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Genome-Wide Association Study of Age-Related Macular Degeneration Reveals 2 New Loci Implying Shared Genetic Components with Central Serous Chorioretinopathy

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Purpose: To investigate the genetic architecture of age-related macular degeneration (AMD) in a Japanese population.

Design: Genome-wide association study (GWAS).

Participants: Three thousand seven hundred seventy-two patients with AMD and 16 770 control participants from the Japanese population were enrolled in the association analyses.

Methods: We conducted a meta-analysis of 2 independent GWASs that included a total of 2663 patients with AMD and 9471 control participants using the imputation reference panel for genotype imputation specified for the Japanese population (n = 3541). A replication study was performed using an independent set of 1109 patients with AMD and 7299 control participants.

Main Outcome Measures: Associations of genetic variants with AMD.

Results: A meta-analysis of the 2 GWASs identified 6 loci significantly associated with AMD ($P < 5.0 \times 10^{-8}$). Of these loci, 4 were known to be associated with AMD (*CFH*, *C2/FB*, *TNFRSF10A*, and *ARMS2*), and 2 were novel (rs4147157 near *WBP1L* and rs76228488 near *GATA5*). The newly identified associations were confirmed in a replication study ($P < 0.01$). After the meta-analysis of all datasets, we observed strong associations in these loci ($P = 1.88 \times 10^{-12}$ and $P = 1.35 \times 10^{-9}$ for meta-analysis for rs4147157 and rs76228488, respectively). When we looked up the associations in the reported central serous chorioretinopathy (CSC) GWAS conducted in the Japanese population, both loci were associated significantly with CSC ($P = 4.86 \times 10^{-3}$ and $P = 4.28 \times 10^{-3}$ for rs4147157 and rs76228488, respectively). We performed a genetic colocalization analysis for these loci and estimated that the posterior probabilities of shared causal variants between AMD and CSC were 0.39 and 0.60 for *WBP1L* and *GATA5*, respectively. Genetic correlation analysis focusing on the epidemiologically suggested clinical risk factors implicated shared polygenic architecture between AMD and smoking cessation (r_g [the measure of genetic correlation] = −0.33; $P = 0.01$; false discovery rate, 0.099).

Conclusions: Our findings imply shared genetic components conferring the risk of both AMD and CSC.

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Age-related macular degeneration (AMD) is a progressive retinal degeneration in the elderly and is a leading cause of blindness worldwide.[1] Age-related macular degeneration susceptibility is influenced by a variety of clinical and genetic factors.[1] To date, > 40 genetic loci have been reported to be associated with AMD[2–4]; most were identified in genome-wide association studies (GWASs) conducted by the International Age-Related Macular Degeneration Genetics Consortium (IAMDGC).[2,4] Integration of the results of genetic association analyses with other biological resources revealed new aspects and implications for pathogenesis of AMD.[5–7] Despite the successful identification of AMD-associated genetic components, few GWASs have been performed in Asian populations.[3,8,9] Although the sample sizes were rather smaller, the previous GWASs of East Asian individuals (EAS) revealed novel genetic variants associated with AMD,[3,10] emphasizing the importance of such studies on non-European populations. Considering that the GWAS of IAMDGC studied principally European individuals (EUR), further investigations of Asian individuals might identify novel AMD-associated loci.

Comprehensive genetic analyses can elucidate shared biological mechanisms underlying multiple complex traits (including diseases) by identifying overlapping genetic components and by evaluating shared polygenic architectures.[11,12] For example, the overlapped genetic components have been determined between Crohn's disease and ulcerative colitis[13] and among psychiatric diseases.[14] Genome-wide association studies of AMD revealed that genetic components are shared between exudative and atrophic AMD[15] and between intermediate and advanced AMD.[4] Polypoidal choroidal vasculopathy (PCV) and typical neovascular AMD (tAMD) share a polygenic architecture.[16] These findings indicate that subtypes of AMD share their cause and support the previous clinical and epidemiologic findings.[1,17] A recent study suggested that the genetic components overlap between AMD and central serous chorioretinopathy (CSC),[18] which is characterized by serous retinal detachment around or involving the fovea.[19] Our recent GWAS of CSC conducted in a Japanese population revealed 2 associated loci (*TNFRSF10A* and *GATA5*).[18] Of these, the genetic association of *TNFRSF10A* with AMD originally was identified by the GWAS for AMD in the Japanese population.[8] Moreover, we recently reported that certain genetic loci associated with AMD also were associated with macular neovascularization in patients with CSC.[20] Although such previously reported associations in both AMD and CSC imply a shared genetic architecture, this has not been elucidated fully.

In this study, to identify associated loci and gain biological insights into the pathogenesis of AMD, we conduct large-scale GWASs in the Japanese population. After the meta-analysis of 2 independent GWASs, we sought to replicate the associations discovered using an independent dataset. We further evaluated the effects of identified loci between tAMD and PCV and the associations with CSC by looking up the summary statistics of the published GWAS results.[18] We also evaluate the shared polygenic architecture with epidemiologically suggested clinical risk factors to characterize the genetic architecture of AMD.

Methods

Participants

We used 3 independent datasets to assess genetic associations with neovascular AMD (Table S1, available at www.aaojournal.org). In brief, we reanalyzed the GWAS (first set) that we reported previously[8] and constructed a new case–control dataset for an additional GWAS (second set). After performing a meta-analysis of 2 GWAS datasets, the associated variants were evaluated in the independent dataset (third set). The first set originally included 827 patients with AMD and 3323 control participants.[8] To control strictly for bias caused by population stratification, we performed a principal component analysis of the genotypes and excluded individuals of the Ryukyu cluster.[21] Finally, the first set contained 821 patients with AMD and 3202 control participants who were included in the first set. The patients with AMD (n = 1938 before quality control) of the second set were recruited from 3 hospitals (Kyushu University Hospital, Kyoto University Hospital, and Yokohama City University Medical Center). The control genotype data were those of the 8121 individuals (before quality control) of the Biobank Japan (BBJ) project.[22,23] The third set comprised patients with AMD recruited from the 7 hospitals (Kansai Medical University Hirakata Hospital, Kobe University Hospital, Kyushu Hospital, Nagoya University Hospital, Nagoya City University Hospital, Surugadai Nihon University Hospital, and University of Tokyo Hospital) and the BBJ control participants. The diagnosis of the AMD and its subtypes was described in our previous report.[8] The control participants from the 3 datasets did not overlap. Although clinical information on glaucoma and cataract in some control participants was available because these diseases were targeted in the BBJ, not all ophthalmic examination information, including retinal condition, was available. We obtained written informed consent from all participants. This study was approved by the ethics committee of RIKEN and each collaborative hospital (Kyushu University Hospital, Kyoto University Hospital, Yokohama City University Medical Center, Kansai Medical University Hirakata Hospital, Kobe University Hospital, Kyushu Hospital, Nagoya University Hospital, Nagoya City University Hospital, Surugadai Nihon University Hospital, and University of Tokyo Hospital) and adhered to the tenets of the Declaration of Helsinki.

