Genome-Wide Association Study to Identify a New Susceptibility Locus for Central Serous Chorioretinopathy in the Japanese Population

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PURPOSE. Central serous chorioretinopathy (CSC) is a retinal disorder that often affects the vision of middle-aged people yet the molecular mechanisms of CSC remain unknown. This study was conducted to identify genetic factors influencing individual differences in susceptibility to CSC.

METHODS. A two-stage genome-wide association study (GWAS) was conducted with a total of 320 unrelated Japanese idiopathic CSC cases and 3245 population-based controls. In a discovery stage, 137 unrelated Japanese idiopathic CSC cases and 1174 population-based controls were subjected to GWAS, followed by a replication study using an additional 183 individuals with idiopathic CSC and 2071 population-based volunteers. The results of the discovery and replication stages were combined to conduct a meta-analysis.

RESULTS. In the two-stage GWAS, rs11865049 located at SLC7A5 in chromosome 16q24.2 was identified as a novel disease susceptibility locus for CSC, as evident from the discovery and replication results using meta-analysis (combined \( P = 9.71 \times 10^{-3} \), odds ratio = 2.10).

CONCLUSIONS. The results of the present study demonstrated that SLC7A5 might be the potential candidate gene associated with CSC, indicating a previously unidentified molecular mechanism of CSC.

Keywords: central serous chorioretinopathy, genome-wide association study, Japanese, SLC7A5

Central serous chorioretinopathy (CSC) is an idiopathic retinal disorder that often affects the vision of middle-aged people (mainly 40-60 years old).1,2 The prevalence of CSC has been reported to be approximately 1:10,000 with a male-to-female sex ratio of approximately 3:1 to 6:1.1,3-5 The clinical aspects of CSC are well described and are characterized by serous retinal detachment, retinal pigment epithelial (RPE) detachment, and RPE atrophy mainly at the posterior pole of the fundus. In addition, pinpoint or diffuse dye leakage is usually found by fluorescein angiography (FA), and choroidal vessel dilation and choroidal vascular hyperpermeability (CVH) are often found by indocyanine green angiography (ICGA).6,7 Although the molecular basis of this disease is poorly understood, a number of clinical reports have suggested the correlation of glucocorticoid, adrenergic hormones, and psychopharmacologic medication with the pathophysiology of CSC.6-8 Moreover, several reports describing familial cases of CSC have suggested the existence of a genetic susceptibility to this disease.9,10 which influences the response to the external stimuli including psychological stresses and hormonal imbalance. Our previous genetic association study using a candidate gene approach revealed the association of single nucleotide polymorphism (SNP) in complement factor H (CFH) gene with CSC.11 CFH is known to bind with adrenomedullin,12 which could influence the status of choroidal vessels.13 Several genetic association studies have reported other possible candidate genes that affect the susceptibility to chronic CSC.14-17 However, the genetic predisposition to CSC has not been fully elucidated, and one of the reasons for this may be the lack of a genome-wide association study (GWAS) to generally identify potential suscepti-
Materials and Methods

Ethics Statement

This study was approved by the Institutional Review Board at the Kobe University Graduate School of Medicine (protocol No. 93 and No. 852), Tokushima University, University of Yamanashi, Nihon University, Kyushu University, and the Aichi Cancer Center Research Institute, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Study Cohorts

The present study included a total of 320 unrelated idiopathic CSC cases and 3245 population-based controls. All CSC patients in the present study received detailed ophthalmic examinations, including slit-lamp biomicroscopy, color fundus photography, FA, ICGA (HRA2; Heidelberg Engineering, Heidelberg, Germany), and spectral-domain optical coherence tomography (OCT) (Spectralis; Heidelberg Engineering). In this study, we defined idiopathic CSC, which represents central serous retinal detachment without subretinal hemorrhage or suspected choroidal neovascularization in ICGA or OCT.

