Association of *EAAT1* and *EAAT2* Genes Polymorphism With Schizophrenia in Chinese Han Population

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Abstract

Background: Previous studies have linked schizophrenia to *EAAT1* and *EAAT2* genes. The aim of this study was to investigate the relationship between polymorphism of these two genes and schizophrenia in Chinese Han population.

Methods: A total of 233 schizophrenic patients and 342 healthy controls were enrolled. Six SNPs including rs2269272、rs2731880、rs12360706、rs3088168、rs12294045、rs10836387 were genotyped by SNaPshot. Clinical features were collected using a general demographic questionnaire. Symptoms were measured by the Positive and Negative Syndrome Scale (PANSS), and cognitive function was assessed by Matrics Consensus Cognitive Battery (MCCB).

Results: The allele frequency of *EAAT1* rs2731880 was significantly different between patients and controls (*c2*=4.205, R=0.040). The allele and genotype frequency of EAAT2 rs12294045 were significantly different between case and control group (allele:c^2 = 8.144、*R* = 0.004; genotype: c^2 = 8.054, *R* = 0.02). Patients with family history of psychosis were more likely to have the CT genotype of rs12294045 than patients without family history of psychosis、while patients with CT genotype of rs12294045 had significantly lower scores of verbal learning and working memory.

Conclusions: The polymorphism of *EAAT1* and *EAAT2* genes in Chinese Han population may be associated with schizophrenia. The CT genotype of *EAAT2* rs12294045 may be one of the risk factors for verbal learning memory and working operational memory dysfunction in patients.

Introduction

Schizophrenia is a common psychiatric disorder of unclear etiology characterized by positive and negative symptoms, cognitive symptoms [[i]]. Cognitive dysfunction is now generally recognized as a core symptom of schizophrenia, which has its own pathologic mechanisms and related to the abnormal development of the nervous system [[ii]].

The glutamate(Glu) hypothesis of schizophrenia holds that the abnormal transmission of glutamate neurotransmitters in the brain of schizophrenics leads to cognitive and behavioral abnormalities related to schizophrenia [[iii]-[iv]]. The changes of glutamate concentration in the central nervous system (CNS) may damage the structural connection and integrity of neurons, cause programmed cell death and cell proliferation. Such changes may affect the ability to adapt to environmental changes and resist various physiological injuries. Approximately 40% of the synapses in the central nervous system are glutamatergic synapses [[v]-[vi]]. When Glu is at normal level, it plays an important role in synaptic plasticity, learning and memory, neurodevelopment and so on [6]. The high levels of glutamate in the glutamatergic synaptic gap will cause excessive activation of the corresponding glutamate receptors, resulting in excessive increase of intracellular calcium concentration in neurons, leading to excitatory neuron damage or even nerve cell death [[vii]-[viii]]. Relevant studies have shown that this excitotoxicity may be associated with the occurrence and development of a variety of nervous system diseases and mental diseases, including schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, epilepsy, stroke, amyotrophic lateral sclerosis, and idiopathic tremor [[ix]-[x]-[xi]-[xii]],and the level of glutamate in the synaptic space is mainly regulated by excitatory amino acid transporters (EAATs) [[xiii]-[xiv]-[xv]].

Existing studies have confirmed that *EAAT1* and *EAAT2* genes are associated with schizophrenia. Researches in patients with schizophrenia have shown the partial deletion of *EAAT1* gene [[xvi]-[xvii]].Several studies have found that the expression levels of EAAT2 protein in the patients' brain are lower than that of norma –l [[xviii]-[xix]-[xx]], and the impaired cognitive functions such as speech fluency and verbal learning function in patients are related to the decreased expression level of this gene [13,[xxi]]. Besides, EAAT2 gene polymorphisms were found to be associated with schizophrenia in Japanese population [[xxii]]. According to the glutamate hypothesis of schizophrenia [[xxiii]-[xxiv]-[xxv]-[xxvi]], the researchers suggested that decreased levels of glutamate in schizophrenia may be associated with impaired cognitive and social functioning [[xxvii]-[xxviii]]. In schizophrenic patients, the G allele of *EAAT2* SNP rs4354668 was associated with poor clinical manifestations in abstract thinking and working memory [[xxix]-[xxx]].