Genotyping and Imputation

The genotyping and quality control of the first set have been described.[8] As mentioned above, we further excluded 6 patients with AMD and 21 control participants to control conservatively for population stratification. For the second set, patients with AMD were genotyped using Illumina HumanOmniExpress BeadChips (n = 1362) or Illumina Infinium Omni2.5 BeadChips (n = 576), and the control participants were genotyped using Illumina HumanOmniExpress BeadChips. As the quality control for samples in the second set, we excluded individuals who met 1 or more of the following criteria: (1) call rate of < 98%, (2) heterozygosity rate of 0.32 or more, (3) closely related sample (PI_hat ≥ 0.125), and (4) principal component analysis outlier from the main Japanese cluster.[21] Details regarding the quality control of variants in the second set are shown in the Supplemental Note (available at www.aaojournal.org). Finally, 1842 patients with AMD and 6269 control participants were

included. For the second set, we excluded variants meeting the following criteria in either genotyping array: (1) call rate of $< 98\%$, (2) $P < 1.0 \times 10^{-5}$ for Hardy–Weinberg equilibrium, and (3) minor allele count of < 5. To reduce the bias caused by the differences between genotyping arrays, we also excluded variants with allele frequency differences of $> 10\%$ between arrays. PILNK, version 1.90b3.26,[24] was used to handle the genotype data. To evaluate the associations of candidate loci suggested by GWAS in the third dataset, we genotyped an additional 1112 patients with AMD and 7342 BBJ control participants using our previously described multiplex polymerase chain reaction–based sequencing method.[25] We excluded variants with a call rate of $< 90\%$ and then individuals with a call rate of $< 95\%$; a total of 1109 patients with AMD and 7299 control participants were used for the association analysis.

We phased the haplotypes using SHAPEIT, version 2.r837,[26] and imputed variants using Minimac3, version 2.0.1,[27] using the previously constructed imputation reference panel for genotype imputation specified for the Japanese population.[28] Briefly, the reference panel contained whole-genome sequence data from 1037 Japanese individuals and those of the 1000 Genomes Project[29] (1KGP Phase3, version 5; n = 2504).[28]

Genetic Association Analysis

We performed a GWAS using rvtest[30] for the imputed allele dosages in each dataset by logistic regression analysis using the top 10 principal components as covariates. The meta-analysis of GWAS was performed using the inverse variance method for variants for which the imputation quality scores of R^2 were 0.3 or more in both GWASs. After meta-GWAS, we selected lead variants of the associated loci ($P < 1.0 \times 10^{-6}$) that had not been reported as AMD susceptibility loci for the evaluation in the third set. We considered variants exhibiting $P < 5.0 \times 10^{-8}$ on meta-analysis of all datasets to be associated significantly with AMD.

We performed a subtype analysis for tAMD and PCV by subdividing the patients with AMD of the GWASs (first and second sets) into patients with tAMD (n = 1199) and patients with PCV (n = 1343). The same control participants were used. We estimated the statistical differences in effect sizes between subtypes using the Cochran Q test. R, version 3.3.3, software (R Foundation for Statistical Computing) was used for all evaluations.

We assessed colocalization of the association signals using the coloc[31] package in R, version 5.1.0. This evaluates the probability of colocalization of causal variants within the regions evaluated in 2 genetic association analyses. We used the summary statistics of the CSC GWAS in the Japanese population[18] and the association results of the AMD meta-GWAS (first and second sets) to perform the analysis. We noted a low possibility of overlaps in control participants between GWAS and CSC GWAS because the hospitals in which participants were enrolled were not overlapped in these studies. Variants located within ± 500 kb from the lead variant of each newly identified locus were used in the analysis. To assess difference of probability of colocalization between tAMD and PCV, we tested associations of variants evaluated in the colocalization analysis using a dataset for subtype analysis and performed colocalization analysis in each subtype.

Prioritization of Candidate Genes and Bioinformatics Analysis

We sought to prioritize AMD candidate genes from positional candidates of newly identified loci. First, we determined positional candidate genes by their positions with respect to the lead variants (< 250 kb). Second, variants in linkage disequilibrium (LD) were annotated using annovar software.[32] Then, we looked up the expression quantitative trait locus (eQTL) data of the Genotype-Tissue Expression (GTEx) project[33] using the GTEx portal, version 8 (https://gtexportal.org/home/), and the Eye eQTL browser (eye-eqtl.com)[7] to assess overlaps of identified loci and eQTL across various tissues, including the retina and retinal pigment epithelium. Given the LD difference of LD between our GWAS of East Asian individuals and the eQTL results obtained from a European population, we regarded significant overlap if the lead variants of AMD GWAS and eQTLs were in LD in both populations ($r^2 > 0.7$ for both EAS and EUR of 1KGP). Finally, we performed a gene-set enrichment analysis using MAGMA, version 1.10,[34] using the result of meta-analysis of AMD GWAS (first and second datasets). We used 6366 curated gene sets (c2.all.v7.5.1.entrez.gmt) of the MSigDB collections (http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp). In the gene-set enrichment analysis, we considered pathways satisfying false-discovery rates (FDRs) estimated by the Benjamini–Hochberg procedure with values $< 5\%$ as significant.

Genetic Correlation Analysis

To assess shared genetic components between AMD and epidemiologically suggested clinical factors, we estimated genetic correlations using the summary statistics of our meta-analysis of AMD GWASs and those of the BBJ project.[35–37] We selected 10 traits across 4 categories, including blood pressure traits (systolic blood pressure and diastolic blood pressure), lipid-related traits (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol levels), smoking-related traits (cigarettes per day and smoking initiation and cessation), and alcohol-related traits (alcoholic drinks per day). Genetic correlations were estimated by LD score regression using ldsc software.[12]

Results

Genome-Wide Association Study and Replication Study

After meta-analyzing 2 independent GWAS datasets, we finally evaluated 22 317 264 autosomal variants in 2663 patients with AMD and 9471 control participants. We did not observe any inflation of the meta-GWAS statistics ($\lambda = 1.00$; Fig S1, available at www.aaojournal.org). We detected 6 loci significantly associated with AMD (*CFH*, *C2/FB*, *TNFRSF10A*, *WBP1L*, *ARMS2*, and *GATA5*; $P < 5.0 \times 10^{-8}$). Of these, *WBP1L* and *GATA5* have not been reported previously (Fig 1 and Table 1).

