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Relationship of common variants in *CHRNA5* with early-onset schizophrenia and executive function

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ABSTRACT

Altered cholinergic neural transmission is hypothesized to increase susceptibility to cognitive deficits in psychotic disorders such as schizophrenia (SCZ). The nicotinic acetylcholine receptor α5 subunit gene (*CHRNA5*) is reported to be associated with cognitive function in nicotine-dependent populations and SCZ in non-smoking SCZ patients. Nevertheless, it is still not clear whether the *CHRNA5* gene contributes to susceptibility to the cognitive deficits of SCZ without smoking. To further clarify the role of *CHRNA5*, we designed a two-stage, case-control study to examine the association between *CHRNA5* and SCZ and its clinical features adjusted for smoking status in early-onset SCZ patients. A total of 15 tag single nucleotide polymorphisms (SNPs) on *CHRNA5* were genotyped in the discovery stage, which included 485 early-onset SCZ patients and 1018 controls, and then, we replicated this association in a confirmatory population of 674 patients and 1886 controls. The rs16969968 SNP was identified as significantly associated with SCZ in both datasets. In addition, the severity of psychotic symptoms and cognitive deficits was assessed using the Positive and Negative Syndrome Scale (PANSS) and the Wisconsin Card Sorting Test (WCST). The rs16969968 SNP was associated with psychotic symptoms in patients and with cognitive function in patients and controls. Our results show that rs16969968 on *CHRNA5* is tightly linked to genetic susceptibility, psychotic symptoms and cognitive deficits in SCZ in an early-onset Chinese population, suggesting that *CHRNA5* may play an important role in the etiology of SCZ.

1. Introduction

Schizophrenia (SCZ) is a common and devastating mental disorder of unknown etiology that affects approximately 1% of the world population. The disease is mainly characterized by severe disruption in cognition and emotion, thought, and sense of self (Goghari et al., 2010). So far, previous etiological studies showed that both environmental factors and genetic factors play an important role in the onset and development of SCZ (Ujike et al., 2008). The heritability of SCZ was estimated to be approximately 80–85% by twin, adoption and family studies, which results from multiple loci with small effects (Cirulli and Goldstein, 2010). Cognitive impairment is one of the core symptoms of SCZ (Heinrichs and Zakzanis, 1998) and was associated with poor functional outcomes in these patients (Ahnallen et al., 2015). To date, the cholinergic system in the brain is considered to be the most likely pathway associated with cognitive function, in which neuronal nicotinic acetylcholine receptors (nAChRs) play an essential role (Haense et al., 2012; Ochoa and Lasaldedominicci, 2007). Clinical studies, behavioral studies, and electrophysiological studies have suggested that the decline in nAchR function is closely related to cognitive deficits (Kai et al., 2011; KH et al., 2013). Cognitive impairment is one of the core symptoms of SCZ (Heinrichs and Zakzanis, 1998) and was associated with poor functional outcomes in these patients (Ahnallen et al., 2015). Both the dopaminergic and the cholinergic system in the brain is closely related to cognitive function (Haense et al., 2012; Boggs et al., 2014), in which neuronal nicotinic acetylcholine receptors (nAChRs) can regulate both of these system (Ochoa and Lasaldedominicci, 2007). Moreover, several studies have demonstrated that nAChR agonists can treat SCZ-associated

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cognitive deficits (D'Souza and Markou, 2012), which further demonstrated that nAChR may be involved in cognitive impairment in SCZ. However, the clear molecular mechanism remains unknown.

