

Genome-wide Survival Analysis for Macular Neovascularization Development in Central Serous Chorioretinopathy Revealed Shared Genetic Susceptibility with Polypoidal Choroidal Vasculopathy

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Purpose: To identify susceptibility genes for macular neovascularization (MNV) development in central serous chorioretinopathy (CSC).

Design: Genome-wide survival analysis using a longitudinal cohort study.

Participants: We included 402 and 137 patients with CSC but without MNV at their first visit from the Kyoto CSC Cohort and Kobe CSC dataset, respectively. All patients underwent detailed ophthalmic examinations, including multimodal imaging, such as fundus autofluorescence, spectral-domain OCT, and fluorescein angiography/indocyanine green angiography or OCT angiography.

Methods: We conducted a genome-wide survival analysis using the Kyoto CSC Cohort. We applied the Cox proportional hazard model to adjust for age, sex, and the first principal component. Single nucleotide polymorphisms (SNPs) with P values $< 1.0 \times 10^{-5}$ were carried forward to the replication in the Kobe CSC dataset. Moreover, we evaluated the contribution of previously reported age-related macular degeneration (AMD) susceptibility loci. We used FUMA and ToppFun for the functional enrichment analysis.

Main Outcome Measures: The association between SNPs and MNV development in patients with CSC.

Results: Rs370974631 near *ARMS2* displayed a genome-wide significant association in the meta-analysis of discovery and replication result (hazard ratio [HR]_{meta}, 3.63; $P_{\text{meta}} = 5.76 \times 10^{-9}$). Among previously reported AMD susceptibility loci, we additionally identified *CFH* rs800292 (HR, 0.39, $P = 2.55 \times 10^{-4}$), *COL4A3* rs4276018 (HR, 0.26, $P = 1.56 \times 10^{-3}$), and *B3GALT* rs9564692 (HR, 0.56, $P = 8.30 \times 10^{-3}$) as susceptibility loci for MNV development in CSC. The functional enrichment analysis revealed significant enrichment of 8 pathways (GO:0051561, GO:0036444, GO:0008282, GO:1990246, GO:0015272, GO:0030955, GO:0031420, and GO:0005242) related to ion transport.

Conclusions: *ARMS2*, *CFH*, *COL4A3*, and *B3GALT* were identified as susceptibility genes for MNV development in CSC. These 4 genes are known as susceptibility genes for AMD, whereas *COL4A3* and *B3GALT* were previously reported to be polypoidal choroidal vasculopathy (PCV)-specific susceptibility genes. Our findings revealed the shared genetic susceptibility between PCV and MNV secondary to CSC. *Ophthalmology* 2022;129:1034-1042 © 2022 by the American Academy of Ophthalmology



Supplemental material available at www.aaojournal.org.

Central serous chorioretinopathy (CSC) is characterized by serous retinal detachment around the fovea and is one of the common causes of vision loss, principally in young to middle-aged men.¹ Central serous chorioretinopathy consists of 2 clinically distinct entities, namely, acute CSC and chronic CSC.² Acute CSC resolves spontaneously within 3 months such that its visual prognosis is generally good. However, a recent long-term observational study by

Mrejen et al³ reported that chronic CSC lasting > 6 months resulted in legal blindness in 12.8% of the patients with an average 11.3 years of follow-up. In this report, 24% of the patients developed macular neovascularization (MNV), which was strongly associated with visual acuity at their last visit. Therefore, MNV is considered a major cause of poor visual prognosis in patients with CSC. The prevalence of MNV secondary to CSC ranges from 2% to 28.7%.⁴⁻⁷

Researchers have reported on several susceptibility genes for CSC. *CFH* was the first reported CSC susceptibility gene, which is also an established susceptibility gene for age-related macular degeneration (AMD); however, the effect direction is opposite between CSC and AMD.⁸ We have also identified additional susceptibility genes for CSC through 2 genome-wide association studies (GWASs): (1) an intermediate phenotype 2-staged GWAS of 6110 healthy Japanese individuals, which led to the identification of *VIPR2*,⁹ and (2) a 2-staged GWAS of 1546 CSC samples and 13 029 controls from Asian and White participants that identified *TNFRSF10A* and *GATA5*.¹⁰ Despite reports on these susceptibility genes for CSC development, researchers have not yet explored a detailed phenotype-genotype correlation.

Among a variety of clinical phenotypes for CSC, MNV development is most important. Macular neovascularization secondary to CSC is paid more attention after being located within the novel disease concept, pachychoroid spectrum diseases, and named *pachychoroid neovascularopathy* (PNV).¹¹ Despite speculating that the mechanical damage of choriocapillaris and Sattler's layer due to dilated choroidal vessels may cause MNV,¹² its molecular biological susceptibility is unknown. We aimed to conduct a genome-wide survival analysis for MNV development in patients with CSC, followed by replication in an independent dataset.

Methods

This study followed the tenets of the Declaration of Helsinki and was approved by the ethics committee of the Kyoto University Graduate School of Medicine and Kobe University Graduate School of Medicine. Written informed consent was obtained from all participants.

Discovery Dataset

We used the Kyoto CSC Cohort¹⁰ for the discovery stage. It consists of 610 unrelated patients with CSC who visited the Macular Service of the Kyoto University Hospital (Kyoto, Japan) and were recruited for genetic studies on disease development and clinical course with written informed consent. All patients underwent detailed ophthalmic examinations, including a measurement of the best-corrected visual acuity (BCVA), intraocular pressure, slit-lamp biomicroscopy, color fundus photography, infrared reflectance, fundus autofluorescence, fluorescein angiography (FA), indocyanine green angiography (ICGA), and spectral-domain OCT, with or without OCT angiography. Central serous chorioretinopathy was diagnosed as subretinal fluid and choroidal thickening in the macula on OCT with leakage on FA regardless of choroidal hyperpermeability on ICGA. Two retina specialists (M.M. and Y.H.) independently diagnosed the patients. The discrepancies were resolved by a face-to-face discussion. We excluded patients with MNV or characteristics suggestive of AMD, such as confluent soft drusen, pseudodrusen, or choroidal thinning. Moreover, we excluded those without follow-up visits and any visits after 2013.

Replication Dataset

For the replication stage, we used the Kobe CSC dataset.¹³ It consists of 137 idiopathic patients with CSC recruited at the Kobe University Hospital. All patients underwent detailed ophthalmic examinations, including slit-lamp biomicroscopy, color fundus photography, FA, ICGA, and spectral-domain OCT.

Central serous chorioretinopathy was defined as eyes with central serous retinal detachment without subretinal hemorrhage or suspected MNV in ICGA or OCT. The exclusion criteria were as follows: (1) a history of corticosteroid treatment; (2) central choroidal thickness < 250 μ m; (3) age > 80 years; and (4) a history of retinal vessel occlusion or uveitis.

Genotyping

The Kyoto CSC Cohort was genotyped using a series of BeadChip DNA arrays (Illumina), namely, OmniExpress (N = 250) and Asian Screening Array (N = 360). We performed genotype imputation using the Michigan imputation server (<https://imputationserver.sph.umich.edu/index.html#!pages/home>) and used the East Asian population of the 1000 Genomes dataset (phase III, version 5 release) as a reference for each dataset. In each dataset, single nucleotide polymorphisms (SNPs) with a call rate < 90% or a minor allele frequency (MAF) < 1% were excluded before the genotype imputation. After the imputation, we also excluded the following imputed SNPs from the subsequent association analysis: those with $R^2 < 0.9$, a call rate < 90%, MAF < 1%, or significant deviation ($P < 1.0 \times 10^{-5}$) from the Hardy–Weinberg equilibrium. The SNPs included in both datasets after imputation and quality control were used for the analysis.

The Kobe CSC dataset was genotyped using the Human OmniExpress BeadChips (Illumina).¹³ We performed genomic imputation using the BEAGLE 4.1 with the 1000 Genomes dataset (phase III, version 5 release) as reference panels. Imputed SNPs with $R^2 < 0.7$ were excluded. In the current study, the coordinates and variant identifiers were based on GRCh37/hg19.