We selected 11 variants that were independently associated with AMD ($P < 1.0 \times 10^{-6}$) in the meta-GWAS for the replication study with 1109 patients with AMD and 7299 control participants. Of these 11 variants, 10 were genotyped successfully. We observed that rs4147157 (the lead variant of the *WBP1L* locus) exhibited a significant association with AMD at the Bonferroni-corrected level ($P = 1.20 \times 10^{-3}$; odds ratio [OR], 1.16; $\alpha = 5.0 \times 10^{-3}$ [= 0.05/10]) and that rs76228488 of the *GATA5* locus was associated nominally ($P = 6.29 \times 10^{-3}$; OR, 1.13) in the replication study (Table 2; Table S2, available at www.aaojournal.org). After the meta-analysis of the meta-GWAS and the replication study, we confirmed that these variants satisfied genome-wide significance without any evidence of heterogeneity ($P = 1.88 \times 10^{-12}$ and $P =$

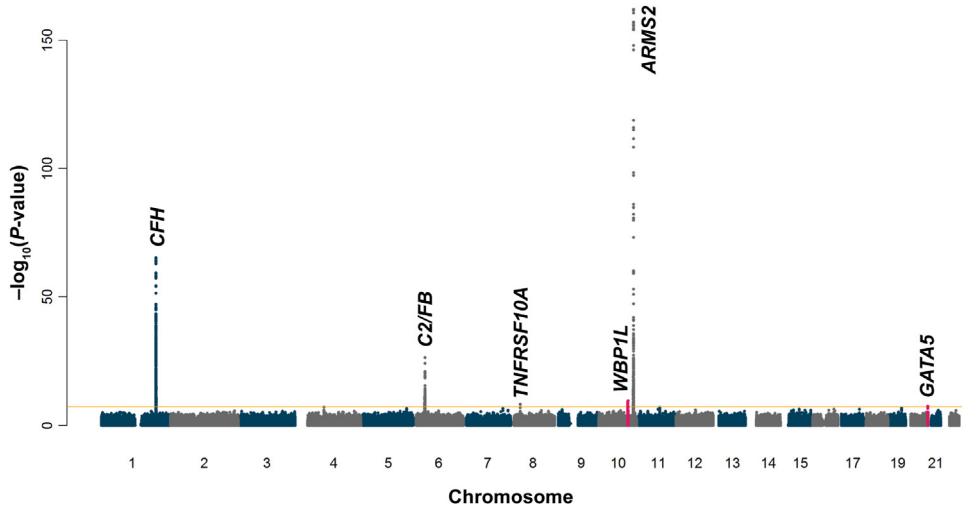

Figure 1. Manhattan plot showing the meta−genome-wide association study. The associations in the meta-analysis were illustrated. The y-axis indicates association strengths on a $-\log_{10}$ scale. The x-axis denotes chromosomal positions. The orange line is the genome-wide significance threshold ($P = 5.0 \times 10^{-8}$). Newly identified loci (*WBP1L* and *GATA5*) are highlighted in pink.

1.35×10^{-9} for meta-analysis for rs4147157 and rs76228488, respectively; $P > 0.05$ for heterogeneity).

Associations of Previously Reported Loci

Of the 34 lead variants reported by IAMDGC[4] and the 4 variants identified by the GWAS in East Asian individuals,[3] 30 were evaluated in our GWAS. Eight loci (*ADAMTS9, CFI, VEGFA, TGFBR1, CETP, C60rf223, SLC44A4*, and *APOE*) showed a Bonferroni-corrected level of significant associations (Table S3, available at www.aaojournal.org; $\alpha = 1.67 \times 10^{-3}$ [= 0.05/30]). In addition to the 6 loci that reached genome-wide significance, we considered that these 8 loci also were associated with AMD in the Japanese population.

Subtype Analysis

We next assessed the differences in the effects of the 14 AMD-associated loci between patients with tAMD (n = 1199) and patients with PCV (n = 1343) using the same control participants. In line with the previous reports,[16] a significant effect size heterogeneity of the associated variant at *ARMS2* was observed between tAMD and PCV ($P = 6.80 \times 10^{-5}$ for difference; OR, 2.83 and 2.18 for tAMD and PCV, respectively; Table S4, available at www.aaojournal.org). The impacts of the newly identified loci on 2 major subtypes of exudative AMD were comparable ($P = 0.79$ and 0.58 for difference for *WBP1L* and *GATA5*, respectively).

Associations in CSC GWAS

Next, we explored the associations of the 14 AMD-associated variants with CSC. Among the 14 loci, 9 were evaluated in the CSC GWAS conducted in the Japanese population.[18] We observed significant associations at 3 loci (Table S5, available at www.aaojournal.org; *TNFRSF10A*, *WBP1L*, and *GATA5*) in the same directional effects (Fig 2,

Table 1. Six Loci That Reached Genome-Wide Significance in the Meta−Genome-Wide Association Study

Lead Variant	Chromosome	Position	Reference Allele/Alternative Allele	Locus Name	Odds Ratio (95% Confidence Interval)	*P* Value	
						*Meta-analysis**	*Heterogeneity*†
Previously reported loci							
rs10922109	1	196 704 632	C/A	*CFH*	0.58 (0.55−0.63)	4.83×10^{-55}	0.89
rs116503776	6	31 930 462	G/A	*C2-CFB*	0.60 (0.53−0.68)	3.43×10^{-16}	0.21
rs79037040	8	23 082 971	G/T	*TNFRSF10A*	1.22 (1.14−1.30)	5.02×10^{-9}	0.02
rs3750846	10	124 215 565	T/C	*ARMS2*	2.48 (2.32−2.65)	3.18×10^{-161}	0.34
Newly reached genome-wide significance							
rs4147157	10	104 536 360	G/A	*WBP1L*	1.22 (1.15−1.31)	2.34×10^{-10}	0.09
rs76228488	20	61 022 329	T/G	*GATA5*	1.20 (1.13−1.29)	3.47×10^{-8}	0.84

The position is based on those of National Center for Biotechnology Information build 37. The meta-analysis was performed using the inverse variance method.
*In the meta−genome-wide association study estimated using the inverse variance method.
†Heterogeneity between genome-wide association studies.

Table 2. Associations of the Newly Identified Loci

Variant (Nearest Gene)	Chromosome Position (Reference Allele/Alternative Allele)	Stage	Alternative Allele Frequency Patient/Control Participant	Odds Ratio (95% Confidence Interval)	P Value	P Value (Heterogeneity)*
rs4147157 (*WBP1L*)	10	GWAS (first set)	0.535/0.482	1.34 (1.29−1.51)	1.48×10^{-6}	
	104 536 360	GWAS (second set)	0.523/0.476	1.18 (1.10−1.28)	9.29×10^{-6}	
	(G/A)	Replication	0.514/0.477	1.16 (1.06−1.27)	1.20×10^{-3}	
		Meta-analysis		1.20 (1.14−1.27)	1.88×10^{-12}	0.14
rs76228488 (*GATA5*)	20	GWAS (first set)	0.436/0.401	1.22 (1.08−1.37)	1.71×10^{-3}	
	61 022 329	GWAS (second set)	0.442/0.398	1.20 (1.11−1.30)	4.81×10^{-6}	
	(T/G)	Replication	0.426/0.395	1.13 (1.04−1.24)	6.29×10^{-3}	
		Meta-analysis		1.18 (1.12−1.24)	1.35×10^{-9}	0.58

GWAS = genome-wide association study.
The position is based on those of National Center for Biotechnology Information build 37. The meta-analysis was performed using the inverse variance method.
*Of the heterogeneity among the 3 datasets.

