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ORIGINAL RESEARCH

The *NCAN* gene: schizophrenia susceptibility and cognitive dysfunction

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Background: Cognitive dysfunction has been recognized as a cardinal feature of schizophrenia. Elucidating the neurobiological substrates of cognitive dysfunction in schizophrenia would help identify the underlying mechanism of this disorder. The rs1064395 single nucleotide polymorphism, within the gene encoding neurocan (*NCAN*), is reported to be associated with schizophrenia in European populations and may influence brain structure in patients with schizophrenia.

Methods: In this study, we aimed to explore whether *NCAN* rs1064395 confers some risk for schizophrenia and cognitive dysfunction in Han Chinese. We recruited 681 patients with schizophrenia and 699 healthy subjects. Two hundred and fifty-four patients were evaluated according to Repeatable Battery for the Assessment of Neuropsychological Status (RBANS).

Results: There were no significant differences in genotype or allele distributions of the rs1064395 polymorphism between the schizophrenia and control groups. Patients showed significantly poorer performance than controls on immediate memory, visuospatial skill, language, attention, delayed memory, and total RBANS score. Patients with the A/A or A/G genotype of rs1064395 had lower scores of immediate memory, visuospatial skill, attention, and total RBANS score than those with the G/G genotype. We performed an expression quantitative trait loci analysis and observed a significant association between rs1064395 and *NCAN* expression in the frontal (P=0.0022, P=0.022 after Bonferroni correction) and cerebellar cortex (P=0.0032, P=0.032 after Bonferroni correction).

Conclusion: Our findings indicate that this single nucleotide polymorphism may be a risk factor for cognitive dysfunction in patients with schizophrenia. Further investigations are warranted for validation purposes and to identify the precise mechanism by which rs1064395 influences cognitive performance in patients with schizophrenia.

Keywords: *NCAN*, schizophrenia, cognitive function, polymorphism, eQTL

Introduction

Schizophrenia is a severe and chronic psychiatric disorder that affects ~1% of the population worldwide. Although its pathophysiological mechanism remains unclear, there is compelling evidence from family, twin and adoption studies supporting the involvement of a genetic predisposition to schizophrenia, with an estimated heritability up to 80%. While multiple susceptibility loci for schizophrenia diagnosis have been identified, only few have been widely replicated. One suggestion for such a dilemma is that novel phenotypes be defined to reflect neurobiological processes in schizophrenia.[1]

Cognitive dysfunction is recognized as a cardinal feature of schizophrenia that implicates functional outcome.[2,3] There is a realm of evidence showing that patients with schizophrenia exhibit impairment across a range of cognitive domains, such as

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working memory, language function, executive function, episodic memory, processing speed, attention, inhibition, and sensory processing.[4] The neurodevelopmental hypothesis of schizophrenia is therefore based, at least in part, on the presence of cognitive dysfunction in schizophrenic patients.[5] Therefore, elucidating the neurobiological substrates of cognitive dysfunction in schizophrenia would help to identify the underlying mechanism of this disorder.

Neurocan is a member of the lectican/chondroitin sulfate proteoglycan protein family and consists of a neurocan core protein and chondroitin sulfate. Neurocan is an important constituent of the brain extracellular matrix that is involved in neural crest cell migration, neurite outgrowth, neuronal cell adhesion, and synaptic plasticity.[6] The gene encoding neurocan (*NCAN*) is located on chromosome 19p13 and has been reported to be a genetic susceptibility region for schizophrenia.[7] A recent meta-analysis employed three independent studies and showed a single nucleotide polymorphism (SNP), rs1064395, in the 3′-untranslated region (3′-UTR) of the *NCAN* gene that is significantly associated with schizophrenia in European populations.[8] To describe the effect of rs1064395 on brain structure, Schultz et al[9] performed an imaging genetics study and found an implication of this SNP with increased cortical folding (a putative marker of increased neuronal efficiency) in the occipital and prefrontal cortex, but not in healthy subjects, which may establish schizophrenia susceptibility during neurodevelopment. Taken together, the *NCAN* rs1064395 may be a functional polymorphism that influences the brain structure that results in neurodevelopmental risk for schizophrenia.

To the best of our knowledge, no genetic study to date has addressed the association between *NCAN* and schizophrenia in Han Chinese. Building on this premise, we aimed to explore whether *NCAN* rs1064395 confers some risk of susceptibility to schizophrenia among a Chinese Han population. Given the important neurobiological function of *NCAN*, we subsequently aimed to determine whether the rs1064395 polymorphism influences cognitive function in patients with schizophrenia.