Genome-wide Survival Analysis

We reviewed the medical records to obtain phenotypic information of all participants. The duration from CSC diagnosis to the event (MNV development) or censoring (final visit in August 2019 or lost to follow-up) was recorded daily. The age at the first visit was used for statistical analysis.

We conducted a 2-staged genome-wide survival analysis. In the discovery stage, using the Kyoto CSC Cohort, we conducted a survival analysis on the MNV-free period for each SNP with an MAF $\geq 10\%$ using the Cox proportional hazard regression model to adjust for the age at first visit, sex, and the first principal component. We carried SNPs with a suggestive association ($P < 1.0 \times 10^{-5}$) forward to the replication stage. In the replication stage, using the Kobe CSC dataset, we performed a similar analysis adjusted for the age at first visit and sex. Eventually, both Cox regression analysis results were meta-analyzed using the METAL software¹⁴ (<http://www.sph.umich.edu/csg/abecasis/Metal/>), with the inverse variance-based method and the fixed effect model.

Association of AMD Susceptibility Genes

We investigated the contribution of AMD susceptibility genes to MNV development in CSC. To evaluate the 34 AMD susceptibility SNPs reported by the International AMD Genome Consortium,¹⁵ we extracted the results of all SNPs with an MAF $\geq 1\%$ correlated with the aforementioned susceptibility SNPs with linkage disequilibrium (LD) $r^2 \geq 0.5$, as previously reported.¹⁶

Functional Enrichment Analysis

To investigate the functional background of MNV development in CSC, we conducted a functional enrichment analysis. First, we performed a gene mapping of the SNPs that demonstrated a suggestive association ($P < 0.05$) with an MAF $\geq 1\%$ in our genome-wide survival analysis by using the SNP2GENE function of FUMA, version

Table 1. Summary of Patients' Baseline Characteristics Based on Development of MNV

Items	Kyoto				Kobe			
	All	MNV+	MNV-	P Value [‡]	All	MNV+	MNV-	P Value [‡]
n	402	45	357	-	137	11	126	-
Sex, male:female	307:95	34:11	273:84	1.00 [§]	114:23	9:2	105:21	1.00 [§]
Age at the first visit, yrs*	55.1 ± 12.5	58.4 ± 12.1	54.6 ± 12.5	0.054	48.3 ± 10.4	50.1 ± 15.2	48.2 ± 10.0	0.70
Follow-up period, mos [†]	34.4 (12.3–72.0)	81.9 (50.6–117.1)	32.2 (12.1–72.1)	<0.001 [¶]	15.7 (5.4–64.0)	102.5 (77.5–139.8)	13.2 (4.4–55.0)	<0.001 [¶]

MNV = macular neovascularization.

*Mean ± standard deviation.

[†]Median (first quantile to third quantile).

[‡]Difference between patients who developed MNV and patients who did not was tested.

[§]Chi-square test.

^{||}Unpaired *t* test.

[¶]Mann–Whitney *U* test.

1.3.6a (<https://fuma.ctglab.nl/>). FUMA is an often-used online software for genetic research^{17–19} and integrates 18 biological databases and tools for processing GWAS summary statistics.²⁰ Detailed settings are described in Table S1 (available at www.aaojournal.org). Subsequently, we performed a functional enrichment analysis using the mapped genes by the ToppFun (transcriptome, ontology, phenotype, proteome, and pharmacome annotations-based gene list functional enrichment analysis) function of ToppGene (<https://toppgene.cchmc.org/>),²¹ a well-known online software^{22,23} that detects the functional enrichment of a gene list based on functional annotations and protein interactions network. We focused on 3 categories of pathway as follows: GO, biological process; GO, cellular component; and GO, molecular function. To search for pathways specifically related to the mapped genes, we excluded those consisting ≥ 50 genes. To reduce false-positives, we excluded pathways associated with only 1 of the mapped genes.

Statistical Analysis

The R 4.1.0 software (The R Foundation for Statistical Computing) was used for the statistical analyses. For a

comparison among the patients' backgrounds, we used the unpaired *t* test, Mann–Whitney *U* test, and chi-square test for age at their first visit, follow-up period, and sex distribution, respectively.

The genome-wide survival analysis in the discovery stage was performed using the SurvivalGWAS_SV, version 1.3.2, software (<https://www.liverpool.ac.uk/population-health/research/groups/statistical-genetics/survival-gwas-sv/>, University of Liverpool Institute of Translational Medicine, Liverpool, United Kingdom).²⁴ Cox proportional hazard regression analysis in the replication stage and the Kaplan–Meier method with estimation of survival curves using the Wilcoxon test were performed using the survival package, version 3.2-11, for R (<https://CRAN.R-project.org/package=survival>). Pairwise measures of LD r^2 in European and Japanese (in Tokyo, Japan) were obtained via LDlink 5.1 (<https://ldlink.nci.nih.gov/?tab=home>) using the LDlinkR package, version 1.1.2, for R.^{25,26}

A *P* value < 0.05 was considered statistically significant. A *P* value < 5.0×10^{-8} was considered genome-wide significant. In the functional enrichment analysis, a Bonferroni-corrected *q*-value < 0.05 was considered statistically significant.

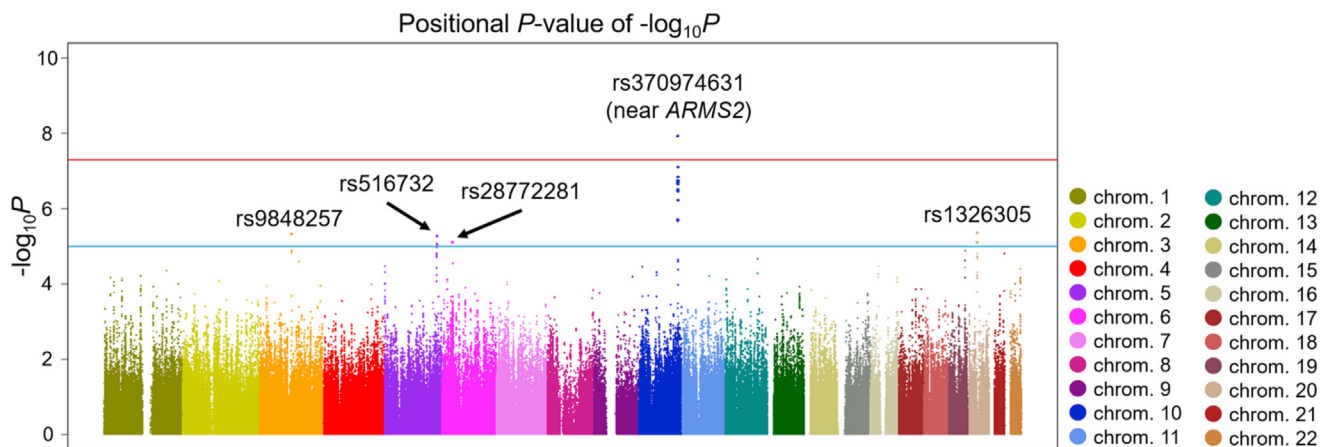


Figure 1. Manhattan plots of the discovery stage genome-wide survival analysis for macular neovascularization in central serous chorioretinopathy. The plots represent the $-\log_{10}$ transformed *P* values for all single nucleotide polymorphisms. The red and blue horizontal lines represent the genome-wide significant threshold ($P = 5.0 \times 10^{-8}$) and the suggestive threshold ($P = 1.0 \times 10^{-5}$), respectively.