$P = 5.94 \times 10^{-7}$, $P = 4.86 \times 10^{-3}$, and $P = 4.28 \times 10^{-3}$; OR, 1.38 [95% confidence interval, 1.22−1.57], 1.20 [95% confidence interval, 1.06−1.35], and 1.20 [95% confidence interval, 1.06−1.35] for rs13278062 [*TNFRSF10A*], rs4147157 [*WBP1L*], and rs76228488 [*GATA5*], respectively; $\alpha = 5.56 \times 10^{-3}$ [= 0.05/9]). We also compared the association signals between AMD and CSC (Fig 3). Although the lead variants for each disease lay close (106 kb and 2 kb for both *WBP1L* and *GATA5*, respectively), the LDs of the lead variants were weak ($r^2 = 0.05$ and $r^2 = 0.11$ for *WBP1L* and *GATA5* in the whole-genome sequences of 1037 Japanese people, respectively). Next, we assessed the colocalization of causal variants between AMD and CSC using coloc software.[31] The posterior probabilities (PPs) of colocalization were 99%, 39%, and 60% for the loci at *TNFRSF10A*, *WBP1L*, and *GATA5*, respectively (Fig 4; Table S6, available at www.aaojournal.org). We also assessed differences of colocalization with CSC at these loci by subdividing the patients with AMD into tAMD and PCV and observed high PP of shared causal variants at *TNFRSF10A* and *GATA5* in both typical AMD and PCV (PP > 80%; Fig S2, available at www.aaojournal.org). However, PP at *WBP1L* seemed to be low in tAMD, whereas that in PCV was similar to that in all patients with AMD (PPs in tAMD and PCV were 11% and 37%, respectively). Notably, the lead variants of *CFH* and *CFI* exhibited nominal associations with CSC ($P < 0.05$).

Candidate Gene Prioritization and Bioinformatics Analysis

We sought to prioritize candidate AMD-relevant genes from the positional candidates of newly identified loci. First, we determined 28 candidate genes (18 genes around rs4147157 and 10 genes around rs76228488) according to their positions (±250 kb) from the variants in LD ($r^2 > 0.7$) with the lead variants of each locus (Table S7, available at www.aaojournal.org). Second, we annotated variants in LD ($r^2 > 0.8$) with identified lead variants; however, these variants were not in LD with nonsynonymous variants of the positional candidate genes. Third, we evaluated the overlaps with the eQTLs of GTEx project.[33] We found no significant eQTL for rs76228488, but rs4147157 was registered as a significant eQTL in various tissues in the GTEx portal. Therefore, we evaluated the LDs between rs4147157 and the lead variants of eQTLs and found that rs999867 satisfied our criteria ($r^2 > 0.7$ for both EAS and EUR; $r^2 = 0.90$ and $r^2 = 0.97$ between rs4147157 and rs999867 in EAS and EUR samples of 1KGP, respectively). rs999867 was registered as a significant eQTL for *WBP1L* in the thyroid. We also sought eQTL of the retina and retinal pigment epithelium using the Eye eQTL browser[7] (www.eye-eqtl.com) and observed a strong association of rs4147157 with the expression level of *SFXN2* in the retina at the nonmacular region ($P = 1.25 \times 10^{-8}$; FDR = 1.31×10^{-5}). However, the lead variant of this eQTL (rs2902548) was in weak LD only with rs4147157 ($r^2 = 0.4$ and $r^2 = 0.3$ in EUR and EAS samples of 1KGP, respectively). Finally, we performed gene-set enrichment analysis using MAGMA[34]; however, we found no significantly associated gene sets (FDR < 0.05; Table S8, available at www.aaojournal.org).

Pleiotropic Associations

To characterize the biological roles played by the identified variants, we evaluated their associations of identified variants with the 220 complex traits of the GWAS of BBJ[38] (https://pheweb.jp/; Fig S3 and Table S9, available at www.aaojournal.org). At the Bonferroni-corrected level ($\alpha = 1.14 \times 10^{-4}$ [= 0.05/ (220 × 2)]), we observed significant pleiotropic associations of rs4147157 with blood pressure-related traits (systolic blood pressure, pulse pressure, and mean arterial pressure), body weight, body mass index, and myocardial infarction. rs76228488 was associated significantly with renal function-related traits (estimated glomerular filtration rate [$\beta = -0.010$; standard error = 0.002; $P = 2.6 \times 10^{-5}$], serum creatinine level [$\beta =$ 0.012; standard error = 0.029; $P = 7.7 \times 10^{-5}$]) and use of calcium channel blockers ($\beta = -0.034$; standard error = 0.009; $P = 5.1 \times 10^{-5}$). We also searched for reported