Methods

Subjects

We recruited 681 patients with schizophrenia from four mental hospitals in Eastern China, including Shanghai Mental Health Center, Tongde Hospital of Zhejiang Province, Jinhua Second Hospital, and Wenzhou Kangning Hospital. All patients met the diagnoses of schizophrenia according to the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (*DSM*-IV) and had no other observable physical disease or other psychiatric disorder aside from schizophrenia. Among them, 254 schizophrenic patients were used to test cognitive function, whose inclusion criteria were based on our previous publications:[10,11] 1) duration of illness <5 years; 2) has a minimum education of primary middle school; 3) receives atypical antipsychotic monotherapy; 4) has maintained a stable condition for >6 months before entry into the study; and 5) has a positive and negative syndrome scale total score <60.

Six hundred and ninety-nine healthy controls were recruited from hospital staff and students of School of Medicine in Shanghai, and then interviewed by a specialized psychiatrist using the *Structured Clinical Interview for DSM-IV-TR Axis I Disorders* – Patient Edition. Aside from the controls enrolled for the genetic analysis, we recruited 72 healthy subjects undergoing cognitive evaluation from the Shanghai region.

All procedures for this study were reviewed and approved by the Institutional Review Boards of Shanghai Mental Health Center and other participating institutions. This study was performed in strict accordance with the Declaration of Helsinki and other relevant national and international regulations. Written informed consent was obtained from each participant prior to any procedures related to this study being performed.

Evaluation

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was the primary outcome instrument used in this study.[12] The 12-item RBANS consists of five subsets, corresponding to five domains of the neuropsychological process: 1) immediate memory (list learning and story memory); 2) visuospatial/constructional (figure copy and line orientation); 3) language (picture naming and semantic fluency); 4) attention (digit span and coding); and 5) delayed memory (list learning free recall, list learning recognition, story memory free recall, and figure free recall). RBANS has good validity and reliability in Chinese people[13] and works well in genetic studies on schizophrenia.[14–16]

Genotyping

Genomic DNA from all participants was extracted from peripheral blood using a Tiangen DNA Isolation Kit (Tiangen Biotech, Beijing, People's Republic of China). Genotyping of the rs1064395 polymorphism was carried out according to the methods described in our previous studies.[17,18] Table S1 details the primer information. Briefly, polymerase chain

reaction (PCR) amplification was performed in a volume of 25 μL containing one primer pair for each SNP. PCR primers were also used for sequencing. Sequencing results were analyzed using DNAStar package (DNAStar Inc., Madison, WI, USA), and the original sequencing chromatograms of each sample were checked manually.

Psychiatric genomics consortium data analysis

To validate the association between the rs1064395 polymorphism and schizophrenia, we extracted schizophrenia genetic association data from the Psychiatric Genomics Consortium (PGC, http://www.broadinstitute.org/mpg/ricopili/) database[19] and reanalyzed the data set as an independent sample.

Brain expression quantitative trait loci analysis

Schizophrenia originates from structural and functional brain abnormalities,[20] and brain samples are presumably appropriate for expression quantitative trait loci (eQTL) analysis of risk SNP(s).[21,22] Here, we performed an eQTL analysis to determine whether the rs1064395 polymorphism is associated with *NCAN* expression in the brain using the brain eQTL database (http://caprica.genetics.kcl.ac.uk/BRAINEAC/), a large exon-specific eQTL data set covering ten human brain regions. More detailed information can be found in the original study.[23]

Statistical analysis

Demographic variables between case and control groups were compared by use of independent *t*-tests for quantitative variables and Fisher's exact test for qualitative variables. Analysis of covariance (ANCOVA) was used to compare RBANS scores between case and control groups, controlling for demographic characteristics. Hardy–Weinberg equilibrium testing, and allele and genotype frequency analyses were conducted using SHEsis (http://analysis.bio-x.cn).[24] SNPStats (http://bioinfo.iconcologia.net/snpstats/start.htm) was used to examine the association between rs1064395 and schizophrenia under four inheritance models, including codominant, dominant, recessive, and log-additive models.[25] The possible effects of the rs1064395 genotypes on cognitive function were examined using ANCOVA by comparing the mean RBANS scores of each genotype. Variables that affect cognitive parameters (ie, age, gender, education, and duration of illness) were included as covariates. Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All *P*-values were two-tailed, and those <0.05 were considered statistically significant after Bonferroni correction.