Table 2. Summary of the Discovery Stage Genome-wide Survival Analysis, Replication Stage, and Meta-analysis

SNP	Chr	BP	EA/Non-EA	EAF	Nearby Genes	Discovery Stage (N = 402)		Replication Stage (N = 137)		Meta-analysis (N = 539)	
						HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
rs9848257	3	99404989	T/C	0.167	COL8A1*	2.93 (1.85–4.64)	4.70×10^{-6}	0.94 (0.32–2.75)	0.91	2.46 (1.61–3.75)	3.12×10^{-5}
rs516732	5	165107100	T/C	0.348	RN72KP60-LOC574080	3.05 (1.89–4.93)	5.44×10^{-6}	0.90 (0.37–2.19)	0.82	2.31 (1.51–3.52)	1.01×10^{-4}
rs28772281	6	32674086	A/G	0.268	MTCO3P1*	2.87 (1.81–4.56)	7.92×10^{-6}	0.96 (0.35–2.65)	0.94	2.38 (1.56–3.62)	5.51×10^{-5}
rs370974631	10	124213143	C/CAA	0.374	ARMS2†	4.07 (2.51–6.60)	1.19×10^{-8}	2.24 (0.83–6.05)	0.11	3.63 (2.35–5.61)	5.76×10^{-9}
rs1326305	20	24488340	G/A	0.239	SYNDIG1*	3.13 (1.92–5.09)	4.40×10^{-6}	1.11 (0.22–5.71)	0.90	2.87 (1.80–4.58)	9.11×10^{-6}

BP = base pair; Chr = chromosome; CI = confidence interval; EAF = effect allele frequency; HR = hazard ratio; non-EA = noneffect allele; SNP = single nucleotide polymorphism. Boldface indicates genome-wide significantly associated SNP.

*These SNPs were located in these genes.

rs370974631 was located near ARMS2.

BP = base pair; Chr = chromosome; CI = confidence interval; EA = effect allele; EAF = effect allele frequency; HR = hazard ratio; non-EA = noneffect allele; SNP = single nucleotide polymorphism. Boldface indicates genome-wide significantly associated SNP.

*These SNPs were located in these genes.

†Rs370974631 was located near ARMS2.

Results

Patient Background

A total of 402 and 137 patients with CSC met the inclusion criteria for the Kyoto CSC Cohort and Kobe CSC dataset, respectively, and 45 patients among the Kyoto CSC Cohort and 11 patients among the Kobe CSC dataset developed MNV during the follow-up period. Table 1 summarizes their baseline characteristics. Their ages at the first visit in the Kyoto CSC Cohort and Kobe CSC dataset were 55.1 ± 12.5 years and 48.3 ± 10.4 years, respectively. The median (interquartile range [IQR]) follow-up periods of the Kyoto CSC Cohort and Kobe CSC dataset were 34.4 (IQR, 59.7) months and 15.7 (IQR, 58.6) months, respectively. In both datasets, the follow-up period was significantly longer for patients who developed MNV than it was for those who did not (all $P < 0.001$).

Two-Stage Genome-wide Survival Analysis for MNV Development in CSC

We used a total of 2 281 924 SNPs from 402 CSC samples for the genome-wide survival analysis. Figure 1 depicts the Manhattan plot of the results. The inflation factor (λ_{GC}) was 1.035. We identified a genome-wide significant association for 1 locus (Table 2); rs370974631 near ARMS2 (hazard ratio [HR], 4.07; 95% confidence interval [CI], 2.51–6.60; $P = 1.19 \times 10^{-8}$) and suggestive association for 4 loci, namely, rs9848257 at COL8A1 (HR, 2.93; 95% CI, 1.85–4.64; $P = 4.70 \times 10^{-6}$), rs516732 at RN7SKP60-LOC574080 (HR, 3.05; 95% CI, 1.89–4.93; $P = 5.44 \times 10^{-6}$), rs28772281 at MTCO3P1 (HR, 2.87; 95% CI, 1.81–4.56; $P = 7.92 \times 10^{-6}$), and rs1326305 at SYNDIG1 (HR, 3.13; 95% CI, 1.92–5.09; $P = 4.40 \times 10^{-6}$). Table S2 (available at www.aaojournal.org) summarizes all results of SNPs with $P < 1.0 \times 10^{-5}$.

Table 2 outlines the results of the replication stage and meta-analysis. In the replication stage, 2 SNPs displayed the same direction of effect while being statistically insignificant (rs370974631 near ARMS2, HR, 2.24; 95% CI, 0.83–6.05; $P = 0.11$; rs1326305 at SYNDIG1, HR, 1.11; 95% CI, 0.22–5.71; $P = 0.90$). However, the meta-analysis of the discovery and replication sets revealed that rs370974631 near ARMS2 was genome-wide significantly associated with MNV development (HR, 3.63; 95% CI, 2.35–5.61; $P = 5.76 \times 10^{-9}$). Figures 2 and 3 represent the regional association and forest plots for the rs370974631 near ARMS2, respectively. The Kaplan–Meier curves are provided in Figures S1 and S2 (available at www.aaojournal.org).

Of the 34 susceptibility loci for AMD,¹⁵ CFH, CLO4A3, ARMS2-HTRA1, and B3GALT1 were significantly associated with MNV development in CSC (Table 3). Table S3 (available at www.aaojournal.org) summarizes the comprehensive results.

Functional Enrichment Analysis

We mapped a total of 243 inputted SNPs to 23 candidate genes in 13 genomic risk loci. Figure S3 (available at www.aaojournal.org) depicts the distributions of the mapped SNPs and candidate genes. Of the 23 candidate genes, 17 were included in the database of Human Genome Organization Gene Nomenclature Committee and were forwarded to ToppFun (Table S4, available at www.aaojournal.org). Eventually, 8 pathways were significantly associated with MNV development in CSC (Table 4). All pathways were associated with ion transport at the cellular level; 5 pathways (GO:0008282, GO:0015272, GO:0030955, GO:0031420, and GO:0005242) were associated with K^+ , whereas 3 (GO:0051561, GO:0036444, and GO:1990246) were associated with Ca^{2+} .

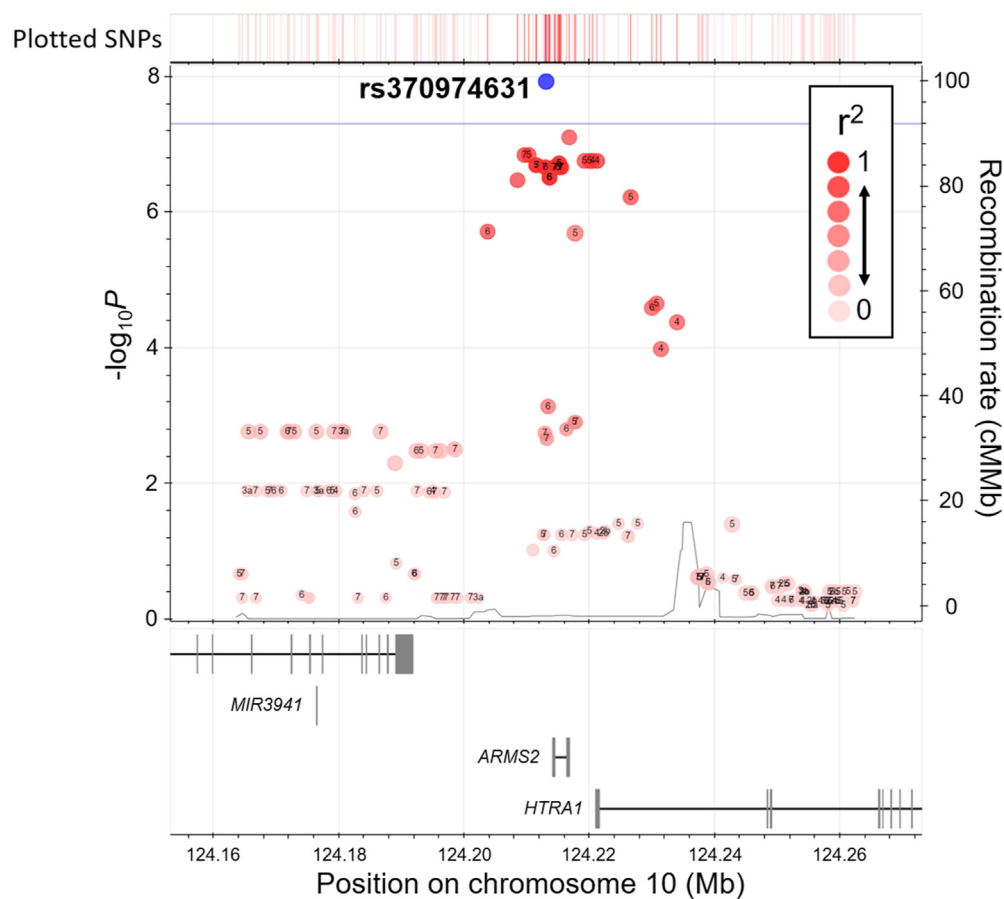


Figure 2. Regional association plots for genotyped single nucleotide polymorphisms (SNPs) around rs370974631 near ARMS2. The plots represent the $-\log_{10}$ transformed P values for SNPs located within 50 000 base pairs of rs370974631. The blue horizontal line represents the genome-wide significance threshold ($P = 5.0 \times 10^{-8}$).

