS2 A69S. The C3a levels were high in these patients. In other words, eyes with the ARMS2 risk allele and high C3a level exhibited different MNV phenotypes. We speculated that additional factors, such as ischemia or cell death, might affect the phenotypes of MNV. That speculation should be validated in a large number of patients in the future.

Study Limitations

The current study had several limitations. First, we did not measure other complement factors (B, D, H, and I) and components (C3, C4, and C5). More complement proteins should be measured to determine the mechanism of complement pathway activation. Second, all proteins associated with MNV under the retina may not reflect the aqueous

humor profiles, although we previously confirmed the correlations of the anaphylatoxin levels between the vitreous and aqueous humor.¹⁹ We should also consider protein production from other parts of the eye when interpreting the complement protein concentrations in the aqueous humor. Third, we recruited control eyes with cataracts, which may be associated with intraocular inflammation; however, it is unethical to collect aqueous humor from healthy eyes. Fourth, there were significant differences in age and gender among the drusen-associated nAMD, PCV, RAP, and PNV groups, which may have affected the results. The differences should be considered when interpreting the differences among the 4 categories. Fifth, the number of patients with RAP was small compared with other subtypes. Research with a larger number of RAP patients is needed in the future for more accurate comparisons.

Conclusions

The C3a concentration in the aqueous humor was significantly higher in the nAMD patients with the ARMS2 A69S risk allele, whereas it was not elevated in the patients with CFH I62V. The ARMS2 A69S polymorphism may be strongly associated with local complement activation in Japanese patients with nAMD. The CFH I62V variant has little association with intraocular complement activation.

Footnotes and Disclosures

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Author Contributions:

Conception and design: Kato, Oguchi, Omori, Sekiryu

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Analysis and interpretation: Kato, Oguchi, Machida, Sekine, Sekiryu

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Abbreviations and Acronyms:

AMD = age-related macular degeneration; **ARMS2** = age-related maculopathy susceptibility 2; **CFH** = complement factor H; **C3a** = complement component 3a; **C4a** = complement component 4a; **C5a** = complement component 5a; **FA** = fluorescein angiography; **ICGA** = indocyanine green angiography; **MNV** = macular neovascularization; **nAMD** = neovascular age-related macular degeneration; **PCV** = polypoidal choroidal vasculopathy; **PNV** = pachychoroid neovascularization; **RAP** = retinal angiomatous proliferation; **RPE** = retinal pigment epithelium.

Keywords:

Aqueous humor, ARMS2, CFH, C3a, C4a, C5a, Polymorphism.

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