Results

The genotypic distribution of the rs1064395 polymorphism in the control group was in accordance with Hardy–Weinberg equilibrium. The results from single marker analysis are shown in Table 1. There were no significant differences in genotype or allele distributions of the rs1064395 polymorphism between the schizophrenia and control groups. Unconditional logistic regression was performed to further detect an association between rs1064395 and schizophrenia after adjusting for age and gender. We did not observe any significant association under four inheritance models (Table S2). Next, we extracted the schizophrenia genetic association data from the PGC database and observed a significant association between rs1064395 and schizophrenia (P=0.000256) (Figure S1).

Demographic characteristics of the cognitive evaluation samples from the schizophrenia and control groups are summarized in Table S3. There were significant differences in terms of sex and years of education between the two groups (Ps<0.01). Consequently, we used these parameters as covariates to compare the RBANS scores between groups. Table S4 shows that patients had significantly poorer performance than controls on immediate memory, visuospatial skill, language, attention, delayed memory, and total RBANS score. We then examined whether rs1064395 plays an important role in cognitive dysfunction in patients with schizophrenia. ANCOVA was carried out with the rs1064395 genotypes (A/A + A/G vs G/G) as independent variables, the cognitive scores (RBANS total score and five index scores) as dependent variables, and age, gender, years of education, and duration

Table 1 Distribution of rs1064395 genotype and allele in schizophrenia patients and controls

SNP rs1064395	N	Genotype, n (%)			*P*-value	Allele, n (%)		*P*-value	OR (95% CI)	*P*-value[a]
		A/A	G/A	G/G		A	G			
Case	681	21 (3.1)	163 (23.9)	497 (73.0)	0.21	205 (15.1)	1,157 (84.9)	0.078	1.21 (0.98–1.51)	
Control	699	14 (2.0)	150 (21.5)	535 (76.5)		178 (12.7)	1,220 (87.3)			0.36

Note: [a]Hardy–Weinberg *P*-values in the control group.
Abbreviation: OR, odds ratio.

Table 2 RBANS performance comparisons of rs1064395 genotypic groups in patients with schizophrenia

RBANS	A/A + G/A (n=71)	G/G (n=183)	*F*-value[a]	*P*-value[b]	*P*-value[c]
Total score	291.45±40.11	320.42±30.91	46.03	<0.01	<0.01
Immediate memory	49.52±14.40	64.39±7.60	114.88	<0.01	<0.01
Visuospatial skill	56.58±8.85	59.49±5.66	12.10	0.001	0.006
Language	55.70±5.50	55.44±4.53	0.12	0.73	
Attention	64.37±18.99	73.19±18.09	14.52	<0.01	<0.01
Delayed memory	65.28±11.74	67.90±10.35	5.35	0.02	0.12

Notes: Data presented as $\bar{x} \pm s$. [a]*F*-values adjusted for age, gender, years of education, and duration of illness. [b]*P*-values not corrected for multiple testing. [c]*P*-values corrected after Bonferroni correction.
Abbreviation: RBANS, repeatable battery for the assessment of neuropsychological status.

of illness as covariates. Table 2 shows that patients with the A/A or A/G genotype of rs1064395 have lower scores of immediate memory, visuospatial skill, attention, and total RBANS score than those with the G/G genotype.

We then performed an eQTL analysis to investigate whether rs1064395 influences *NCAN* expression in the brain. As shown in Figure 1, we observed a significant association between rs1064395 and *NCAN* expression in the frontal cortex (P=0.0022, P=0.022 after Bonferroni correction) and cerebellar cortex (P=0.0032, P=0.032 after Bonferroni correction). Data show that carriers of the A allele have significantly higher levels of *NCAN* expression in the frontal and cerebellar cortex than those without the A allele.

Discussion

Cognitive dysfunction has been widely reported to stably present in either patients with first-onset schizophrenia or those with chronic situation.[26] Considerable evidence indicates that cognitive dysfunction is a core feature of schizophrenia, which can also serve as an endophenotype for the illness in genetic studies.[27,28] In our previous work, we found that genes involved in neurodevelopment, such as brain-derived neurotrophic factor and ankyrin 3, have modulatory effects on executive function and working memory in patients with first onset schizophrenia.[5,29] The gene and cognitive dysfunction associations strengthen the neurodevelopmental hypothesis of schizophrenia. A risk conferring allele A at locus rs1064395 within *NCAN* has been reported in the association with neurodevelopment, as well as the development of schizophrenia. The present study aimed to determine the role of the *NCAN* gene in shaping the developmental trajectories leading to schizophrenia.