Case Presentation

Figure 4 shows a representative case of CSC that subsequently developed MNV. A 47-year-old man visited the Macular Service at the Kyoto University Hospital with a chief symptom of

micropsia and blurred vision in his right eye. At the initial visit, the right eye had a BCVA of 20/22. OCT, FA, and ICGA revealed the typical presentations of CSC but no sign of MNV. At 4.5 years after the initial visit, the serous retinal detachment was resolved, and BCVA was increased to 20/16. However, OCT revealed retinal

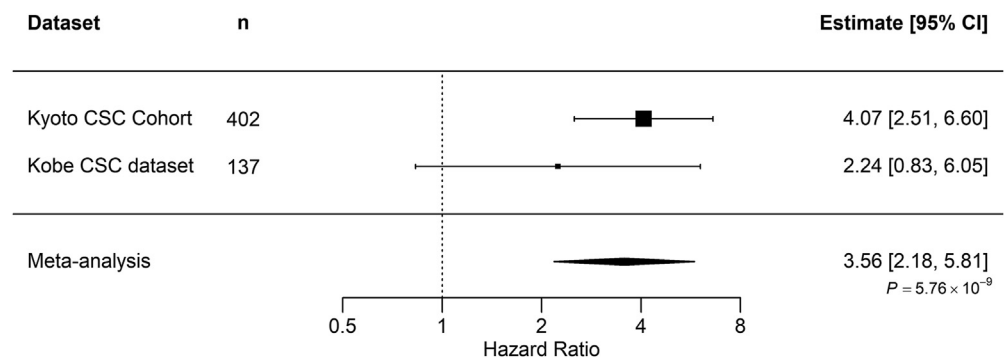


Figure 3. A forest plot depicting the effect of rs370974631 on macular neovascularization (MNV) development in central serous chorioretinopathy (CSC). In the discovery stage genome-wide survival analysis, the hazard ratio (HR) for MNV developing is highest for rs370974631 (risk allele: C) near ARMS2 (HR, 4.07; 95% confidence interval [CI], 2.51–6.60; $P = 1.19 \times 10^{-8}$). A similar trend is observed in the replication dataset from the Kobe University (HR, 2.24; 95% CI, 0.83–6.05; $P = 0.11$), and the meta-analysis demonstrates genome-wide significant association (HR, 3.56; 95% CI, 2.18–5.81; $P = 5.76 \times 10^{-9}$).

Table 3. Contribution of AMD Susceptibility Loci on MNV Development in CSC

AMD Susceptibility Locus	Chr	Genome-wide Survival Analysis for MNV Development in CSC					
		SNP	BP	r ²	EA/Non-EA	HR (95% CI)	P Value
CFH	1	rs800292*	196642233	0.74	A/G	0.39 (0.24–0.65)	2.55 × 10 ⁻⁴
COL4A3	2	rs4276018†	228092758	0.84	G/T	0.26 (0.11–0.60)	1.56 × 10 ⁻³
ARMS2-HTRA1	10	rs370974631	124213143	0.79	C/CAA	4.07 (2.51–6.60)	1.19 × 10 ⁻⁸
B3GALTL	13	rs9564692‡	31821240	1.00	T/C	0.56 (0.36–0.86)	8.30 × 10 ⁻³

Only loci that displayed significant associations were extracted. Comprehensive results are summarized in Table S3.

AMD = age-related macular degeneration; BP = base pair; Chr = chromosome; CI = confidence interval; CSC = central serous chorioretinopathy; EA = effect allele; HR = hazard ratio; MNV = macular neovascularization; non-EA = noneffect allele; r² = correlation between the lead SNPs from this study and from European AMD study; SNP = single nucleotide polymorphism.

*Rs514591, rs551397, and rs559350 displayed similar results.

†Rs4263106 displayed similar result.

‡Rs2137075 displayed similar result.

pigment epithelium elevation with a double-layer sign, and OCT angiography revealed MNV in the corresponding area.

Discussion

In the current study, we successfully identified *ARMS2* as a strong susceptibility gene for MNV development in CSC via a genome-wide survival analysis. In addition, an analysis of AMD susceptibility loci revealed 3 additional susceptibility genes, namely, *CFH*, *COL4A3*, and *B3GALTL*. *COL4A3* and *B3GALTL* are known as AMD susceptibility genes that are more strongly associated with the polypoidal choroidal vasculopathy (PCV) subtype than typical AMD is;¹⁶ thus, our results would facilitate the clarification of the molecular biological relationship between CSC and PCV.

ARMS2 is the most significant susceptibility gene for AMD.¹⁵ However, its molecular biological mechanism for developing AMD is unclear. A recent molecular biological study suggested that an insertion/deletion at *ARMS2-HTRA1* increases the systemic blood circulation of secreted serine protease HTRA1, which eventually leads

to Bruch's membrane degradation and MNV in AMD.²⁷ Regarding AMD, *ARMS2* is associated with not only MNV development but also geographic atrophy and soft drusen.¹² In addition, the gene is associated with the development of pachydrusen, a recently depicted drusen subtype that is correlated with pachychoroid spectrum diseases.^{28,29} Contrarily, the gene is not associated with myopic choroidal neovascularization, which is generally not accompanied by drusen.³⁰ Taken together, *ARMS2* would contribute to the molecular biological pathway of MNV pathogenesis via drusen formation regardless of background characteristics, such as choroidal thickness. Poor understanding of the association between the pachydrusen and the development of PNV warrants further evaluation.

Macular neovascularization with pachychoroid phenotype, including thickened choroid^{31,32} and choroidal vascular hyperpermeability,³³ was termed PNV in 2013.^{12,31} Because CSC is the most representative disease of pachychoroid spectrum, MNV secondary to CSC is also diagnosed as PNV. Despite limited information on its genetic background, several studies, including ours,

Table 4. Results of the Functional Enrichment Analysis

Pathway		No. of SNPs Constituting the Pathway	Mapped Genes in the Pathway	Bonferroni-Corrected <i>q</i> -Value
Category of Pathway				
ID	Name			
GO: Biological Process				
GO:0051561	Positive regulation of mitochondrial calcium ion concentration	11	MCU, MICU1	0.021
GO:0036444	Calcium import into the mitochondrion	14	MCU, MICU1	0.034
GO: Cellular Component				
GO:0008282	Inward rectifying potassium channel	4	ABCC8, KCNJ11	<0.001
GO:1990246	Uniplex complex	6	MCU, MICU1	0.001
GO: Molecular Function				
GO:0015272	ATP-activated inward rectifier potassium channel activity	6	ABCC8, KCNJ11	<0.001
GO:0030955	Potassium ion binding	12	KCNJ11, HDAC4	0.004
GO:0031420	Alkali metal ion binding	19	KCNJ11, HDAC4	0.010
GO:0005242	Inward rectifier potassium channel activity	24	ABCC8, KCNJ11	0.017

ATP = adenosine triphosphate; SNP = single nucleotide polymorphism.