In order to investigate whether *EAAT1* and *EAAT2* gene polymorphism related to the clinical characteristics, symptom severity and cognitive impairment of Chinese Han patients with schizophrenia, our research team conducted this case-control study.

Methods

Participants

The inclusion of patients (a total of 233 patients were hospitalized in the Shandong Mental Health Center from November 2015 to March 2018) was based on a Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) diagnosis of schizophrenia. In addition, they ranged in age from 18 to 60; biology parents were the Chinese Han population and patients were required to be able to cooperate in completing the Matrics Consensus Cognitive Battery (MCCB) cognitive test, have not been on antipsychotic medication for at least one month with no modified electric convulsive therapy for six months, had no history of drug or cigarette or alcohol abuse. Furthermore, we included a total of 342 healthy physical examinees from November 2015 to March 2018 in Shandong Province aged 18 to 60. Their biology parents were the Chinese Han population, and had no family history of mental illness, had no substance abuse and no pregnancy plan in the near future. The Positive and Negative Syndrome Scale (PANSS) was used to assess the severity of schizophrenia. MATRICS Consensus Cognitive Battery (MCCB) was used to assess cognitive function in patients with schizophrenia.

SNPs selection

The target genetic location was obtained by using the database of the National Center for Biological Information. The SNPs of *EAAT1* and *EAAT2* genes in the Chinese Han population (CHB) were downloaded from the International Genome Database and The Genome Reference Consortium Human Genome Build 38 (GRch38) edition and then were imported into Haploview 4.2 software. Tag SNPs were screened according to MAF > 0.05 and ¡^{2} ≥ 0.8. Two loci of *EAAT1* (rs2269272 and rs2731880) and four loci of *EAAT2* (rs12360706, rs3088168, rs12294045 and rs10836387) were selected.

DNA extraction and SNPs genotyping

The fasting peripheral venous blood of at least 5ml was collected from the case group and control group members was drawn and placed in anticoagulant tubes (containing ethylenediamine teacetic acid). The collected samples were then centrifuged at the speed of 3000 RPM for 10 minutes. After centrifugation, leukocytes were extracted and placed in EP tubes in -80℃ refrigerator for use. Then the deoxyribonucleic acid (DNA) of the blood sample was extracted using the modified potassium iodide method.

The primers were designed using the online primer design tool and synthesized by The Beijing Genomics Institute (Beijing, China). The PCR was carried out in a 30-ml volume containing 2 ml genomic DNA, 3 ml of 10 Ex Taq buffer (Takara), 1ml of MIX Primer (Table 1), 2 ml of dNTPs (2.5mM each), 20.8 ml of H_2O and 0.2 ml of Ex Taq [5U/ml] (Takara). The reaction conditions of PCR were as follows: after an initial step of 2 min at 96 °C, denaturation at 96 °C for 20 s, annealing at 54 °C for 10 s, extended at 72 °C for 30 s; 35 cycles in total. Then the amplified products were digested and purified. The SNaPshot extension reactions were performed with the SNaPshot Multiplex PCR Kit (Applied Biosystems), and the 5ml volume containing 3 ml PCR product, 1 ml of MIX Primer (5 PM), 0.5 ml of SNaPshot MIX(ABI) and 0.5 ml of ddH_2O. After an initial step of 2 min at 95 °C, denaturation at 95 °C for 10 s, annealing at 50 °C for 5 s, extended at 60 °C for 30 s then followed by 35 cycles. After the above reaction, 5ul of SNaPshot PCR product was taken, added 0.5U of SAP, purification was performed at 37 °C for 60 min and 75 °C for 15 min. Finally, genetic analysis of an 8ml volume containing 1 ml SNaPshot product, 6.5 ml of Hi-Di Formamide and 0.5 ml of GS-120 LIZ (ABI) was carried out using the PRISM 3730 XI Genetic Analyzer (Applied Biosystems), and the results were analyzed with GeneMapper v4.1 software (Applied Biosystems).