NCAN rs1064395 has been reported to be a common risk factor for schizophrenia in Europeans.[30,31] Here, we further confirmed rs1064395 as a risk locus for schizophrenia by utilizing a large-scale schizophrenia sample of European ancestry from PGC genome-wide association study. In the Han Chinese samples tested in this study, we failed to detect significance for rs1064395 in the susceptibility to schizophrenia. This suggests that rs1064395 is probably not a risk SNP for schizophrenia in Han Chinese. The A allele of rs1064395 in our control subjects was 12.7%,

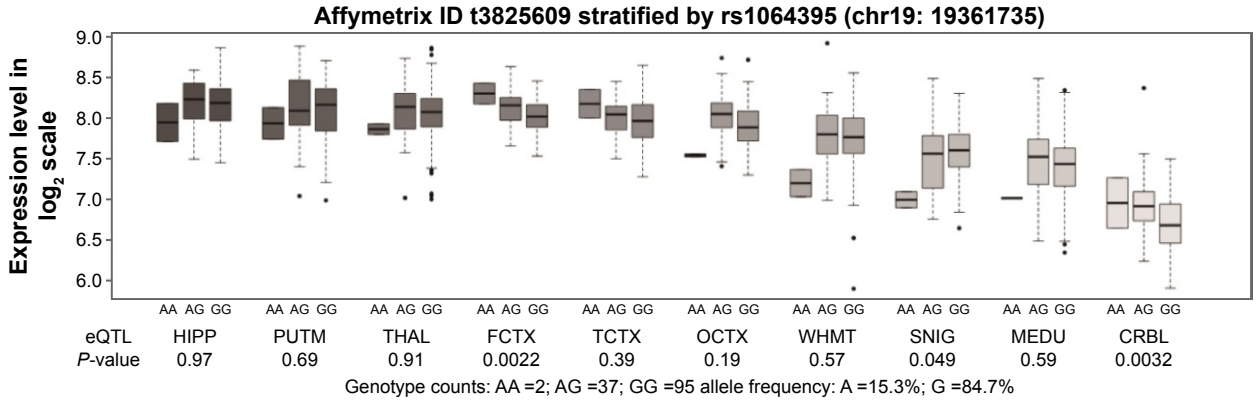

Figure 1 Association of rs1064395 with the *NCAN* mRNA expression level in ten brain regions (Affymetrix ID t3825609).
Abbreviations: CRBL, cerebellar cortex; FCTX, frontal cortex; HIPP, hippocampus; MEDU, the inferior olivary nucleus (sub-dissected from the medulla); OCTX, occipital cortex; PUTM, putamen (at the level of the anterior commissure); SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus (at the level of the lateral geniculate nucleus); WHMT, intralobular white matter; Data were extracted from the BRAINEAC database (http://caprica.genetics.kcl.ac.uk/BRAINEAC/). Ramasamy A, Trabzuni D, Guelfi S, et al. Genetic variability in the regulation of gene expression in ten regions of the human brain. *Nat Neurosci.* 2014;17(10):1418–1428.[23]

which is similar to the frequency in Han Chinese from the HapMap database (12.8%). We noticed that the frequency of the rs1064395 A allele in Europeans is reported to range from 14.7% to 15.2%.[30,31] The inconsistent association of rs1064395 with schizophrenia between Chinese and European populations implies genetic heterogeneity of *NCAN* sequence variations across continental populations. As such, the population-specific factors, such as environmental exposure, diet, differences in genetic structure, population history, and culture, are likely to play an important role in the observed inconsistent replications.[32]

A recent neuroimaging study reported that *NCAN* risk status (carried with the A allele of rs1064395) is associated with higher folding in the right lateral occipital region and at a trend level for the left dorsolateral prefrontal cortex in patients with schizophrenia, but not in matched control subjects,[9] implying that rs1064395 may be involved in cognitive disruption in schizophrenia. In this study, we found that schizophrenic patients with the A allele of *NCAN* rs1064395 have poorer cognitive processes, such as immediate memory, visuospatial skill, and attention, compared with those without the A allele. This result is in agreement with a recent study reporting that rs1064395A allele carriers exhibit poorer immediate and delayed verbal memory performance than those with the G/G genotype, implying that this SNP influences both neural processing and cognitive performance.[33] We then carried out an eQTL analysis and found that rs1064395 is significantly associated with *NCAN* expression in the frontal and cerebellar cortex. Our findings suggest that *NCAN* rs1064395 may be a risk factor for the development of cognitive dysfunction in schizophrenia, whose regulatory effects are likely mediated via the frontal cortex and cerebellar cortex.