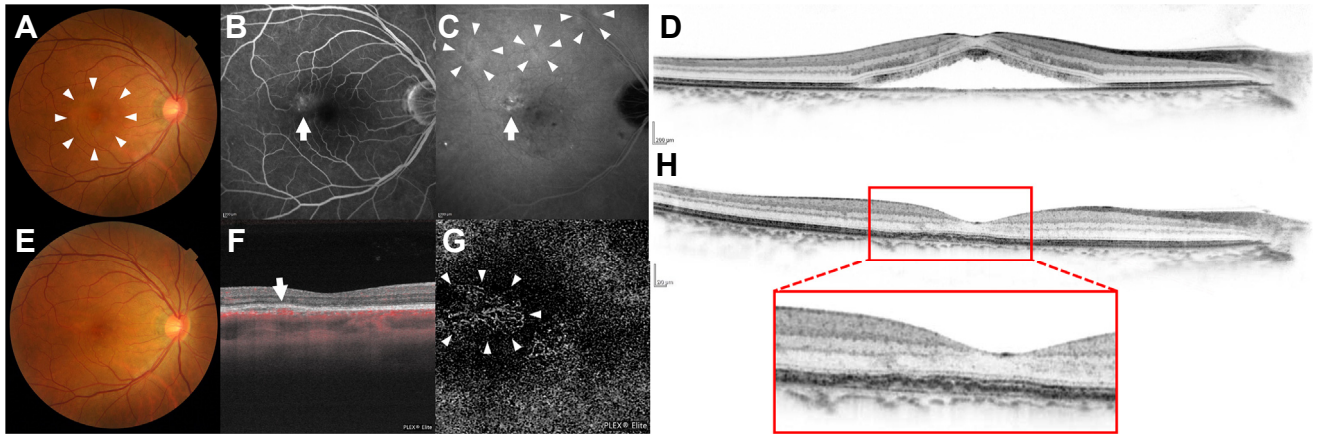


Figure 4. A case of central serous chorioretinopathy (CSC) that subsequently developed macular neovascularization (MNV). **A–D**, Images obtained at the patient's first visit. **A**, The color fundus photograph reveals serous retinal detachment without drusen. **B**, **C**, Fluorescein angiography and indocyanine green angiography (ICGA) reveal late leakage (diffuse leakage pattern; arrows). The ICGA also reveals choroidal vascular hyperpermeability (arrowheads). **D**, The OCT (horizontal scan thorough the center of the fovea) reveals serous retinal detachment. All these findings are compatible with a diagnosis of CSC. **E–H**, Images obtained at 4.5 years after the patient's first visit. **E**, The color fundus photograph reveals no obvious findings. **F**, **G**, OCT angiography reveals decorrelation signals beneath the retinal pigment epithelium elevation in the B-scan image, suggesting the presence of blood flow (arrow). The MNV is clearly depicted in the *en face* image (arrowheads). **H**, The OCT scan reveals no serous retinal detachment; however, it reveals a retinal pigment epithelium elevation with a double-layer sign, raising suspicions for MNV development (in red box, enlarged). These findings led to the diagnosis of MNV secondary to CSC.

reported that the T-allele frequency of *ARMS2* rs10490924 was significantly higher in PNV cases than in controls, whereas not as high as in AMD cases.³⁴ These results were successfully explained by the current longitudinal cohort study, which demonstrated considerably high HR of the *ARMS2* rs10490924 T allele for the MNV development in patients with CSC (3.45; 95% CI, 2.16–5.50). Development of MNV is an important prognostic factor for CSC; thus, our results could significantly contribute to the precise medicine for CSC.

Polypoidal choroidal vasculopathy is categorized as a subtype of AMD; however, its genetic background is partially different from that of AMD.¹² In addition, PCV eyes reportedly manifest thicker choroid and higher prevalence of CVH than AMD eyes.³⁵ This necessitates exploring the association between PCV and pachychoroid spectrum. The Genetics for AMD in Asians consortium evaluated the contribution of known AMD susceptible SNPs to each AMD subtype, namely, typical AMD and PCV. An analysis of 1062 patients with PCV, 1157 with typical AMD, and 5275 controls identified that *COL4A3*, *TNFRSF10A*, and *B3GALT* were more strongly associated with PCV than those with typical AMD.¹⁶ Of note, the current study found that *COL4A3* and *B3GALT* were significantly associated with the MNV development in CSC eyes (Table 3). In addition, we previously reported on *TNFRSF10A* as a significant susceptibility gene for CSC through relatively large-scaled GWAS.¹⁰ These results revealed the shared genetic susceptibility between PCV and pachychoroid spectrum. Detailed genotype–phenotype correlation studies should be conducted in the future.

For complex traits, gene and gene-set analysis provide a valuable biological addition to single-marker analysis. By applying the SNP2GENE function of FUMA, we could successfully map candidate SNPs to 17 genes that were passed to

ToppFun for detecting functional enrichment. The combination of these state-of-the-art bioinformatics programs enabled us to identify 8 possible biological pathways (Table 4). *ABCC8* and *KCNJ11* constitute inward rectifying potassium channel and are associated with the adenosine triphosphate–activated inward rectifier potassium channel (GO:0015272, Bonferroni-corrected *q*-value < 0.001). In addition, *KCNJ11* together with *HDAC4* is also associated with potassium ion binding and alkali metal ion binding (GO:0030955 and GO:0031420). The adenosine triphosphate–sensitive K^+ channel closure in response to their functional deterioration caused by *ABCC8* or *KCNJ11* mutation causes membrane depolarization and the constitutive activation of voltage-dependent Ca^{2+} channels.^{36–38} *MCU* and *MICU1* are recently identified core proteins that form mitochondrial Ca^{2+} uniporter complex (uniplex complex).³⁹ These genes import calcium into the mitochondria for the positive regulation of mitochondrial calcium ion concentration (Table 4, GO:0051561 and GO:0036444). Considering the involvement of the aforementioned pathways, Ca^{2+} might play an important role in MNV development. Increasing Ca^{2+} in photoreceptor mitochondria showed alter cone photoreceptor physiology and metabolic process,⁴⁰ which might influence pachydrusen formation, thereby leading to MNV development.

Study Limitations

The strength of the current study is in the combination of a nonhypothesis-driven genome-wide approach and detailed clinical phenotype; however, it has several limitations. First, the *P* value of the current replication result did not reach the widely accepted cutoff value of 0.05. This might have been because of the relatively small sample size and short follow-up period of the Kobe CSC dataset. However, as described

in the American Statistical Association statement on P values published in 2016,⁴¹ the P value should not be interpreted in a dichotomized fashion. Considering we confirmed the similar trend as the discovery stage in the replication dataset (odds ratio, 2.24, $P = 0.11$) and the meta-analysis enhanced the association between *ARMS2* and MNV development, we could successfully replicate the association identified in the discovery dataset. Second, although the sample size was large for a clinical study, it was insufficient for genome-wide association analysis. A larger sample size would have facilitated the identification of more disease susceptibility genes. However, 1 SNP still exhibited a genome-wide significant association, thus highlighting the clinical significance of this study. Third, this study was retrospective. A confirmation of the reproducibility of our results via a prospective study would enable us to better understand the pathophysiology of pachychoroid disease.

Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at the Kyoto University Graduate School of Medicine and Kobe University Graduate School of Medicine approved the study. All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent.

Conclusions

We reported on the results of a genome-wide survival analysis on MNV development in CSC. This approach not only helped us identify a significant susceptibility SNP with large effect size but also revealed a shared genetic susceptibility between MNV secondary to CSC and PCV. *ARMS2* is a well-known AMD susceptibility gene and was associated with MNV development in CSC. We revealed the overlap of the genetic background between PCV and MNV secondary to CSC, thus supporting the evidence that a certain fraction of PCV had a history of CSC. Further genetic studies will reveal the pathogenesis of pachychoroid spectrum diseases.

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Conception and design: Miyake

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Analysis and interpretation: Mori, Miyake, Hosoda, Miki, Sato, Yamada, Yamashiro, Nakamura, Tajima, Nagasaki, Honda, Tsujikawa

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Abbreviations and Acronyms:

AMD = age-related macular degeneration; **BCVA** = best-corrected visual acuity; **CI** = confidence interval; **CSC** = central serous chorioretinopathy; **FA** = fluorescein angiography; **GWAS** = genome-wide association study; **HR** = hazard ratio; **ICGA** = indocyanine green angiography; **IQR** = interquartile range; **LD** = linkage disequilibrium; **MAF** = minor allele frequency; **MNV** = macular neovascularization; **PCV** = polypoidal choroidal vasculopathy; **PNV** = pachychoroid neovascularization; **SNP** = single nucleotide polymorphism.

