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 $P = 9.71 \times 10^{-9}$, 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 suscep-

Table 1. Description of the Cohorts

Cohorts	Discovery CSC	Discovery Control	Replication CSC	Replication Control
No. of samples	137	1174	183	2071
Sex, male/female	114/23	877/297	152/31	1050/1021
Age, mean ± SD	49.4 ± 10.6	63.1 ± 6.4	51.4 ± 11.1	51.9 ± 11.1

tibility genes associated with CSC to date. In the present study, we conducted a two-stage GWAS for idiopathic CSC in the Japanese population using a total of 320 unrelated idiopathic CSC cases and 3245 population-based controls.

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. 853), 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. Subjects who had received any corticosteroid therapy or showed central choroidal thickness less than 250 μm were excluded. In addition, patients aged over 80 years were excluded to diminish the possibility of age-related macular degenerations. Patients with past histories of retinal vessel occlusion or uveitis were also excluded.

In the discovery stage, 137 individuals with idiopathic CSC recruited at Kobe University Hospital were subjected to the GWAS. Control subjects in the GWAS consisted of 1174 population-based volunteers recruited by Kyushu University. Since it was not clear whether this control cohort included any patients suffering from CSC or other ocular diseases, possible biases might exist in the analysis. Because such biases likely underestimate the association of candidate SNPs with CSC, the true associations might be stronger than those indicated in the present study. In the replication study, 183 individuals with idiopathic CSC were newly recruited at Kobe University, Yamanashi University, Nihon University, and Tokushima University under the same criteria of diagnosis. An imputation data set for 2071 population-based volunteers recruited by the Aichi Cancer Center Research Institute were used as control for the replication study. Then, the results of the discovery and replication stages were combined to conduct the meta-analysis.

The baseline characteristics of participants are presented in Table 1.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a blood DNA kit (QIAGEN, Hilden, Germany). 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 [pi-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 λ 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 Haploview software version 4.2 (https://www.broadinstitute.org/haploview/haploview, provided by the Broad Institute, Cambridge, MA, USA) was used to draw the Manhattan plot.

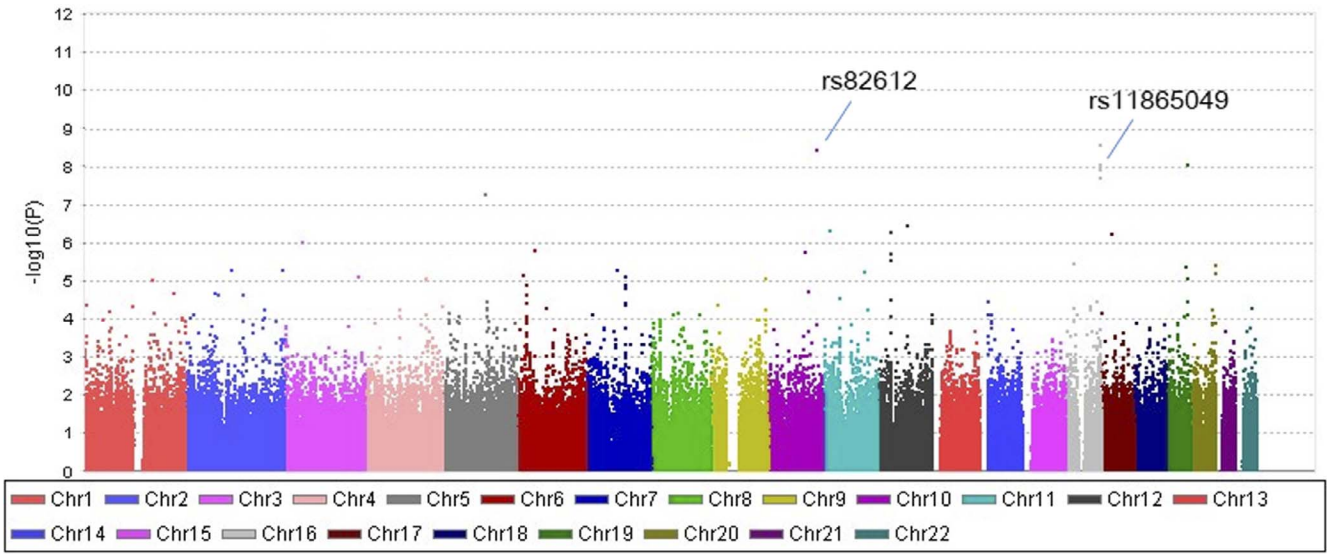

Figure 1. Manhattan plot of the GWAS for CSC. Each plot shows log10-transformed *P* values for all SNPs.