In the discovery phase, 730,525 SNPs were genotyped using Illumina Human Omni Express BeadChips (Illumina, San Diego, CA, USA). We performed a standard quality control procedure to exclude SNPs with a low call rate (<95%), \( P \) value of Hardy-Weinberg equilibrium test of \( <1.0 \times 10^{-5} \) in controls, and minor allele frequency (MAF) of \( <0.05 \) in each stage. Finally, we analyzed 548,653 SNPs in the GWAS and evaluated the association of SNPs with CSC using the Cochran-Armitage trend test (CATT). In the nominal data, we selected the SNPs with a suggestive significance threshold of \( P \) values < \( 5 \times 10^{-6} \) for replication. After visual inspection of cluster plots to exclude the SNPs that did not fit to cluster boundaries, SNPs that were located at annotated genes were further examined in the replication study using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) on StepOnePlus Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer’s instructions. As the second control, an imputation data set was derived from the HumanCoreExome-12 v1.1 BeadChips for 2071 samples (Illumina) of population-based volunteers recruited by the Aichi Cancer Center Research Institute. Before imputation, a sample quality control was performed using PLINK v1.90 (https://www.cog-genomics.org/plink2, in the public domain) and EIGENSOFT 6.0.1 (https://www.hsph.harvard.edu/alkes-price/software, provided by Harvard T. H. Chan, Boston, MA, USA) to exclude cases with a call rate < 98%, duplicate or closely related pairs of samples (pairs of individuals with a relatedness measure [\( \hat{p} \text{-hat} \] > 0.1875) detected by identity-by-descent (IBD) analysis, and cases regarded as the outlier in a principal component analysis with 1000 Genomes Project phase 3. Among 542,585 SNPs that were genotyped with the array, SNPs with a low call rate (<98%), \( P \) value of Hardy-Weinberg equilibrium test of \( <1.0 \times 10^{-6} \) in controls, MAF of \( <0.01 \), and a departure from the allele frequency computed from the 1000 Genomes Project phase 3 EAS samples were excluded. Finally, 248,185 SNPs were selected for imputation. Prephasing and imputation were performed using SHAPEIT2 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html, in the public domain) and Minimac3 (https://genome.sph.umich.edu/wiki/Minimac3, in the public domain), respectively. Postimputation quality control was performed by excluding SNPs with insufficient imputation quality score (IQS) \( r^2 < 0.7 \). Genome build information in the present study was GRCh37/hg19 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13, in the public domain).

Statistical Analysis

All statistical analyses in the present study were performed using PLINK. The association of SNPs with CSC in the discovery stage was tested by the CATT with no adjustment. The genomic inflation factor \( \lambda \) was calculated using all of the tested SNPs in the GWAS. In the replication study, we compared allele frequencies in cases and controls by CATT, in which \( P \) values < 0.05 were considered statistically significant. The Haploviz software version 4.2 (https://www.broadinstitute.org/haploviz/haploviz, provided by the Broad Institute, Cambridge, MA, USA) was used to draw the Manhattan plot.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a blood DNA kit (QIAGEN, Hilden, Germany).
Prediction of Functional Annotation of SNPs

The prediction of functional annotation of SNPs was performed using the SNPinfo web server (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html; provided by the National Institutes of Health, Bethesda, MD, USA) and SNPnexus web server (http://snp-nexus.org/; provided by Barts Cancer Institute, London, UK) based on the HapMap database. The regulatory potential of each SNP was also referred to on the LDlink web server (https://ldlink.nci.nih.gov/?tab¼home, provided by the National Institutes of Health). The Human Genetic Variation database (http://www.hgvd.genome.med.kyoto-u.ac.jp/; provided by Kyoto University, Kyoto, Japan) was referred to for detecting cis- and trans-expression quantitative trait locus (eQTL) associated with candidate SNPs.