Keywords:

Central serous chorioretinopathy, Choroidal neovascularization, Genome-wide association study, Macular neovascularization, Survival analysis.

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Table S1. Detailed settings for positional gene mapping by FUMA

Parameter	Setting	Description
Reference panel	1000G Phase3 EAS	Data from East Asian of 1000 Genome Phase 3 were used as the reference panel for LD r^2 .
Minimum MAF ($\geq$)	0*	No filtering was performed for the results by MAF obtained from the reference panel.
Maximum lead SNP P-value ($<$)	0.00001	SNPs with $P \leq 1 \times 10^{-5}$ were identified as “significant SNPs.”
r^2 threshold for independent significant SNPs ($\geq$)	0.6*	Among the significant SNPs dependent on each other at $r^2 \geq 0.6$, one with the lowest P value was defined as an “independent significant SNP.”
2nd r^2 threshold for lead SNPs ($\geq$)	0.1*	Independent significant SNPs dependent on each other at $r^2 \geq 0.1$ were assigned to similar genomic risk locus, and the one with the lowest P value was defined as a “lead SNP.”
Maximum GWAS P-value ($<$)	0.05*	SNPs with $P < 0.05$ and in LD of $r^2 \geq 0.6$ (set in “ r^2 threshold for independent significant SNPs ($\geq$)”) with each lead SNP were used for gene mapping and involved similar genomic risk locus.
Include variants from reference panel	Yes*	All SNPs in the reference panel in LD of $r^2 \geq 0.6$ (set in “ r^2 threshold for independent significant SNPs ($\geq$)”) with the independent significant SNPs, despite not being included in our results, were involved similar genomic locus. This default setting may provide information on functional variants in LD with the lead SNP.
Maximum distance of LD blocks to merge ($\leq$)	250 kb*	Genomic risk loci with a distance $\leq 250\,000$ base pairs were merged into a single genomic risk locus.
Distance to genes or functional consequences of SNPs on genes to map	Maximum distance 10 kb*	SNPs located within 10 000 base pairs of a gene were mapped to that gene.

EAS = East Asian; GWAS = genome-wide survival analysis; LD = linkage disequilibrium; MAF = minor allele frequency; SNP = single nucleotide polymorphism.

*: Default settings.

Table S2. Results of single nucleotide polymorphisms with $P < 1.0 \times 10^{-5}$ in the genome-wide survival analysis

SNP	Position	EA/NonEA	HR (95% CI)	P value	Genomic locus
rs9848257	3:99404989	T/C	2.93 (1.85–4.64)	4.70×10^{-6}	<i>COL8A1</i>
rs516732	5:165107100	T/C	3.05 (1.89–4.93)	5.44×10^{-6}	<i>RN7SKP60-LOC574080</i>
rs4868759	5:165108256	T/C	2.85 (1.80–4.52)	8.85×10^{-6}	
rs28772281	6:32674086	A/G	2.87 (1.81–4.56)	7.92×10^{-6}	<i>MTCO3P1</i>
rs61871744	10:124203787	C/T	3.11 (1.95–4.96)	1.95×10^{-6}	<i>PLEKHA1-ARMS2</i>
rs59616332	10:124208562	G/GATAAAC	3.34 (2.10–5.30)	3.39×10^{-7}	
rs11200630	10:124209684	C/T	3.52 (2.20–5.62)	1.44×10^{-7}	
rs61871745	10:124210369	A/G	3.52 (2.20–5.62)	1.44×10^{-7}	
rs11200632	10:124211536	G/A	3.46 (2.16–5.52)	2.04×10^{-7}	
rs11200633	10:124211596	T/C	3.46 (2.16–5.52)	2.04×10^{-7}	
rs61871746	10:124212913	C/T	3.45 (2.16–5.50)	2.18×10^{-7}	
rs61871747	10:124213046	T/C	3.45 (2.16–5.50)	2.18×10^{-7}	
rs370974631	10:124213143	C/CAA	4.07 (2.51–6.60)	1.19×10^{-8}	
rs200227426	10:124213671	A/C	3.36 (2.11–5.35)	3.10×10^{-7}	
rs201396317	10:124213674	A/C	3.36 (2.11–5.35)	3.10×10^{-7}	
rs199637836	10:124213677	A/C	3.36 (2.11–5.35)	3.10×10^{-7}	
rs11200634	10:124213680	A/C	3.36 (2.11–5.35)	3.10×10^{-7}	
rs75431719	10:124213688	A/C	3.36 (2.11–5.35)	3.10×10^{-7}	
rs10490924	10:124214448	T/G	3.45 (2.16–5.50)	2.18×10^{-7}	
rs61544945	10:124214600	GGT/G	3.45 (2.16–5.50)	2.18×10^{-7}	
rs36212731	10:124214976	T/G	3.45 (2.16–5.50)	2.18×10^{-7}	
rs36212732	10:124215198	G/A	3.45 (2.16–5.49)	1.91×10^{-7}	<i>ARMS2</i>
rs36212733	10:124215211	C/T	3.45 (2.16–5.49)	1.91×10^{-7}	
rs3750848	10:124215315	G/T	3.45 (2.16–5.50)	2.18×10^{-7}	
rs3750847	10:124215421	T/C	3.45 (2.16–5.50)	2.18×10^{-7}	
rs3750846	10:124215565	C/T	3.45 (2.16–5.50)	2.18×10^{-7}	
rs566108895	10:124216823	T/G	3.60 (2.26–5.75)	7.89×10^{-8}	
rs5788557	10:124217757	GC/G	2.96 (1.89–4.63)	2.05×10^{-6}	<i>ARMS2-HTRA1</i>
rs3793917	10:124219275	G/C	3.41 (2.15–5.40)	1.76×10^{-7}	
rs3763764	10:124220061	G/A	3.41 (2.15–5.40)	1.76×10^{-7}	
rs11200638	10:124220544	A/G	3.41 (2.15–5.40)	1.76×10^{-7}	
rs1049331	10:124221270	T/C	3.41 (2.15–5.40)	1.76×10^{-7}	<i>HTRA1</i>
rs2293870	10:124221276	T/G	3.41 (2.15–5.40)	1.80×10^{-7}	
rs2284665	10:124226630	T/G	3.20 (2.03–5.05)	6.05×10^{-7}	
rs6083539	20:24488084	G/A	3.03 (1.86–4.92)	7.86×10^{-6}	<i>SYNDIG1</i>
rs6049745	20:24488170	C/T	3.03 (1.86–4.92)	7.86×10^{-6}	
rs1326305	20:24488340	G/A	3.13 (1.92–5.09)	4.40×10^{-6}	

CI = confidence interval; EA = effect allele; HR = hazard ratio; NonEA = non-effect allele; SNP = single nucleotide polymorphism.

Table S3. Contribution of all 34 age-related macular degeneration susceptibility loci on macular neovascularization development in central serous chorioretinopathy