Brain nAChRs are pentameric ligand-gated cation channels typically comprising alpha and beta subunit combinations, including α1–7, α9–10, β1–4, δ, ε and γ subunits (Kalantaridehaghi et al., 2015). Through a family-based association study in 117 Canadian families, researchers showed that the *CHRNA4* gene combined with the *CHRNB2* gene may be linked to SCZ (De et al., 2006). A number of studies have shown that the *CHRNA7* gene has a significant association with sensory-gated P50 defects and SCZ (Xu et al., 2001). *CHRNA5* is an accessory subunit co-expressed only with other alpha and beta subunits. When present, the α-5 subunits increase nAChR function (Gotti and Clementi, 2004). Previous studies have found that rs16969968 on the *CHRNA5* gene is closely related to smoking and nicotine dependence (Hartz et al., 2012; Janes et al., 2012). The missense variant rs16969968 changes the encoded amino acid sequence from aspartic acid (G allele) to asparagine (A allele) at position 398 (Asp398Asn) (Saccone et al., 2007). Evidence from GTEx data shows that rs16969968 affects the expression of *CHRNA5* in several brain tissues, which may contribute to dysfunction of cholinergic and dopamine signalling in SCZ. These results further prove that CHRNA5 may involve in the pathogenesis of the SCZ and cognition deficits. Recently, a case-control study of 313 SCZ patients and 525 controls has indicated that the rs16969968 SNP on *CHRNA5* was associated with smoking severity and SCZ in Caucasians and African Americans and, interestingly, also in nonsmokers in two ethnic groups (Hong et al., 2011). Nevertheless, it is still not clear whether the *CHRNA5* gene contributes to SCZ-smoking comorbidity or is directly associated with susceptibility to SCZ. In addition, researchers also found an association between variants of the nAChR gene cluster, *CHRNA5–CHRNA3–CHRNB4*, and cognition in Caucasian and African American nicotine-dependent populations (Winterer et al., 2010), but whether the *CHRNA5* gene is associated with the cognitive deficits in SCZ patients in the Han Chinese population remains unanswered.

Early-onset SCZ (EOS) refers to onset of psychotic symptoms before the age of 18 years. Epidemiological data show that EOS and adult-onset SCZ might share similar pathophysiologic features but that EOS may reflect a more severe form of the disorder associated with a greater genetic predisposition (Kumra and Charles, 2008). Hence, we postulated that if *CHRNA5* variation plays a significant role in SCZ, then a strong relationship between *CHRNA5* and SCZ should be present in EOS. Therefore, we conducted a two-stage, case-control association study using EOS samples from the Chinese Han population adjusted for smoking status to evaluate the role of *CHRNA5* in susceptibility to SCZ. In addition, gene expression data of *CHRNA5* in multiple human tissues were extracted from GTEx database (Lonsdale et al., 2013) for expression quantitative trait loci (eQTL) analyses.

2. Methods

2.1. Study subjects

A two-stage study design was applied in this study. A total of 1503 study subjects including 485 SCZ cases and 1018 healthy controls were recruited for the discovery stage. Another 2560 study subjects including 674 SCZ cases and 1886 healthy controls were enrolled for a replication study in which only significant SNPs in the discovery stage were analyzed. All SCZ patients were EOS, which was defined in the study as onset before 18 years as first manifestation of positive symptoms. All patients were recruited at the Xi'an Mental Health Center based on the following criteria: (a) receiving diagnosis strictly according to the DSM-IV by at least two experienced psychiatrists; (b) having onset before 18 years; (c) receiving antipsychotic treatment and maintaining a stable condition for more than three months before entry into the study; (d) not being first-episode SCZ given that initial diagnoses are often unreliable; and (e) having no substance-induced psychotic disorders, learning disabilities, head injuries, or other symptomatic psychoses except SCZ. All healthy controls were selected from local volunteers. Study subjects with a family history of mental illness in the previous three generations and with current or past evidence of psychoses were ruled out for the present study. In addition, subjects with a history of neurological disorder, history of head injury leading to loss of consciousness, alcohol or substance abuse or an intelligence quotient (IQ) < 70 were excluded from the study. All subjects were recruited from the city of Xi'an in Shaanxi Province. We excluded anyone not born in Xi'an or whose families were not born in Xi'an within the last three generations.

The general characteristics of all two-stage subjects were collected. Subjects in the replication stage completed the cognitive assessments using WCST, and SCZ clinical symptom assessments were performed in the patients using the PANSS. We only evaluated smoking status in the replication samples. Smokers were classified as the subjects who smoked for more than one year and were currently smoking, while non-smokers were defined by lifetime smoking of <30 cigarettes and currently not smoking. All smokers were prohibited from smoking for 6 h at least before tests. Ex-smokers were defined as smokers who had quit smoking for two years at least and were currently not smoking, and they were excluded from the study. Relevant data were obtained and are summarized in Table 1. This study was performed in accordance with the ethical guidelines of the Declaration of Helsinki (version 2002) and was approved by the Medical Ethics Committee of Xi'an Jiaotong University. Informed consent was obtained from all subjects.