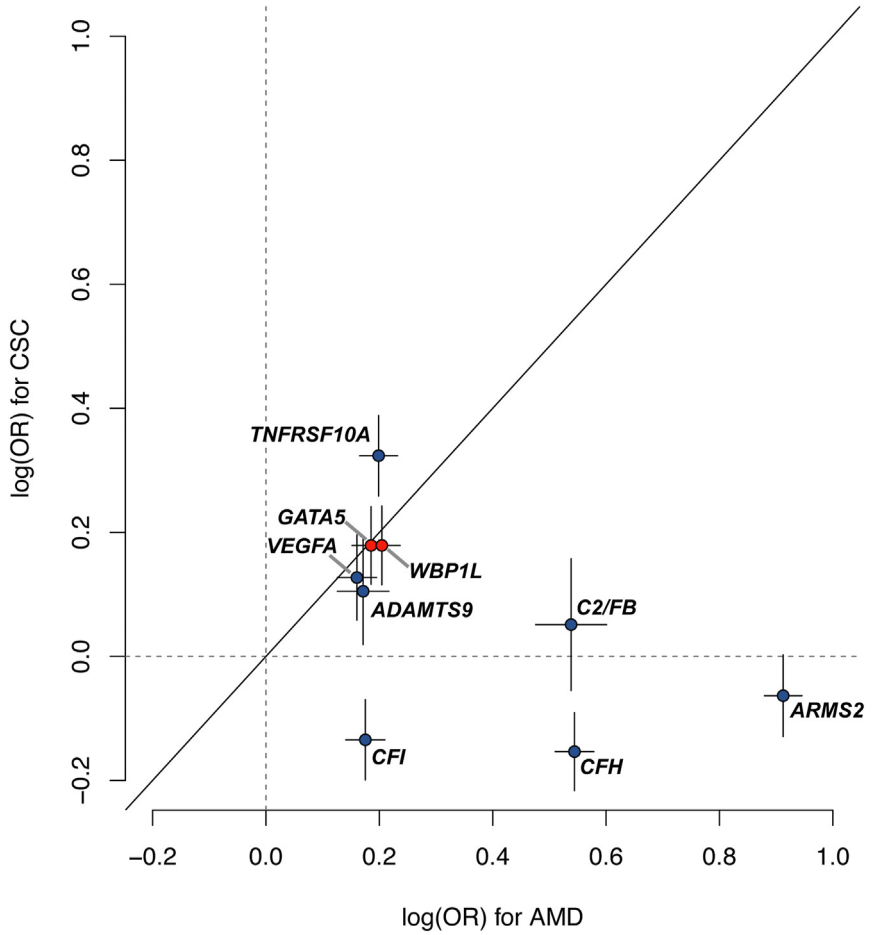

Figure 2. Plots comparing of effect sizes between age-related macular degeneration (AMD) and central serous chorioretinopathy (CSC). We plotted the effect sizes of 9 AMD-associated variants for AMD (x-axis) and CSC (y-axis). The effect sizes for CSC are those from our previous genome-wide association study.[18] Newly identified loci are shown in red. OR = odds ratio.

genetic associations in the GWAS catalog; rs4147157 was reported to be associated with schizophrenia in East Asian individuals.[39]

Genetic Components Shared with Epidemiologic Risk Factors

To assess shared genetic effects with epidemiologically suggested clinical risk factors, we evaluated genetic correlations[12] using the publicly available summary statistics of the BBJ.[35–37] We targeted 11 traits across 4 categories including blood pressure (systolic blood pressure and diastolic blood pressure), serum lipid levels (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and total cholesterol), alcohol consumption (alcohol drinks per week), and smoking-related traits (age at smoking initiation, smoking cessation, cigarettes per week, and smoking initiation) and found a suggestive negative genetic correlation between AMD and smoking cessation (r_g [the measure of genetic correlation] = −0.33; $P = 0.01$; FDR = 0.099; Fig 5).

Discussion

Through a large-scale genetic association analysis in the Japanese population, we identified 2 novel loci (*WBP1L* and *GATA5*) associated with AMD. In addition to *TNFRSF10A* (originally identified by the previous Japanese AMD GWAS[8]), pleiotropic associations with CSC were suggested at the 2 newly identified loci. Our colocalization analysis implied shared causal variants for both diseases underlying these new loci. Genetic correlation analysis implicated a polygenic architecture shared between AMD and smoking cessation.

We identified 2 new AMD-associated loci. When we looked up the results of IAMDGC GWAS data,[4] the lead variant of *WBP1L* (rs4147157) was associated only nominally with advanced AMD ($P_{GC} = 0.038$) in which A allele was the risk for AMD that was consistent with our findings; however, the association of this variant was embedded within those of other variants (Fig S4, available at www.aaojournal.org). The association of rs76228488 was not significant in IAMDGC (P_{GC} [P value after

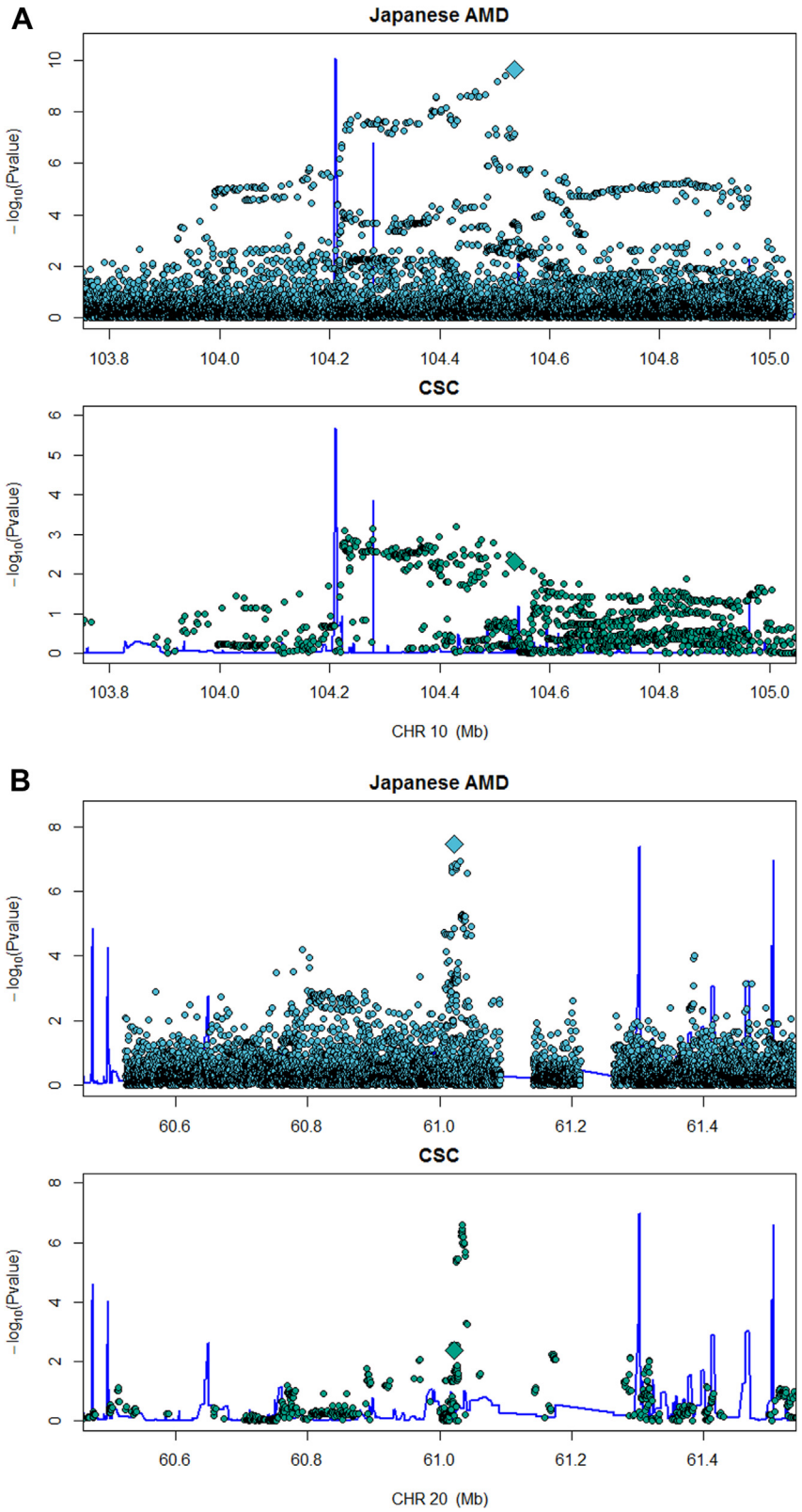

Figure 3. Association plots showing the newly identified loci for age-related macular degeneration (AMD) and central serous chorioretinopathy (CSC). We compared the association signals of the newly identified loci (*WBP1L* **[A]** and *GATA5* **[B]**) in the AMD genome-wide association study (GWAS; upper boxes) and the CSC GWAS[18] (lower boxes) in the Japanese population. The x-axis denotes the chromosomal position. The y-axis indicates the strength of the association on a $-\log_{10}$ scale. CHR = chromosome.