AMD susceptibility Locus	Chr	Genome-wide survival analysis for MNV development in CSC					
		SNP	BP	r ²	EA/NonEA	HR (95% CI)	P value
<i>CFH</i>	1	rs514591	196640320	0.74	G/A	0.39 (0.24–0.65)	2.55×10 ⁻⁴
		rs551397	196642072	0.74	T/C	0.39 (0.24–0.65)	2.55×10 ⁻⁴
		rs800292	196642233	0.74	A/G	0.39 (0.24–0.65)	2.55×10 ⁻⁴
		rs559350	196642533	0.74	C/T	0.39 (0.24–0.65)	2.55×10 ⁻⁴
<i>COL4A3</i>	2	rs4276018	228092758	0.84	G/T	0.26 (0.11–0.60)	1.56×10 ⁻³
		rs4263106	228092899	0.84	T/G	0.26 (0.11–0.60)	1.56×10 ⁻³
<i>ADAMTS9-AS2</i>	3	rs7428936	64710850	1.00	C/T	0.96 (0.55–1.68)	0.900
		rs62247658	64715155	1.00	C/T	0.96 (0.55–1.68)	0.900
<i>COL8A1*</i>	3	-	-	-	-	-	-
<i>CFI</i>	4	rs10033900	110659067	1.00	T/C	0.89 (0.59–1.35)	0.586
<i>PRLR-SPEF2*</i>	5	-	-	-	-	-	-
<i>C9*</i>	5	-	-	-	-	-	-
<i>C2-CFB-SKIV2L</i>	6	rs2284179	31941891	0.58	G/T	1.29 (0.61–2.72)	0.503
<i>VEGFA</i>	6	rs7758685	43825266	0.88	A/G	0.89 (0.54–1.48)	0.663
<i>PILRB-PILRA</i>	7	rs314302	100376549	0.53	A/G	0.64 (0.22–1.86)	0.418
		rs314333	100386001	0.53	A/C	0.64 (0.22–1.86)	0.418
		rs191137	100386900	0.53	T/C	0.64 (0.22–1.86)	0.418
<i>KMT2E-SRPK2</i>	7	rs144245492	104609793	0.63	C/G	0.70 (0.42–1.17)	0.177
<i>TNFRSF10A</i>	8	rs13278062	23082971	1.00	G/T	0.84 (0.54–1.33)	0.462
<i>TRPM3[†]</i>	9	-	-	-	-	-	-
<i>MIR6130-RORB</i>	9	rs10781168	76555517	1.00	T/C	0.92 (0.60–1.40)	0.692
		rs4014108	76564465	1.00	C/T	0.92 (0.60–1.40)	0.692
		rs10781171	76565484	0.98	C/A	0.92 (0.60–1.40)	0.692
<i>TGFBR1</i>	9	rs1888225	101909657	0.76	G/A	0.93 (0.61–1.41)	0.730
		rs3739798	101914812	0.76	A/G	0.93 (0.61–1.41)	0.730
<i>ABCA1</i>	9	rs111919605	107636559	0.57	A/G	1.19 (0.75–1.89)	0.465
<i>ARHGAP21</i>	10	rs71493396	25018251	0.77	T/C	2.11 (0.93–4.76)	7.34×10 ⁻²
<i>ARMS2-HTRA1</i>	10	rs370974631	124213143	0.79	C/CAA	4.07 (2.51–6.60)	1.19×10 ⁻⁸
<i>RDH5-CD63</i>	12	rs3138142	56115585	1.00	T/C	1.19 (0.58–2.46)	0.638
		rs3138141	56115778	1.00	A/C	1.19 (0.58–2.46)	0.638
<i>ACAD10*</i>	12	-	-	-	-	-	-

AMD susceptibility Loci	Chr	Genome-wide survival analysis for MNV development in CSC					
		SNP	BP	r ²	EA/NonEA	HR (95% CI)	P value
<i>B3GALT1</i>	13	rs9564692	31821240	1.00	T/C	0.56 (0.36–0.86)	8.30×10 ⁻³
		rs2137075	31821505	1.00	G/A	0.56 (0.36–0.86)	8.30×10 ⁻³
<i>RAD51B</i>	14	rs149571521	68796698	0.76	CAA/C	0.81 (0.55–1.20)	0.295
<i>LIPC</i>	15	rs1532085	58683366	0.86	G/A	1.22 (0.79–1.87)	0.366
<i>CETP</i>	16	rs9929488	56998572	0.65	C/G	1.58 (0.89–2.81)	0.119
<i>CTRB2-CTRB1</i> [†]	16	-	-	-	-	-	-
<i>TMEM97-VTN</i>	17	rs7210719	26686218	0.67	T/C	1.38 (0.83–2.30)	0.216
		rs730331	26689432	0.67	C/G	1.38 (0.83–2.30)	0.216
		rs2277668	26691760	0.67	C/G	1.38 (0.83–2.30)	0.216
		rs2277667	26691916	0.67	C/T	1.38 (0.83–2.30)	0.216
<i>NPLOC4-TSPAN10</i>	17	rs58976732	79569376	0.53	G/C	0.72 (0.44–1.19)	0.201
<i>CNN2</i> [†]	19	-	-	-	-	-	-
<i>C3</i> [*]	19	-	-	-	-	-	-
<i>APOE</i>	19	rs483082	45416178	0.57	T/G	0.85 (0.47–1.51)	0.572
		rs438811	45416741	0.57	T/C	0.85 (0.47–1.51)	0.572
<i>MMP9</i> [‡]	20	-	-	-	-	-	-
<i>C20orf85</i>	20	rs6026016	56655159	0.66	T/A	0.00 (0.00–∞)	0.999
		rs1334108	56655297	0.66	C/T	0.00 (0.00–∞)	0.999
		rs1334109	56655360	0.66	G/T	0.00 (0.00–∞)	0.999
		rs6015187	56656419	0.66	T/G	0.00 (0.00–∞)	0.999
		rs6026020	56657049	0.66	C/T	0.00 (0.00–∞)	0.999
		rs6099916	56657729	0.66	T/C	0.00 (0.00–∞)	0.999
		rs6026022	56658128	0.66	A/G	0.00 (0.00–∞)	0.999
<i>SYN3-TIMP3</i>	22	rs5754227	33105817	1.00	C/T	0.76 (0.50–1.16)	0.211
<i>SLC16A8</i>	22	rs8135665	38476276	1.00	T/C	1.47 (0.79–2.73)	0.227
		rs561679070	38479774	1.00	C/CACCAGGAGCCGGGGGCTGGGGATAG	1.47 (0.79–2.73)	0.227

AMD = age-related macular degeneration; BP = base pair; Chr = chromosome; CI = confidence interval; CSC = central serous chorioretinopathy; EA = effect allele; HR = hazard ratio; MNV = macular neovascularization; NonEA = non-effect allele; r² = correlation between the lead SNPs from this study and from European AMD study; SNP = single nucleotide polymorphism.

*: Proxy SNPs were undefinable because the lead SNPs of these loci in the genome-wide association study (GWAS) for European AMD were monoallelic in the Japanese population in the 1000 Genome reference panel; †: Proxy SNPs were not available in our results; ‡: Proxy SNPs were undefinable because the lead SNP of this locus in GWAS for European AMD was not available in the 1000 Genome reference panel.

Table S4. Mapped genes used for the functional enrichment analysis

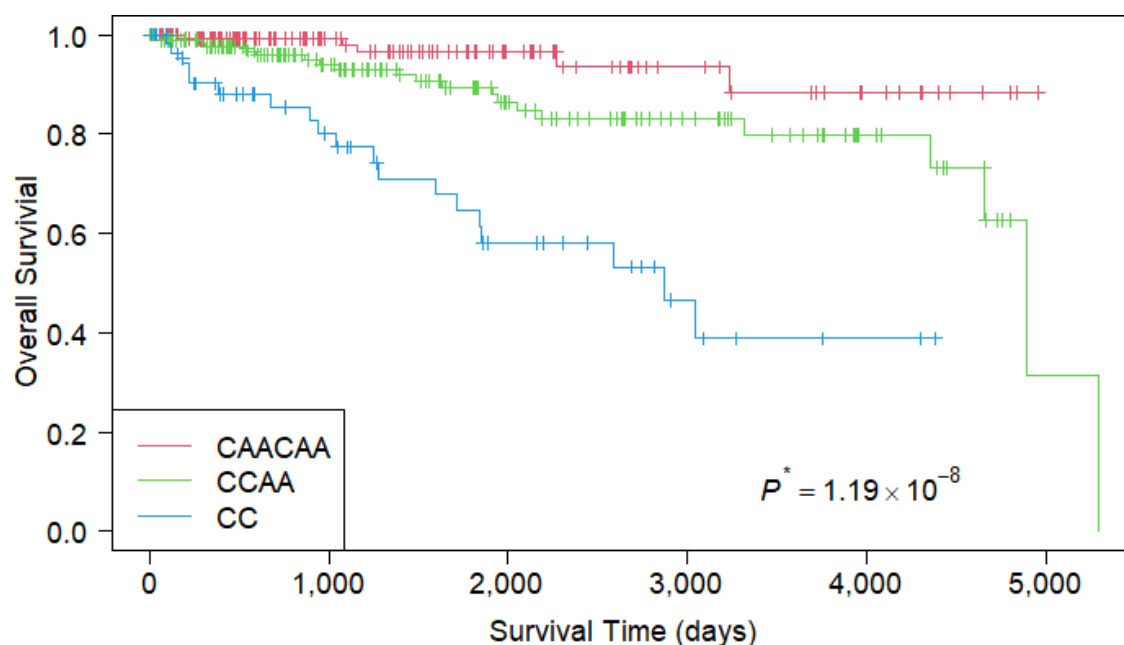
Gene	Chr	Start	End
<i>CAPZB</i>	1	19665267	19812066
<i>RN7SL277P</i>	1	19750878	19751163
<i>HDAC4</i>	2	239969864	240323348
<i>COL8A1</i>	3	99357319	99518070
<i>PGAM5P1</i>	5	109220311	109221124
<i>SLC25A48</i>	5	135170338	135224326
<i>LAMB4</i>	7	107663993	107770801
<i>MICU1</i>	10	74127098	74385899
<i>MCU</i>	10	74451889	74647452
<i>MIR4676</i>	10	74480787	74480858
<i>ARMS2</i>	10	124214169	124216868
<i>HTRA1</i>	10	124221041	124274424
<i>NUCB2</i>	11	17229700	17371521
<i>NCR3LG1</i>	11	17373273	17398888
<i>KCNJ11</i>	11	17407406	17410878
<i>ABCC8</i>	11	17414432	17498449
<i>SYNDIG1</i>	20	24449835	24647252