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/?tab=home, 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 λ 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 ($P = 9.71 \times 10^{-9}$, odds ratio [OR] = 2.10, 95% 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

Table 2. Results of the Replication Study

SNP	ID	Gene	Allele		MAF		IQS for Control	Discovery *P* Value	Replication *P* Value	Corrected *P* Value*	OR
			Major	Minor	Case	Control					
rs82612	10:119295929	*EMX2OS*	T	C	0.09563	0.06494	0.82895	3.53E-09	0.02485	0.0994	1.522
rs6487782	12:29354268	*FAR2*	C	T	0.4481	0.4817	0.86836	5.04E-07	0.2179	0.8716	0.874
rs11050120	12:29356078	*FAR2*	T	C	0.4836	0.4541	0.86296	1.83E-06	0.2778	1	1.126
rs4931166	12:29356263	*FAR2*	C	T	0.4563	0.4812	0.85857	5.04E-07	0.3610	1	0.905
rs2270352	16:87870673	*SLC7A5*	G	A	0.1038	0.07412	0.77281	8.75E-09	0.04045	0.1618	1.447
rs11865049	16:87874140	*SLC7A5*	G	A	0.1066	0.06857	0.85089	1.18E-08	0.006907	0.02763	1.620
rs11117306	16:87878268	*SLC7A5*	G	A	0.09563	0.06857	0.86868	2.67E-09	0.05295	0.2118	1.436
rs6121611	20:61041906	*GATA5*	G	A	0.08743	0.09102	0.83176	3.94E-06	0.8188	1	0.957

* 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 ($r^2 > 0.8$).

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

Chr.	Chr Pos.	SNP ID	Major/Minor Allele	Study	No. of Samples		Meta-Analysis		
					CSC	Control	OR	95%CI	*P* value
16	87840534	rs11865049	G/A	GWAS	137	1174	2.78	1.94–4.00	
				Replication	183	2071	1.62	1.14–2.31	
				Combined	320	3245	2.10	1.62–2.67	9.71×10^{-9}

Chr., chromosome; Chr Pos., chromosome position.

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 = 8.52×10^{-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