Statistical analysis

SPSS 21.0 statistical software was used to establish database for data processing and analysis.

The goodness of fit Chi-square test was used to compare the allelic genotypes of six SNPs and test whether they conform to Hardy Weinberg equilibrium (HWE). Chi-square test and Independent-samples t-test were used to compare gender and age between the two groups. One way ANOVA and nonparametric test were used to test the data with uneven variance. Covariance analysis was used to analyze the association between different genotypes of gene polymorphisms and cognitive function. The difference was statistically significant when $R<0.05$.

Results

A total of 575 subjects were enrolled in this study in which 233 were schizophrenic patients (110 males and 123 females) and 342 were healthy controls (160 males and 182 females). There was no significant difference in gender ($c2$ = 0.041, R= 0.084) and age ($c2$ = 1.858, R= 0.064) distribution between the two groups (Table2). All the 6 SNPs were in Hardy-Weinberg Equilibrium in both case group and control group.

Comparison of allele and genotype frequencies between case group and control group

There was statistically significant difference in the allele frequencies distribution of *EAAT1* rs2731880 ($c2$ = 4.205, R= 0.040) and *EAAT2* rs12294045 ($c2$ = 8.144, R= 0.004) between case and control group, and the mutant allele T was significantly increased in the case group. There were no statistical differences in allele frequencies distribution of rs2269272 of *EAAT1* gene and rs3088168, rs10836387 and rs12360706 of *EAAT2* gene between the two groups. The allele frequencies comparison results of 6 polymorphic loci are shown in table 3.

By comparing the genotype frequencies of the 6 loci in the case and control group, we found that the genotype frequencies distribution of *EAAT2* rs12294045 ($c2$ = 8.054, R= 0.018) were significantly different between the two groups. In the case group, the wild homozygous CC genotype was reduced, while the heterozygous CT genotype was increased. There was no significant difference in genotype frequencies of rs2269272, rs2731880 of *EAAT1* gene and rs3088168, rs10836387, rs12360706 of *EAAT2* gene between the two groups. The results of genotype frequencies comparison of 6 loci are shown in table 4.

Comparison of clinical features and symptom severity in patients with different genotypes of rs12294045

According to the above results, we found that the polymorphism of *EAAT2* rs12294045 was significantly associated with schizophrenia. Therefore, we further examined the differences among three genotypes of rs12294045 in the clinical data and symptom characteristics of patients with schizophrenia. The clinical characteristics included 9 aspects which were gender, age of onset, onset form, duration, family history of psychosis, interpersonal relationship, premorbid characteristics, current marital status and occupational status. The PANSS total score and factor scores were compared as well. We found that there was significant difference among the three genotypes of rs12294045 in family history. Compared with patients without family history, patients with family history of psychosis had lower proportion of CC wild genotype and higher proportion of CT heterozygous genotype. There was no significant difference among the three genotypes of rs12294045 in the other 8 clinical characteristics and the total score and factor scores of PANSS. The comparison of 9clinical characteristics and PANSS scores in patients with different genotypes of rs12294045 is shown in table 5.