2.2. SNP selection and genotyping

We extracted all SNPs with minor allele frequency (MAF) ≥ 0.01 within the region of the *CHRNA5* gene based on data from the 1000 Genomes Chinese Han Beijing population (CHB) database. Pairwise $r^2 \geq 0.8$ was used as the selection criteria for tag SNPs. A total of 15 tag SNPs was included in the subsequent analyses. General information about these 15 selected tag SNPs is summarized in Supplemental Table S1. Genomic DNA was extracted from peripheral blood leukocytes according to the manufacturer's protocol (Genomic DNA kit, Axygen Scientific Inc., California, USA). Genotyping was performed for all SNPs using the Sequenom Mass ARRAY RS1000 system (Sequenom, San Diego, California, USA). The results were processed using Typer Analyzer software (Sequenom), and genotype data were generated from the samples. To

Table 1
Basic characteristics of the SCZ patients in the association study.

Characteristics	Cases	Controls	*P*-value
Discovery stage			
No. of samples	485	1018	/
Gender (male/female)	296/189	616/402	0.866
Age (years), mean ± SD	31.73 ± 9.24	31.94 ± 9.02	0.677
Onset age (years), mean ± SD	14.56 ± 1.96	/	/
Replication stage			
No. of samples	674	1886	–
Gender (male/female)	425/249	1169/717	0.644
Age (years), mean ± SD	34.82 ± 10.19	35.71 ± 11.39	0.059
Onset age (years), mean ± SD	14.23 ± 1.93	/	/
Smoking history (yes/no)	214/460	584/1302	0.735
Total IQ, mean ± SD	88.14 ± 8.80	112.51 ± 8.74	<0.001
Perseverative errors, mean ± SD	31.55 ± 5.18	5.49 ± 2.20	<0.001
Non-perseverative errors, mean ± SD	38.80 ± 3.98	13.58 ± 2.60	<0.001
Total errors, mean ± SD	70.35 ± 7.16	19.07 ± 3.49	<0.001
Trials to compete first category, mean ± SD	68.22 ± 9.83	21.94 ± 4.45	<0.001
Positive subscore, mean ± SD	13.39 ± 4.62	/	/
Negative subscore, mean ± SD	17.93 ± 4.91	/	/
General subscore, mean ± SD	32.31 ± 7.67	/	/
Total score, mean ± SD	63.63 ± 10.42	/	/

SD: standard deviation; IQ: intelligence quotient.

ensure the accuracy of genotyping, we randomly chose 5% of our study subjects and repeated the genotyping process for those individuals. The concordance rate of this process was 100%, which indicated that the genotyping results in our study were reliable.

2.3. Cognitive test

The Wisconsin Card Sorting Test (WCST) is used for evaluate cognitive function, which has been employed in working memory and executive functions of prefrontal lobe activity. WCST has four stimulus cards and 128 response cards. The participants are told to match the response cards to stimulus cards (e.g., color, number or shape of items) during 15–20 min. The test scores include perseverative error, non-perseverative errors, total errors and trials to compete first category, which could be a measure of the important indicators of cognitive function.

2.4. Statistical methods

Statistical power for genetic association tests was determined with the GAS Power Calculator (http://csg.sph.umich.edu/abecasis/gas_power_calculator/). The main parameters are summarized in Supplemental Table S2. The results are shown in Supplemental Fig. S1. As we can see, our sample size provides adequate statistical power (80%) to detect a SNP with a relative risk ≥ 1.5. χ^2 tests were used with our selected SNPs to identify the potential genetic associations in the discovery stage. For the replication stage, logistic models were fitted to evaluate the genetic risk of significant SNPs in the discovery stage. Genetic association analyses were also conducted for the significant SNPs and SCZ PANSS scale scores using linear regression. Smoking status was included in both logistic and linear models as a covariate to adjust for this potential confounding factor. Stratification analyses were also performed to examine the effects of significant SNPs in smokers and non-smokers group respectively. In addition, the cognitive function of individuals with different genotypes at significant SNPs was also compared among the SCZ cases and controls. Linkage disequilibrium (LD) blocks based on data from the discovery stage were constructed, and haplotype-based analyses were performed using Plink (Chang et al., 2015). Bonferroni corrections were applied to address multiple comparisons.

2.5. Expression quantitative trait loci (eQTL) and bioinformatics analyses

We explored the potential functional consequences of significant SNPs using Polyphen2 (Adzhubei et al., 2010). In addition, eQTL data on significant SNPs from multiple human tissues were extracted from the GTEx database (Lonsdale et al., 2013). Gene expression levels of *CHRNA5* in multiple human tissues were compared among different genotype groups of SNP significantly associated with SCZ.