RESULTS

In the discovery stage, multidimensional-scaling (MDS) analysis was performed using PLINK and MDS plot was generated using R, which showed no remarkable population substructure (Supplementary Fig. S1), and the quantile–quantile plot showed the genomic inflation factor \( k \) to be 1.031, suggesting a minimal impact of population stratification (Supplementary Figure S2). We also performed IBD analysis to evaluate the relatedness by selecting a set of SNPs by LD-based SNP pruning using PLINK, and no pair of individuals with pi-hat value >0.25 calculating by PLINK was included in the cohort analyzed. After the quality control, 10 SNPs reached a genome-wide significance level \( (P < 5 \times 10^{-8}) \) in the nominal data (Supplementary Table S1; Fig. 1). When setting a suggestive significance threshold of \( P \) values < 5 \( \times 10^{-6} \), 24 SNPs were identified in the discovery stage. After a visual inspection for those SNPs, 11 SNPs were excluded from further analysis. In addition, five SNPs were excluded due to unavailability of TaqMan probe or insufficient imputation quality (IQS < 0.7) in the control data set. The remaining eight SNPs were subjected to the replication study, and rs11865049 located at SLC7A5 in chromosome 16q24.2 (nominal \( P = 0.006907 \)) was found to be significantly associated with CSC after Bonferroni correction (Table 2). When we combined the discovery and replication results using meta-analysis, rs11865049 achieved genome-wide significance level \( (P = 9.71 \times 10^{-9}, \text{odds ratio [OR]} = 2.10, 95\% \text{confidence interval [95\%CI]} 1.61–2.67) \) (Table 3).

The regional association plots for the SLC7A5 region show SNPs in high linkage disequilibrium with rs11865049 (Fig. 2).

The functional annotation for rs11865049 was searched in the SNPinfo web server and regulatory potential scores for the SNPs in noncoding region were indicated as 0.061. The SNPnexus web server indicated that the functional annotation of SNPs rs11865049 (771-61C > T) is indicated as an intronic SNP at the fourth intron of SLC7A5 gene. In addition, RegulomeDB, which is available at LDlink, indicates the

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**Table 2. Results of the Replication Study**

<table>
<thead>
<tr>
<th>SNP</th>
<th>ID</th>
<th>Gene</th>
<th>Allele</th>
<th>MAF</th>
<th>IQS for</th>
<th>Discovery</th>
<th>Replication</th>
<th>Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
<td>P Value</td>
<td>P Value</td>
<td>P Value</td>
</tr>
<tr>
<td>rs82612</td>
<td>10:119295929</td>
<td>EMX2OS</td>
<td>T</td>
<td>C</td>
<td>0.0963</td>
<td>0.06949</td>
<td>0.82895</td>
<td>0.02485</td>
</tr>
<tr>
<td>rs6487782</td>
<td>12:29354268</td>
<td>FAR2</td>
<td>C</td>
<td>T</td>
<td>0.4481</td>
<td>0.4817</td>
<td>0.86836</td>
<td>0.0363</td>
</tr>
<tr>
<td>rs11050120</td>
<td>12:29356078</td>
<td>FAR2</td>
<td>T</td>
<td>C</td>
<td>0.4836</td>
<td>0.4541</td>
<td>0.86296</td>
<td>0.0278</td>
</tr>
<tr>
<td>rs931166</td>
<td>12:29356263</td>
<td>FAR2</td>
<td>C</td>
<td>T</td>
<td>0.4563</td>
<td>0.4812</td>
<td>0.85857</td>
<td>0.0361</td>
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<tr>
<td>rs2270352</td>
<td>16:87870673</td>
<td>SLC7A5</td>
<td>G</td>
<td>A</td>
<td>0.1038</td>
<td>0.0942</td>
<td>0.77281</td>
<td>0.04045</td>
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<td>rs11865049</td>
<td>16:87874140</td>
<td>SLC7A5</td>
<td>G</td>
<td>A</td>
<td>0.1066</td>
<td>0.06857</td>
<td>0.85089</td>
<td>1.18E-08</td>
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<td>rs11117306</td>
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<td>SLC7A5</td>
<td>G</td>
<td>A</td>
<td>0.09563</td>
<td>0.09102</td>
<td>0.86868</td>
<td>0.05295</td>
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<td>rs6121611</td>
<td>20:61041906</td>
<td>GATA5</td>
<td>G</td>
<td>A</td>
<td>0.08743</td>
<td>0.09102</td>
<td>0.83176</td>
<td>0.0346</td>
</tr>
</tbody>
</table>

* Bonferroni correction for four LD blocks in the replication results. SNPs rs6487782, rs1105012, and rs4931166 are in high LD \( (r^2 > 0.9) \). SNPs rs2270352, rs11865049, and rs11117306 are in high LD \( (r^2 > 0.8) \).
regulatory potential score 4 (minimal binding evidence) for rs11865049. The Human Genetic Variation database available for cis- and trans-expression quantitative trait locus (eQTL) in the Japanese population indicates the strongest association of rs11865049 with SYT6 gene in chromosome 1p13.2 ($P = 1.43 \times 10^{-8}$).