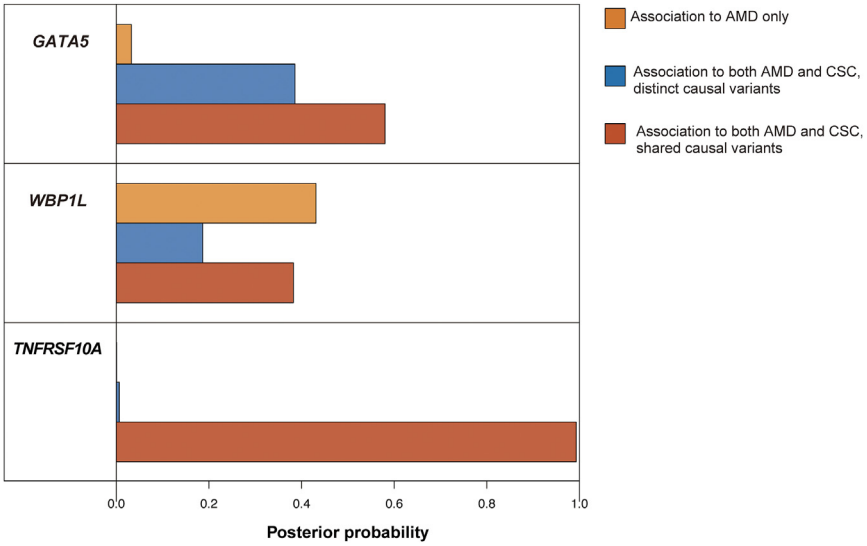

Figure 4. Bar graphs showing posterior probabilities of causal variants colocalization. We used coloc software[31] to estimate the colocalization of causal variant(s) for 3 loci significantly associated with central serous chorioretinopathy (CSC). We illustrated the estimated posterior probabilities for 3 hypotheses as follows: (1) association to age-related macular degeneration (AMD) only (yellow), (2) association to both AMD and CSC (however, causal variants are distinct [blue]), and (3) association to both AMD and CSC and causal variants are shared (red).

genomic control correction] = 0.584 for advanced AMD). Although minor allele frequencies of rs4147157 were relatively rare in a European population (9% in EUR of 1KGP), rs76228488 was common across populations in 1KGP. We also visually confirmed that genetic associations of these newly identified loci were not strong in IAMDGC (Fig S4). Because the IAMDGC evaluated principally European individuals, these observations imply that identified loci may have a greater impact on Japanese neovascular AMD or that true causal variants are uncommon in European individuals. As the other difference between our GWAS and that of IAMDGC, we did not observe significant enrichment of the gene set relevant to the complement system, although "REACTOME_COMPLEMENT_CASCADE" showed strong association ($P = 5.27 \times 10^{-4}$; FDR = 0.3). Given that we observed significant associations of previously reported loci that were relevant to complement cascade (*CFH*, *CFI*, and *C2/FB*), we interpreted that the power of our GWAS might be insufficient to reveal significantly associated gene sets.

We sought to interpret the identified loci using publicly available biological resources. A general limitation of GWASs is that GWASs cannot directly pinpoint a gene associated with phenotypes.[40] In the present study, we used eQTL data provided by GTEx[33] and found significant overlaps between GWAS signals of the *WBP1L* locus (near rs4147157) and eQTL results for *WBP1L*, suggesting that changes in the expression levels of *WBP1L* may influence the susceptibility to AMD. Our analysis of pleiotropic effects across a comprehensive set of human phenotypes indicates that rs4147157 is associated with metabolic-related (blood pressure and body weight) and cardiovascular-related traits, suggesting that *WBP1L* locus confers an AMD risk via systemic involvement. A recent study reported that the genetic risk for cardiovascular disease was associated with that of AMD.[41] At the *GATA5* locus, we found no candidate genes. Additional functional studies are warranted to determine the precise biological mechanisms influencing the susceptibility to AMD.

Our results show that, in addition to the known AMD susceptibility variant located in *TNFRSF10A*, our newly identified loci also may be associated with CSC ($P < 0.01$). Considering the relatively weak associations in the CSC GWAS, we could not conclude that these loci confer the risk of CSC. Although a future large-scale GWAS of CSC is warranted to clarify this point, our colocalization analysis provided additional supports to assess genetic overlaps between AMD and CSC. Of the 2 loci, *GATA5* was reported to be associated with CSC in the Japanese GWAS.[18] Although the LD between the lead AMD and CSC variants was not strong LD ($r^2 = 0.11$), our genetic colocalization analysis indicated that the causal variants for AMD and CSC at this locus were more likely to be shared than not (estimated PPs for distinct vs. shared causal variants, 36% vs. 60%, respectively; Fig 4). However, the association of another locus (*WBP1L*) in the CSC GWAS was not strong (Fig 2A) and has not been reported previously. Given that the PP estimated by colocalization analysis (that for an association with AMD only compared with that of shared causal variants, 44% vs. 39%), it is possible that *WBP1L* features shared causal variant(s) for both AMD and CSC. Our subtype analysis showed high PP (> 80%) for colocalization with CSC at *TNFRSF10A* and *GATA5* in both tAMD and PCV. However, PP at *WBP1L* seemed to be low in tAMD, whereas that in PCV was similar to that in all patients with AMD. Considering these together, we interpreted that colocalizations at *TNFRSF10A* and *GATA5* were not influenced by AMD subtypes; however, that at