3. Results

3.1. General characteristics of the subjects

The distribution of demographic characteristics and potential confounding/risk factors in this study are summarized in Table 1. The mean age of onset among SCZ patients was approximately 14 years old both in the discovery and replication stages. No significant variation in age or sex was found between the SCZ patients and controls. A significant difference was found comparing the total intelligence quotient (IQ) in stage 2 (Table 1). Moreover, there was no significant difference in smoking status between the patients and controls ($P = 0.735$).

3.2. Genetic associations between rs16969968 and SCZ

All 15 SNPs in the discovery stage met Hardy-Weinberg equilibrium (HWE) with MAF > 0.01 (Supplemental Table S1). We observed a missense SNP, rs16969968 (OR = 2.09, $P = 0.0001$), to be significantly associated with SCZ between early-onset schizophrenic patients and controls after applying Bonferroni correction in the discovery stage (Table 2). Then, we performed single-SNP association analysis for this rs16969968 in the replication dataset (674 SCZ cases and 1886 controls). A significant result was also obtained for rs16969968, with an OR = 1.79 and $P = 0.0002$ (Table 2) in early-onset schizophrenic patients. Our results indicated that after adjusting for smoking status, the A allele of rs16969968 on *CHRNA5* was still a significant risk factor for EOS. Results of stratification analyses were summarized in Supplemental Table S3. Then, we examined the LD structure of the genotype data from 15 SNPs in the discovery dataset and identified a two-SNP LD block (rs601079-rs77487352) to be significantly associated with SCZ status (Supplemental Fig. S2).

3.3. Genetic association between SNPs and SCZ-related clinical symptoms

We found significant differences between early-onset schizophrenic patients and controls in total, positive, negative and general scores for different genotypes of rs16969968 (Table 3) using the PANSS. The A allele of rs16969968 was associated with increased positive symptom scores ($P < 1 \times 10^{-4}$) and negative symptom scores ($P < 1 \times 10^{-4}$). We also identified significant differences in cognitive measurements

Table 2
Results of single marker based association analyses.

Dataset	Chr.	SNP	Position	Tested allele	Allele frequency		Odd ratios	*P* values
					Cases (%)	Controls (%)		
The discovery dataset	15	rs503464	78857896	T	474 (48.87)	999 (49.07)	0.99	0.9180
	15	rs684513	78858400	G	246 (25.36)	521 (25.59)	0.99	0.8931
	15	rs871058	78858491	A	114 (11.75)	234 (11.49)	1.03	0.8353
	15	rs201599362	78859605	G	174 (17.94)	359 (17.63)	1.02	0.8375
	15	rs634662	78860818	A	93 (9.59)	181 (8.90)	1.09	0.5344
	15	rs61012457	78865694	G	149 (15.36)	301 (14.78)	1.05	0.6785
	15	rs112973744	78866444	T	67 (6,91)	134 (6.58)	1.05	0.7382
	15	rs58651598	78866811	G	53 (5.46)	118 (5.80)	0.94	0.7135
	15	rs12903839	78867042	G	41 (4.23)	77 (3.78)	1.12	0.5571
	15	rs601079	78869579	T	221 (22.78)	460 (22.59)	1.01	0.9073
	15	rs77487352	78873963	A	228 (23.51)	486 (23.87)	0.98	0.8259
	15	rs1700006	78875623	G	135 (13.92)	293 (14.39)	0.96	0.7284
	15	rs17408276	78881618	C	158 (16.29)	343 (16.85)	0.96	0.7011
	15	**rs16969968**	**78882925**	**A**	**55 (5.67)**	**57 (2.80)**	**2.09**	**0.0001**
	15	rs76474922	78884553	C	71 (7.32)	159 (7.81)	0.93	0.6367
The replication dataset	**15**	**rs16969968**	**78882925**	**A**	**69 (5.12)**	**110 (2.92)**	**1.79**	**0.0002**

The replication dataset in the logistic regression model analysis excluding the extraordinary relevance of smoking history variables. Significant differences ($P < 0.05$) are noted in bold.

Table 3
The quantitative trait test for association of rs16969968 with PANSS in SCZ patients.