We additionally examined the association of CFH variants with CSC in the present cohorts since we previously reported these variants to be associated with CSC using target gene approach.11 In the discovery stage, the association of rs1329428 and rs800292 showed nominal $P$ value $= 8.52 \times 10^{-4}$, OR = 1.534 and $P = 2.88 \times 10^{-3}$, OR = 1.462, respectively. The replication study confirmed the association of these SNPs (nominal $P = 4.66 \times 10^{-3}$, OR = 1.362 and $P = 1.88 \times 10^{-4}$, OR = 1.501, respectively). Meta-analysis which combined the results of two stages revealed a significant association of both SNPs with CSC ($P = 1.73 \times 10^{-5}$, OR = 1.432 and $P = 1.98 \times 10^{-6}$, OR = 1.484, respectively).

### DISCUSSION

In the present study, we conducted a two-stage GWAS for idiopathic CSC in the Japanese population and found that SLC7A5 might be among the potential candidate genes associated with CSC.

SLC7A5 consists of 11 exons that code large neutral amino acid transporter small subunit 1 (LAT1), one of the major System L amino acid transporters that mediate the transport of large neutral amino acids with branched or aromatic side chains in a Na$^+$-independent manner.18 LAT1 is predominantly expressed in brain, placenta, and testis.19 In the eye, LAT1 is expressed in the retinal pigment epithelium (RPE),20,21 retinal vascular endothelial cells,22–26 Müller cells,27 and ciliary nonpigmented epithelium.28 In polarized epithelial and endothelial cells, LAT1 is considered to play an important role in transportation of various neutral amino acids at the basolateral plasma membrane.29 LAT1 is also an exchanger and can exchange intracellular glutamine for external large neutral amino acids.30

### Table 3. Summary of the GWAS, Replication, and Meta-Analysis

<table>
<thead>
<tr>
<th>Chr</th>
<th>Chr Pos.</th>
<th>SNP ID</th>
<th>Major/Minor Allele</th>
<th>Study</th>
<th>No. of Samples</th>
<th>Meta-Analysis</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CSC</td>
<td>Control</td>
</tr>
<tr>
<td>16</td>
<td>87840554</td>
<td>rs11865049</td>
<td>G/A</td>
<td>GWAS</td>
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<td>1174</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Replication</td>
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<td>2071</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>320</td>
<td>3245</td>
</tr>
</tbody>
</table>

Chr., chromosome; Chr Pos., chromosome position.
A recent GWAS with European cohorts demonstrated the most significant association of rs1329428 with chronic CSC. The authors found that some genes involved in the complement system are also significantly associated with CSC, but failed to detect significant association of SLC7A5 with CSC. This might due to the difference in race since MAF of rs11865049 in Europeans is 0.0310 in the HapMap database and 0.0457 in Asians. Since our study indicated that OR for the variant rs11865049 was 2.10 in the meta-analysis, it might be a reason why CSC is more prevalent in Asians than in Caucasians. A recent GWAS study revealed that CFH variants are associated with thickened choroid, which is often observed in CSC. Although it remains to be concluded whether CSC is a disorder of choroid origin or RPE origin, the present study suggests that some complex mechanisms may underlie the pathogenesis of CSC.

With respect to the limitations of this study, we used imputed data in the replication study, which might cause false-positive or false-negative-results despite high IQS. The sample size might not provide sufficient power to find more SNPs possibly associated with CSC. Because this was a study of a single race, replication studies with cohorts from other races are anticipated. In addition, DNA sequencing or imputation related to the SNP identified was not performed. Hence, it is possible for SNPs that were not genotyped to be associated with CSC, which could be another limitation of this study.