There is a large body of evidence from functional magnetic resonance imaging studies suggesting that cognitive deficits in patients with schizophrenia are associated with decreased activity of the frontal region, especially the dorsolateral prefrontal cortex, the ventrolateral prefrontal cortex, and the medial frontal cortex.[34,35] In contrast, neuroimaging studies have shown that cortical cerebellar and global cerebellar volumes are significantly reduced in patients with schizophrenia compared to healthy controls.[36] As the cerebellum represents at least 10% of the brain volume that contains 50% of its neurons,[37] it has been established that cerebellar cortical volume reduction is implicated in cognitive dysfunction in schizophrenia.[36] Moreover, the aberrant network connecting the frontal cortex and cerebellum is believed to be involved in the development of cognitive dysfunction,[38] because cerebellar stimulation dynamically influences the medial frontal cortex in mice and clinically alleviates cognitive dysfunction in patients with schizophrenia.[39,40] Both human and animal investigations suggest an important role for *NCAN* in the developing mammalian brain, and its expression is localized within the cognition-relevant brain regions, such as the cortex.[41] The rs1064395 SNP is located within the 3′-UTR of *NCAN*. While the effect of the 3′-UTR on gene expression or function is not well known, it is possible that variants in this region could produce changes in messenger RNA processing.[42,43] If accurate, rs1064395 may result in an alteration in *NCAN* expression, and by extension, account for the results we encountered in this study. However, no evidence supports the genuine effect of rs1064395 on *NCAN*, although some preliminary results indicate an effect on *NCAN* expression in the frontal cortex and cerebellum. Therefore, further research is required to verify this supposition.

When interpreting the results of this study, we would be remiss in not noting some limitations. First, the lack of a significant association between rs1064395 and schizophrenia may be caused by the modest sample size, possibly resulting in a type II error.[44] Accordingly, our findings should be considered only preliminary and will require further investigations for validation in independent samples. Second, this case–control association study has the potential for population stratification, although all participants were of Han Chinese origin and from Eastern China. Therefore, we could not completely exclude the possibility of a population structure effect in our sample. Third, although we found that rs1064395 influences cognitive performance in patients with schizophrenia, the genuine effect of rs1064395 on *NCAN* is unclear. As such, the possibility that the observed effect was driven by another variation within *NCAN* that is in linkage disequilibrium with rs1064395 could not be fully excluded.[45]

Conclusion

Although we failed to replicate the findings of an association between rs1064395 and schizophrenia in Han Chinese. Our results indicate that this SNP may be a risk factor for cognitive dysfunction in patients with schizophrenia. While intriguing, these findings are still only suggestive, and further investigations are warranted to validate our findings to identify the precise mechanism by which rs1064395 influences cognitive performance in patients with schizophrenia.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Primers for the genotyping of rs1064395

SNP ID	Primer	Primer sequences (5′>3′)
rs1064395	PCRU	AGCCCAGTGCACATACCCAGTC
	PCRL	GGGGAGGAAGGCAAGGTGAG
	SNP	$t(gact)_{16}$ AACACTGAGCATCTCTCTACAATATGAC

Notes: PCR amplification primers were marked by "PCRU" and "PCRL", whereas SNP-specific oligonucleotide primer was marked by "SNP". In the "$(gact)_n$", n means the number of "gact" repeats.
Abbreviations: PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Table S2 Logistic regression analysis of rs1064395 between schizophrenia and control groups

SNP	Inheritance model	OR (95% CI)	*P*-value
rs1064395	Codominant		
	G/A vs G/G	0.85 (0.66–1.10)	0.21
	A/A vs G/G	0.62 (0.31–1.23)	
	Dominant		
	G/A + A/A vs G/G	0.83 (0.65–1.05)	0.13
	Recessive		
	A/A vs G/G + G/A	0.64 (0.32–1.27)	0.2
	Log-additive		
	G/G vs G/A vs A/A	0.83 (0.67–1.03)	0.083

Note: Results adjusted by age and gender.
Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