PANSS	Genotypes		β	t	*P*-value
	Non-GG	GG			
Positive subscore	16.13 (0.49)	13.08 (0.19)	2.875	5.141	$<1 \times 10^{-4}$
Negative subscore	21.10 (0.54)	17.17 (0.20)	3.404	5.750	$<1 \times 10^{-4}$
General subscore	38.88 (0.98)	31.59 (0.30)	6.812	7.531	$<1 \times 10^{-4}$
Total score	76.12 (1.15)	62.25 (0.39)	13.090	11.110	$<1 \times 10^{-4}$

Data were shown as mean, and all standard error (SE) were indicated in the parenthesis. *P* values were adjusted for PANSS (Positive and Negative Syndrome Scale) in patients, and they were excluding the extraordinary relevance of smoking history variables.

(perseverative errors, non-perseverative errors, total errors and trials to compete first category) between individuals with GG and non-GG genotypes on rs16969968 within groups of EOS cases and controls (Table 4). Our results indicated that the genotype of rs16969968 was significantly associated with cognitive function in EOS.

3.4. Functional consequences and the eQTL of rs16969968

The rs16969968 SNP had a score of 0.045 (a benign change) according to polyPhen2, which indicated that the functional consequences of this SNP are very limited despite the altered amino acid sequence of the *CHRNA5* gene. Based on data extracted from GTEx, we identified a widespread pattern of eQTL for rs16969968 from multiple human tissues (Fig. 1). The genotype of rs16969968 was significantly associated with the gene expression of *CHRNA5* in several brain-related tissues, including the cortex, hippocampus, and caudate (Supplemental Table S4). Nevertheless, eQTL data solely extracted from public database is insufficient to describe the underlying functional consequences of a specific SNP, and it should be carefully interpreted the results from our study.

4. Discussion

In this study, we demonstrated a significant association between the rs16969968 SNP on *CHRNA5* and SCZ in two independent samples of early onset Chinese Han people. To the best of our knowledge, this study is the first study to demonstrate the significant association between the SNP (rs16969968, G/A) on *CHRNA5* and the risk of EOS in the Chinese population, which adjusted the effects of smoking status. Our findings further proved that the A allele of rs16969968 on the *CHRNA5* gene can directly increase the risk of EOS without the effect of smoking. Further analysis showed that the rs16969968 SNP on *CHRNA5* was associated with the clinical symptoms of EOS, particularly cognitive function ($P < 2.2 \times 10^{-16}$). More severe cognitive deficiency was observed with the A allele of rs16969968. Psychiatric genomics consortium (PGC) has united investigators around the world to conduct meta- and mega-analyses of genome-wide genomic data for psychiatric disorders including schizophrenia. GWAS data aggregated from multiple studies on schizophrenia were recorded in the website of PGC. We examined SNP rs16969968 in the SCZ data from PGC (http://www.med.unc.edu/pgc/). The odds ratio of the A allele was 1.06, with a *P*-value of 4.63×10^{-7}. This *P*-value was not below the 10^{-8} genome-wide significance level, and therefore, it was not reported to be significant in the PGC paper. Compared to that of the PGC data, the association with rs16969968 identified in our study was in the same direction, but the odds ratio for the A allele in our study was much larger (OR = 2.09).

As mentioned in the introduction, nAChR polymorphisms are closely related to the risk of SCZ. Recently, a meta-analysis of seven SCZ and three bipolar datasets demonstrated that four variants in the 15q25 gene cluster, including rs16969968, were associated with the risk for SCZ and bipolar disorder (Jackson et al., 2013), which is similar to our results. More importantly, nAChRs have been shown to have a major role in SCZ and are therefore viewed as potential therapeutic targets for drugs designed to lessen cognitive deficits (Boggs et al., 2014). The missense variant rs16969968 changes the encoded amino acid sequence from aspartic acid (G allele) to asparagine (A allele) at position 398 (Asp398Asn) (Saccone et al., 2007). Evidence from GTEx data shows that rs16969968 affects the expression of *CHRNA5* in several brain tissues. Studies in European and African populations demonstrated that the A allele resides almost exclusively on a haplotype associated with reduced *CHRNA5* mRNA expression in the brain (Wang et al., 2013). In addition, genetic studies have identified that rs16969968 on *CHRNA5* is not only associated with nicotine dependence but also alcohol dependence and cocaine addiction (Di et al., 2007; Grucza et al., 2008; Wang et al., 2009), which are likely to involve both direct and indirect stimulation of dopamine release in the mesolimbic dopaminergic system (Di et al., 2007). Notably, the availability of dopamine D2 receptors has been reported to have a significant impact on neurocognitive function, including attention and executive function, in healthy subjects (Volkow et al., 1998). Similarly, the blockade of dopamine D2 receptors has a negative correlation with attention in patients with SCZ (Uchida et al., 2009). Recently, through fMRI study, rs1076560 on DRD2 and rs16969968 on CHNRA5 interact to modulate cognitive function and prefrontal physiology during working memory (Di et al., 2014), which further convinced us that rs16969968 on *CHRNA5* may play an important role in the cognitive deficits of SCZ.