Chr = chromosome.

Figure S1. Kaplan-Meier curves by genotypes of rs370974631 near *ARMS2*. The red, green, and blue lines represent non-risk allele homozygotes (CAACAA), heterozygotes (CCAA), and risk allele homozygotes (CC), respectively. **(A)** Kaplan-Meier curves of the discovery dataset (Kyoto CSC Cohort). In contrast to patients with the CAACAA genotype (n = 160), 11.7% of whom developed macular neovascularization (MNV) in 10 years, 20.1% of those with the CCAA genotype (n = 184) and 61.2% of those with the CC genotype (n = 58) developed MNV in the same duration. **(B)** Kaplan-Meier curves of the replication dataset (Kobe CSC dataset). In contrast to patients with the CAACAA genotype (n = 51), 0.0% of whom developed MNV in 10 years, 38.1% of those with the CCAA genotype (n = 73) and 20.0% of those with the CC genotype (n = 13) developed MNV in the same duration.

*: Cox proportional hazard regression model.

A



B

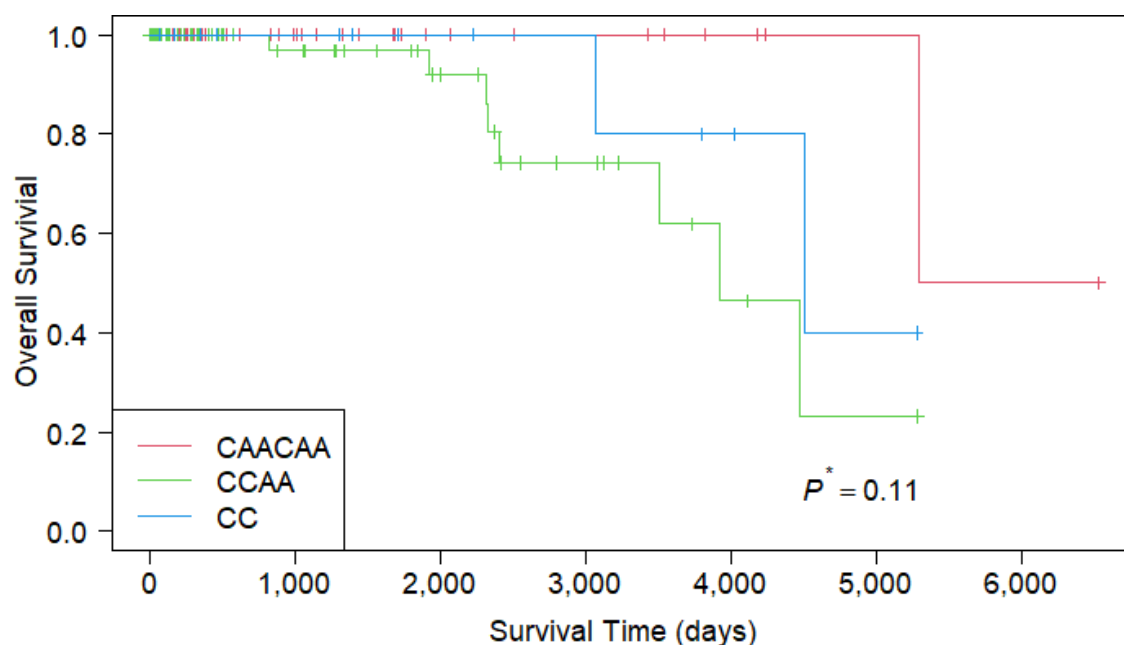


Figure S2. Kaplan-Meier curves, by genotype, of rs370974631 near *ARMS2* in dominant model in the replication stage. The *red* and *blue* lines represent non-risk allele homozygotes (CAACAA) and heterozygotes or risk allele homozygotes (CCAA/CC), respectively. In contrast to patients with the CAACAA genotype (n = 51), 0.0% of whom developed MNV in 10 years, 33.1% of those with the CCAA/CC genotype (n = 86) developed MNV in the same duration.

*: Wilcoxon test.

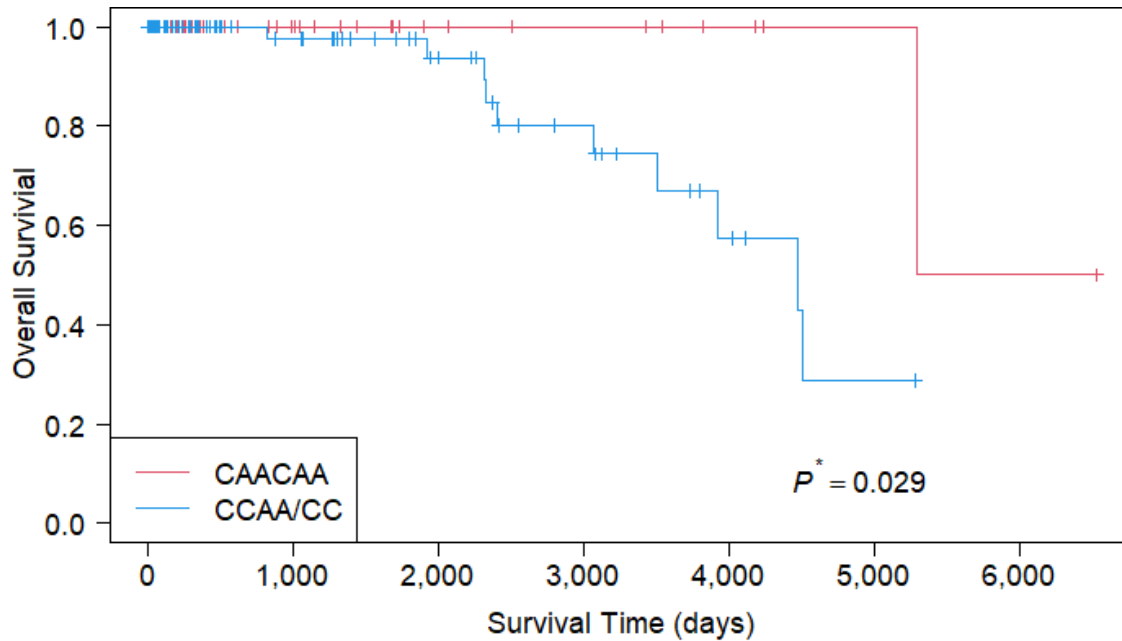


Figure S3. Summary of mapped genes by FUMA

Settings for gene mapping is described in [Table S1](#). A total of 243 single nucleotide polymorphisms (SNPs) (206 candidate SNPs, including 15 independent significant SNPs, of the genome-wide survival analysis and 37 SNPs in the reference panel in strong linkage disequilibrium with any of independent significant SNPs) were mapped into 23 genes in 13 genomic risk loci.