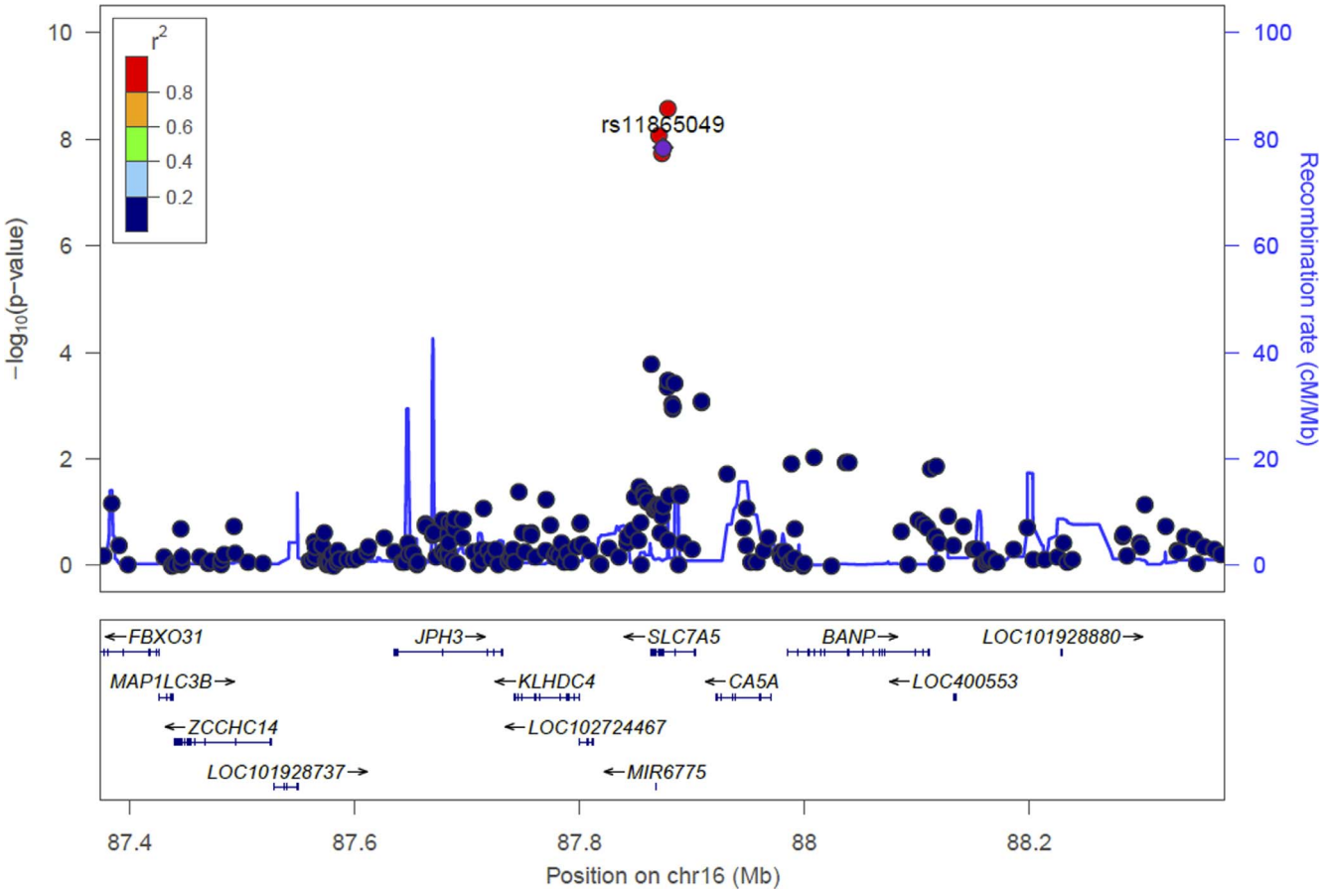

Figure 2. Regional association plots for the *SLC7A5* region. Regional association plots show SNPs in high linkage disequilibrium with rs11865049 indicated in *red*.

amino acids.[30] This transportation of large amino acids through basolateral membrane may move water from the apical side to the basal side of the RPE cells. Since CSC is characterized by an accumulation of fluid in the subretinal space, which is the apical side of RPE, abnormality in the transporting system associated with LAT1 may be involved in the pathogenesis of CSC.

It is unknown whether or not rs11865049 influences the expression or the function of LAT1 since no previous report was found regarding the association of this SNP with the state of LAT1 to date. However, we speculate that some unidentified effects could exist with this SNP. It is interesting that rs11865049 is located 61 base pairs ahead to the next exon, which might affect splicing or integrity of the transcript of *SLC7A5* gene. The cis- and trans-eQTL may provide useful information associated with this SNP, which might be useful to understand the pathogenesis of CSC. In the present study, *SYT6* gene was likely associated with rs11865049 in trans-eQTL. Synaptotagmin 6 coded by *SYT6* gene is involved in calcium-dependent exocytosis of synaptic vesicles. This protein has been shown to be a key component of the secretory machinery involved in acrosomal exocytosis (https://www.ncbi.nlm.nih.gov/gene/148281; provided by the National Institutes of Health), which may be involved in the pathogenesis of CSC. Although no definite information about the functional effects of rs11865049 is available using current SNP assessment programs, further studies would premise the disclosure of functional effects of this SNP on the characteristics of CSC. In addition, rs2270352, rs11865049, and rs11117306 form a haplotype block in high LD ($r^2 > 0.8$) while only rs11865049 was determined to be significant in the replication stage of this study. This may be due to insufficient statistical power in this study, and a larger sample size could disclose the association of this locus with CSC in more detail.

In the meantime, we have confirmed the significant association of rs1329428 and rs800292 in *CFH* gene, though not genome-wide significance level, with CSC in this study. A recent GWAS with European cohorts demonstrated the most significant association of rs1329428 with chronic CSC.[31] 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 be due to the difference in race since MAF of rs11865049 in Europeans is 0.0310 in the HapMap database and 0.0457 in the 1000 Genomes database, which was less than in Asian cohorts including Japanese. Since our study indicated the that OR for the variant rs11865049 was 2.10 in the meta-analysis, the *P* value of this variant was below a suggestive significance threshold according to the power analysis in a previous report.[31] In addition, it might be a reason why CSC is more prevalent in Asians than in Caucasians.[1,32,33] A recent GWAS study revealed that *CFH* variants are associated with thickened choroid, which is often observed in CSC.[34] 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. However, this is the first report of a GWAS identifying a new potential variant and susceptibility gene associated with idiopathic CSC in a Japanese cohort, which likely contributes to the understanding of the pathogenesis of this disease.

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