Investigating cognitive deficits in EOS is particularly informative, as the early stages of SCZ run alongside a critical period of continued brain maturation associated with increased cognitive abilities (Vyas et al., 2012). A previous study found that *CHRNA7* was associated with age of onset of SCZ (Yuanyuan et al., 2013), which means that nAChRs may affect the central cholinergic system. Our results further identified rs16969968 on *CHRNA5* as associated with cognitive function in EOS. Thus, *CHRNA5* and the central cholinergic system may contribute to the cognitive impairment in EOS. Further research should be conducted to evaluate the connection between the *CHRNA5* gene and age of onset of SCZ, which may be more helpful for interpreting the role of *CHRNA5* in the development of SCZ.

Evidence for association between *CHRAN5* polymorphisms and cognitive deficits of SCZ were obtained in this study. Significant signals were captured by all four cognitive measurements. Population stratification is unlikely to occur because all subjects were from a homogeneous population of the same ethnicity in the same geographical region. However, this study still has several limitations that should be addressed. First, this two-stage studied group of patients is a moderate sample size, which was smaller than that of the GWAS samples.

Table 4
Comparisons of cognitive performances of rs16969968 in patients and controls.

Tests of cognitive functions, mean (sd)	Patients ($N = 674$)		*P*-value	Controls ($N = 1886$)		*P*-value
	Non-GG ($N = 67$)	GG ($N = 607$)		Non-GG ($N = 108$)	GG ($N = 1778$)	
PE	37.4 (3.6)	30.9 (4.9)	$<1 \times 10^{-4}$	6.5 (2.1)	5.4 (2.2)	$<1 \times 10^{-4}$
NPE	41.8 (2.0)	38.5 (4.0)	$<1 \times 10^{-4}$	14.3 (2.9)	13.5 (2.6)	0.0103
TE	79.2 (4.6)	69.4 (6.7)	$<1 \times 10^{-4}$	20.8 (3.7)	19.0 (3.5)	$<1 \times 10^{-4}$
TCFC	77.6 (3.6)	67.2 (9.8)	$<1 \times 10^{-4}$	23.0 (4.1)	21.9 (4.5)	0.0082

PE: perseverative errors; NPE: non-perseverative errors; TE: total errors; TCFC: trials to compete first category. Data were shown as mean, and all standard error (SE) were indicated in the parenthesis. *P* values were obtained through *t*-tests.