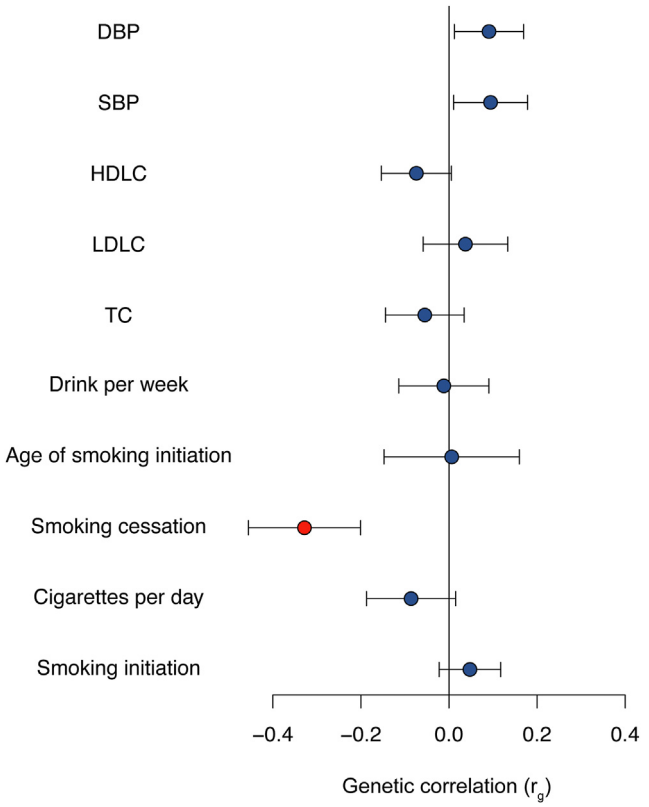

Figure 5. Genetic correlations of age-related macular degeneration (AMD) with epidemiologic risk factors. The genetic correlation between AMD and epidemiologically reported risk factors was estimated using bivariate LD score regression.[12] Error bars indicate the standard errors of the estimated r_g values (that measure genetic correlations). DBP = diastolic blood pressure; HDLC = high-density lipoprotein cholesterol; LD = linkage disequilibrium; LDLC = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol.

WBP1L may differ among AMD subtypes. Because the statistical power for colocalization analysis is influenced by sample sizes in the GWAS[31] and the observed associations of AMD-associated variants with CSC were marginal (if we applied conservative multiple testing correction, such as study-wide Bonferroni correction for look-ups, the observed associations were not significant), further large-scale GWASs for AMD and CSC will clarify this point. Accumulating clinical evidence suggests that some conventional AMD eyes, thus, eyes diagnosed with AMD before the advent of the so-called pachychoroid era, should have been diagnosed with pachychoroid neovasculopathy, a macular neovascularization secondary to principally CSC.[42–46] The pachychoroid neovasculopathy proportion is 19.5% to 46.2% in Japanese patients[43,45] and 15.4% in White patients.[44] Our finding that conventional AMD causal variants are shared with those of CSC is in line with this clinical knowledge.

Many observational epidemiologic studies have reported a number of lifestyle and metabolic risk factors for AMD, although the findings have been inconsistent, which is attributable to biases caused by confounding factors and reverse causation that are inevitable when performing observational studies. To deal with these issues, IAMDGC performed a Mendelian randomization study that can evaluate causation unbiasedly by using genetic predisposition as an instrumental variable.[47] In White people, smoking cessation and initiation and lifetime smoking index were associated significantly with reduced or increased risks of AMD; blood pressure and alcohol consumption were not, consistent with our genetic correlation study except for smoking initiation, which did not show significant genetic correlation with AMD in the current study. Despite the differences, our findings suggested that some AMD heritability can be explained by genetic components that affect Japanese lifestyles.

In conclusion, we performed a GWAS for AMD in the Japanese population and identified 2 new AMD-associated loci that also may affect CSC development. These findings increase our understanding of the genetic architectures of AMD and CSC and suggest that the genetic components in play differ in Japanese and other populations.

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HUMAN SUBJECTS: Human subjects were included in this study. This study was approved by the Ethics Committee of each facility and adhered to the tenet of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were included in this study.

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Obtained funding: N/A

Overall responsibility: Akiyama, Miyake

Abbreviations and Acronyms:
1KGP = 1000 Genomes Project; **AMD** = age-related macular degeneration; **BBJ** = Biobank Japan; **CSC** = central serous chorioretinopathy; **eQTL** = expression quantitative trait locus; **FDR** = false-discovery rate; **GTEx** = Genotype-Tissue Expression; **GWAS** = genome-wide association study; **IAMDGC** = International Age-Related Macular Degeneration Genetics Consortium; **LD** = linkage disequilibrium; **OR** = odds ratio; **PCV** = polypoidal choroidal vasculopathy; **PP** = posterior probability; **tAMD** = typical neovascular age-related macular degeneration.

Keywords:
Age-related macular degeneration, Central serous chorioretinopathy, Genome-wide association study, Polygenic architecture.

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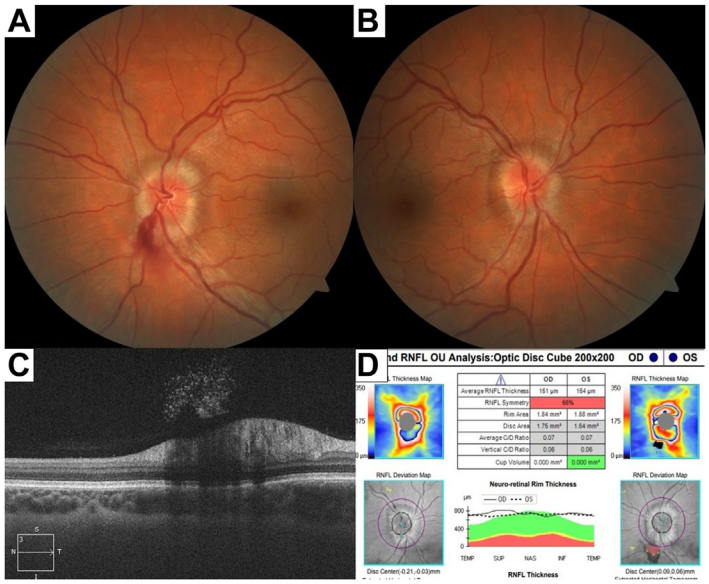
References

1. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet*. 2018;392(10153):1147−1159.
2. Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. *Nat Genet*. 2013;45(4):433−439.
3. Cheng CY, Yamashiro K, Chen LJ, et al. New loci and coding variants confer risk for age-related macular degeneration in East Asians. *Nat Commun*. 2015;6:6817.
4. Fritsche LG, Igl W, Bailey JNC, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2015;48(2):134−143.
5. Wang J, Zibetti C, Shang P, et al. ATAC-Seq analysis reveals a widespread decrease of chromatin accessibility in age-related macular degeneration. *Nat Commun*. 2018;9(1):1−13.
6. Ratnapriya R, Sosina OA, Starostik MR, et al. Retinal transcriptome and eQTL analyses identify genes associated with age-related macular degeneration. *Nat Genet*. 2019;51(4):606−610.
7. Orozco LD, Chen HH, Cox C, et al. Integration of eQTL and a single-cell atlas in the human eye identifies causal genes for age-related macular degeneration. *Cell Rep*. 2020;30(4):1246−1259. e6.
8. Arakawa S, Takahashi A, Ashikawa K, et al. Genome-wide association study identifies two susceptibility loci for exudative age-related macular degeneration in the Japanese population. *Nat Genet*. 2011;43(10):1001−1005.
9. Ruamviboonsuk P, Tadarati M, Singhanetr P, et al. Genome-wide association study of neovascular age-related macular degeneration in the Thai population. *J Hum Genet*. 2017;62(11):957−962.
10. Momozawa Y, Akiyama M, Kamatani Y, et al. Low-frequency coding variants in CETP and CFB are associated with susceptibility of exudative age-related macular degeneration in the Japanese population. *Hum Mol Genet*. 2016;25(22):5027−5034.
11. Solovieff N, Cotsapas C, Lee PH, Purcell SM, Smoller JW. Pleiotropy in complex traits: challenges and strategies. *Nat Rev Genet*. 2013;14(7):483−495.
12. Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. 2015;47(11):1236−1241.
13. Jostins L, Ripke S, Weersma RK, et al. Host−microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491(7422):119−124.
14. Cross-Disorder Group of the Psychiatric Genomics Consortium. Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: A genome-wide analysis. *Lancet*. 2013;381(9875):1371−1379.
15. Sobrin L, Ripke S, Yu Y, et al. Heritability and genome-wide association study to assess genetic differences between advanced age-related macular degeneration subtypes. *Ophthalmology*. 2012;119(9):1874−1885.
16. Fan Q, Cheung CMG, Chen LJ, et al. Shared genetic variants for polypoidal choroidal vasculopathy and typical neovascular age-related macular degeneration in East Asians. *J Hum Genet*. 2017;62(12):1049−1055.
17. Honda S, Matsumiya W, Negi A. Polypoidal choroidal vasculopathy: clinical features and genetic predisposition. *Ophthalmologica*. 2014;231(2):59−74.
18. Hosoda Y, Miyake M, Schellevis RL, et al. Genome-wide association analyses identify two susceptibility loci for pachychoroid disease central serous chorioretinopathy. *Commun Biol*. 2019;2(1):468.
19. Cheung CMG, Lee WK, Koizumi H, et al. Pachychoroid disease. *Eye*. 2019;33(1):14−33.
20. Mori Y, Miyake M, Hosoda Y, et al. Genome-wide survival analysis for macular neovascularization development in central serous chorioretinopathy revealed shared genetic susceptibility with polypoidal choroidal vasculopathy. *Ophthalmology*. 2022;129(9):1034−1042.
21. Yamaguchi-Kabata Y, Nakazono K, Takahashi A, et al. Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am J Hum Genet*. 2008;83(4):445−456.
22. Hirata M, Kamatani Y, Nagai A, et al. Cross-sectional analysis of BioBank Japan clinical data: a large cohort of 200,000 patients with 47 common diseases. *J Epidemiol*. 2017;27(3). S9−S21.
23. Nagai A, Hirata M, Kamatani Y, et al. Overview of the BioBank Japan project: study design and profile. *J Epidemiol*. 2017;27(3):S2−S8.
24. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559−575.
25. Momozawa Y, Akiyama M, Kamatani Y, et al. Low-frequency coding variants in CETP and CFB are associated with susceptibility of exudative age-related macular degeneration in the Japanese population. *Hum Mol Genet*. 2016;25(22):5027−5034.
26. Delaneau O, Marchini J, Zagury J-F. A linear complexity phasing method for thousands of genomes. *Nat Methods*. 2011;9(2):179−181.
27. Li Y, Willer CJ, Ding J, et al. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol*. 2010;34(8):816−834.
28. Akiyama M, Ishigaki K, Sakaue S, et al. Characterizing rare and low-frequency height-associated variants in the Japanese population. *Nat Commun*. 2019;10(1):4393.
29. Auton A, Abecasis GR, Altshuler DM, et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68−74.
30. Zhan X, Hu Y, Li B, et al. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. *Bioinformatics*. 2016;32:1423−1426.
31. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. 2014;10(5):e1004383.
32. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.
33. Ardlie KG, DeLuca DS, Segrè AV, et al. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348(6235):648−660.
34. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. *PLoS Comput Biol*. 2015;11(4):e1004219.
35. Kanai M, Akiyama M, Takahashi A, et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat Genet*. 2018;50(3):390−400.

36. Matoba N, Akiyama M, Ishigaki K, et al. GWAS of smoking behaviour in 165,436 Japanese people reveals seven new loci and shared genetic architecture. *Nat Hum Behav*. 2019;3(5):471−477.
37. Matoba N, Akiyama M, Ishigaki K, et al. GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. *Nat Hum Behav*. 2020;4(3):308−316.
38. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. *Nat Genet*. 2021;53(10):1415−1424.
39. Lam M, Chen CY, Li Z, et al. Comparative genetic architectures of schizophrenia in East Asian and European populations. *Nat Genet*. 2019;51(12):1670−1678.
40. Akiyama M. Multi-omics study for interpretation of genome-wide association study. *J Hum Genet*. 2021;66(1):3−10.
41. Grassmann F, Kiel C, Zimmermann ME, et al. Genetic pleiotropy between age-related macular degeneration and 16 complex diseases and traits. *Genome Med*. 2017;9(1):29.
42. Miyake M, Tsujikawa A, Yamashiro K, et al. Choroidal neovascularization in eyes with choroidal vascular hyperpermeability. *Invest Ophthalmol Vis Sci*. 2014;55(5):3223−3230.
43. Miyake M, Ooto S, Yamashiro K, et al. Pachychoroid neovasculopathy and age-related macular degeneration. *Sci Rep*. 2015;5:16204.
44. Borrelli E, Battista M, Gelormini F, et al. Rate of misdiagnosis and clinical usefulness of the correct diagnosis in exudative neovascular maculopathy secondary to AMD versus pachychoroid disease. *Sci Rep*. 2020;10(1):20344.
45. Hosoda Y, Miyake M, Yamashiro K, et al. Deep phenotype unsupervised machine learning revealed the significance of pachychoroid features in etiology and visual prognosis of age-related macular degeneration. *Sci Rep*. 2020;10(1):18423.
46. Cozzupoli GM, Borrelli E, Capuano V, et al. InCASEOf scoring system for distinction between pachychoroid-associated macular neovascularization and neovascular age-related macular degeneration in patients older than 50 years. *Sci Rep*. 2022;12(1):2938.
47. Kuan V, Warwick A, Hingorani A, et al. Association of smoking, alcohol consumption, blood pressure, body mass index, and glycemic risk factors with age-related macular degeneration: a Mendelian randomization study. *JAMA Ophthalmol*. 2021;139(12):1299−1306.

Pictures & Perspectives



Vitreous Hemorrhage from Papilledema After Neck Massage

A 41-year-old woman presented with left eye floaters after receiving a head and neck massage while prone. The patient had a history of idiopathic intracranial hypertension, treated with acetazolamide. Examination revealed a left inferior optic disc hemorrhage and bilateral optic disc edema (**A**-**B**). OCT of the left optic nerve depicted retinal nerve fiber edema with hyperreflectivity in the preretinal space extending into the vitreous (**C**). OCT retinal nerve fiber layer confirmed bilateral disc edema (**D**). This case represents active disc hemorrhage observed on OCT imaging secondary to idiopathic intracranial hypertension and exacerbated by pressure from massage, which induced venous rupture on the optic disc (Magnified version of Figure **A**-**D** is available online at www.aaojournal.org).

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