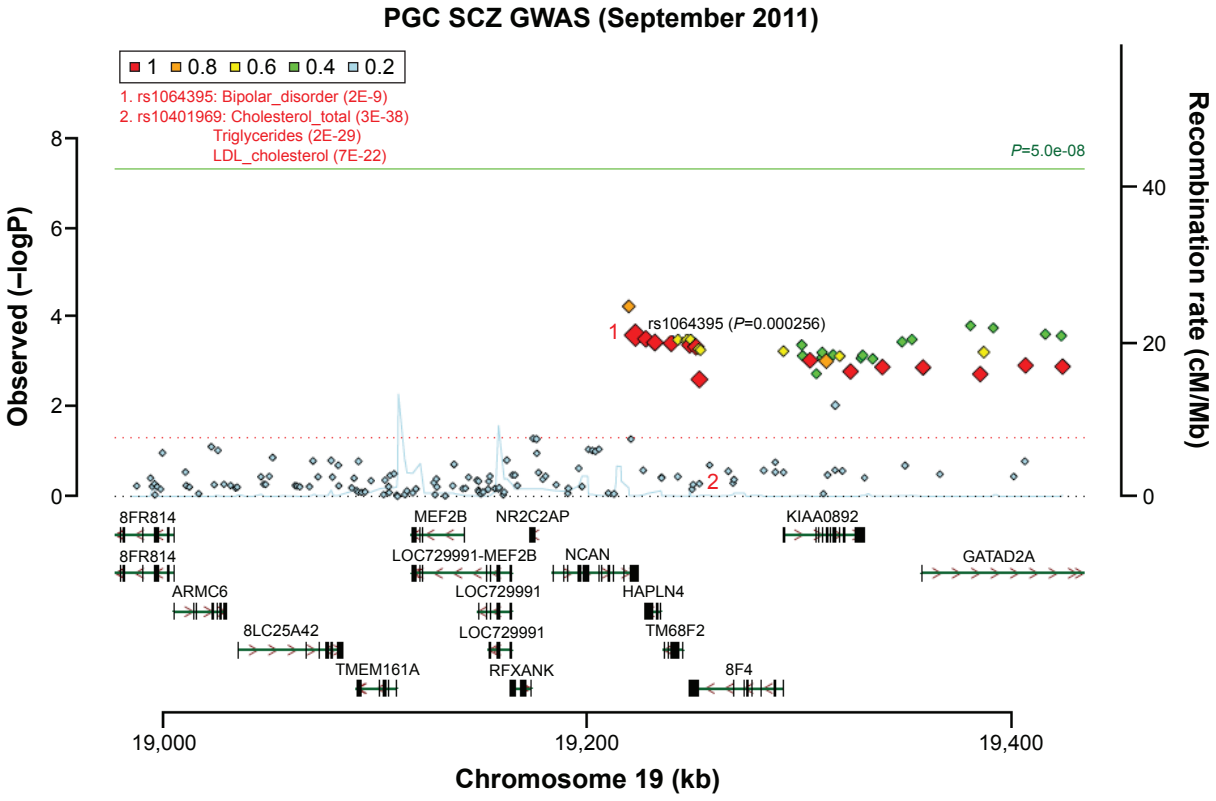

Figure S1 Association of rs1064395 with schizophrenia in PGC database.
Abbreviations: GWAS, genome-wide association study; PGC, psychiatric genomics consortium; SCZ, schizophrenia.

Table S3 Demographic and clinical characteristics between schizophrenia and control groups

	Schizophrenia (n=254)	Controls (n=72)	Statistics	*P*-value
Sex			9.14	<0.01
Male	118	40		
Female	136	32		
Age (years)[a]	34.0±8.7	26.2±5.6	1.86	0.18
Years of education[a]	9.5±1.9	11.9±2.1	−9.39	<0.01
Age at onset (years)[a]	27.7±5.3			
Duration of illness (months)[a]	45.8±16.5			

Note: [a]Data presented as $\bar{x} \pm s$.

Table S4 RBANS scores between schizophrenia and control groups

RBANS		*F*-value	*P*-value
Total score		737.02	<0.01
Cases	312.32±36.09		
Controls	463.69±21.92		
Immediate memory		235.98	<0.01
Cases	60.24±11.99		
Controls	89.21±8.59		
Visuospatial skill		482.55	<0.01
Cases	58.68±6.82		
Controls	88.74±11.51		
Language		1,673.42	<0.01
Cases	55.52±4.81		
Controls	93.63±8.28		
Attention		84.45	<0.01
Cases	70.72±18.73		
Controls	98.74±12.01		
Delayed memory		234.13	<0.01
Cases	67.17±10.80		
Controls	93.39±5.82		

Note: Data presented as $\bar{x} \pm s$.
Abbreviation: RBANS, repeatable battery for the assessment of neuropsychological status.