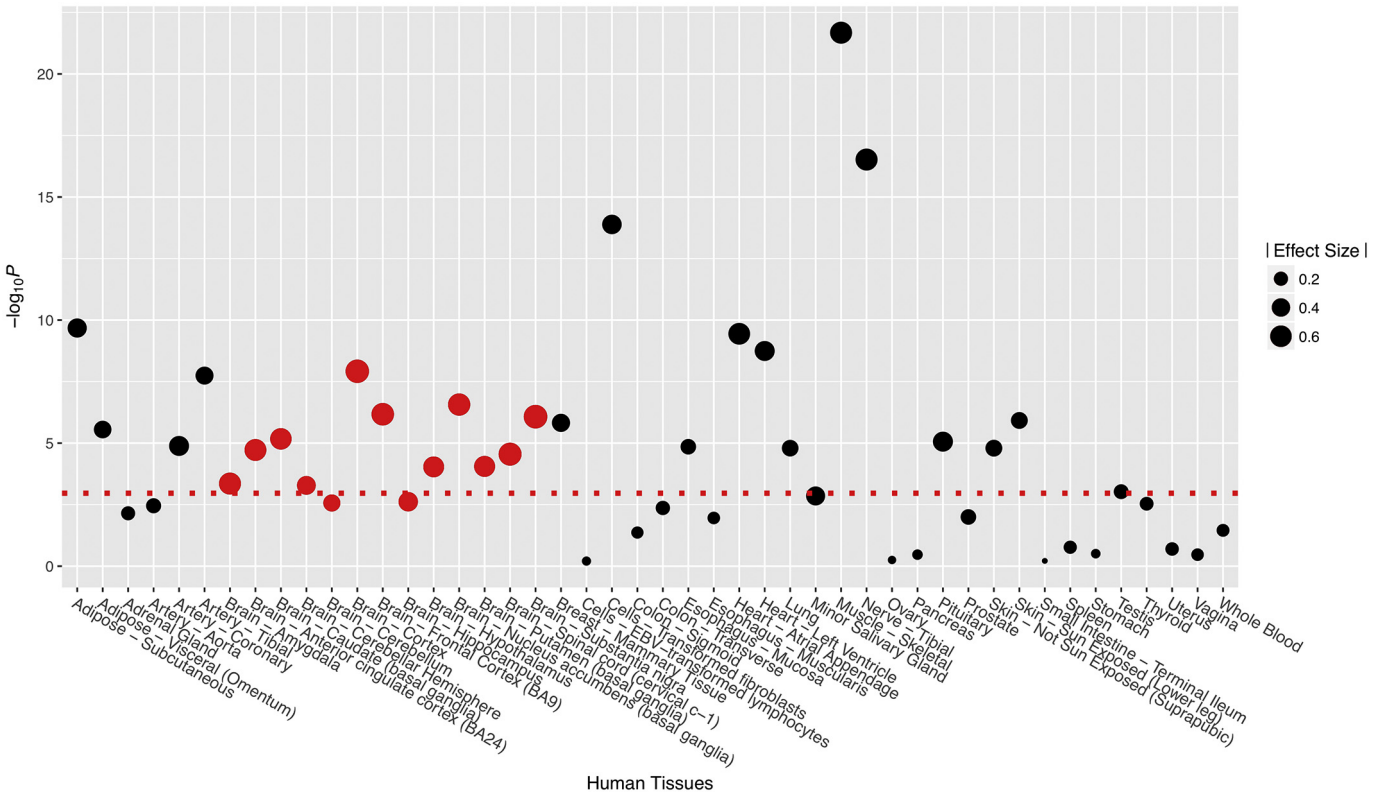

Fig. 1. eQTL pattern of rs16969968 in multiple human tissues. eQTL results extracted from brain tissues were highlighted in red.

Therefore, the statistical power to detect a moderate effect size for SCZ was not very strong. Second, we could not conclusively rule out some other potential confounders of EOS, such as educational attainment, medical history or drug treatments, which could also influence cognitive function. Third, previous studies have reported that antipsychotic medications can influence cognitive deficits in SCZ patients (Ogino et al., 2011). Due to the lack of detailed and complete medication history, the potential influence of medications on this assessment may confound our results. Therefore, our results regarding cognition should be considered to be preliminary and require further confirmation in a larger and well-designed analysis based on different populations. Therefore, our results require further confirmation in a larger and well-designed analysis based on different populations or animal models of SCZ.

In summary, our study provides strong evidence that rs16969968 on *CHRNA5* is tightly linked to genetic susceptibility and cognitive deficits in EOS in the Chinese population. This SNP could reduce α5 nAChR expression and result in central cholinergic system and dopamine system dysfunction, which means that selective pharmacological *CHRNA5* enhancers for this locus may remediate cognitive deficits. These findings contribute to new understanding of the pathogenic mechanisms of SCZ and suggest promising targets for treatment and novel therapeutic strategies for the cognitive deficits of SCZ.

Conflict of interest

All authors have no conflicts of interest to declare.

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Contributors

Guan FL conceived and designed the study. Han W and Zhang TX carried out the computational analyses and candidate SNPs selection. Han W, Ni T, Chen G, Liu D and Lin HL conducted subject screening. Guan FL and Zhang TX performed the statistical analyses. Han W wrote the first draft of the paper, and Guan FL and Chen T revised the current paper. Han W and Lin HL recruited, diagnosed, and gathered patients. Ni T, Zhu L, Chen G and Liu, D contributed to the collection and preparation of control DNA samples.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2018.10.011.

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