Comparison of cognitive test scores in patients with different genotypes of rs12294045 locus

Covariance analysis was then performed to find out whether different genotypes of rs12294045 were related to cognitive function of schizophrenia patients. We regarded family history (Positive = 1, Negative = 2) as a covariate, cognitive test results as the dependent variables, rs12294045 locus of different genotype groups as a fixed factor, and compared them in multiple comparisons. The results showed that there were statistically significant differences in the verbal learning and number sequence among patients with different genotypes. LSD analysis showed that the verbal learning scores of patients with CT genotypes were significantly lower than those of TT genotypes. In addition, patients with CT genotypes had significantly lower scores than those with CC and TT genotypes in terms of number sequence representing working

operational memory. There were no statistical differences in the scores of connection, coding symbol, spatial breadth, maze test, visual test, processing fluency, emotional control, and continuous work among patients with different genotypes of rs12294045. The comparison of cognitive test scores among patients with different genotypes of rs12294045 is shown in table 6.

Discussion

There are two objectives of this study. Firstly, the relationship between *EAAT1* and *EAAT2* genes and schizophrenia was explored. There were two major findings in this regard. First, a statistically significant allele frequencies distribution of *EAAT1* gene SNP rs2731880 between case and control group was discovered. Furthermore, it is found that rs12294045 of *EAAT2* gene was associated with Schizophrenia in Chinese Han population. The second objective of this study was to investigate the relationship between the polymorphism of *EAAT2* gene SNP rs12294045 and clinical characteristics, severity of symptoms and cognitive function in schizophrenic patients. Two major findings were worth mentioning. First, the heterozygous CT genotype of rs12294045 together with family history should increase the risk of schizophrenia. Second the CT genotype of rs12294045 may be one of the risk factors for the dysfunction of verbal learning memory and working operational memory in parents.

In our study, we found *EAAT1* rs2731880 may be associated with schizophrenia. However, the evidence was insufficient. There were differences in the allele frequencies between patients and healthy controls but no differences in the genotype frequencies between patients and healthy controls, a finding partially consistent with previous studies. Spangaro M et al. found that rs2731880 of *EAAT1* gene in European population may be related to schizophrenia [29]. In addition, Smith R E et al. reported significantly higher levels of EAAT1 mRNA expression in the thalamus of subjects with schizophrenia [[i]]. Animal model studies have also found that *EAAT1* gene may be associated with schizophrenia. Karlsson et al [[ii]] found that compared with wild-type mice in the same nest, *EAAT1* knockout mice have negative social behaviors, such as lack of pleasure, social withdrawal and self-neglect. The appearance of phenotypic abnormalities associated with negative symptoms may be related to the deletion of this gene. Those above researches indicate that *EAAT1* gene may be involved in the development of schizophrenia to a certain extent.

This study found certain evidence about the relationship between rs12294045 of *EAAT2* gene and schizophrenia. Previous studies have shown conflicting results. In 2004 [22] a case-control study in Japanese population has found the *EAAT2* gene polymorphism was associated with schizophrenia, and concluded that at least one of the susceptibility locus for schizophrenia may reside in internal or nearby *EAAT2*. Poletti et al. [30] conducted brain MRI and working memory tests on 34 male and 16 female patients with chronic schizophrenia from Milan. It was found that the volume of gray matter in patients with *EAAT2* rs4354668 low activity expression G allele was significantly reduced, and the working memory function was also significantly decreased. However, our study and Merk et al. in 2019 [[iii]] found no association between rs4354668 and schizophrenia.

To our knowledge, this study is the first of its kind to investigate the relationship between family history and the polymorphism of *EAAT2* gene and tried to associate population genetics with molecular genetics. We found that almost half of the patients with a family history of psychosis carried the CT heterozygous genotype of SNP rs12294045 and the proportion(46%) was much higher than that(30%) of patients without family history. Meanwhile, in patients carried the CT genotype, 78% of them (75 out of 96) had a family history which was significantly higher than those without family history. Hence there is robust evidence that the CT heterozygous genotype of *EAAT2* gene rs12294045 together with family history could significantly increase the risk of schizophrenia in Chinese Han population. However, there was no significant difference in PANSS total score and factor scores among three genotypes of rs12294045. This means that this genetic mutation had no effect on the severity of the disease although it could play a role in the etiology of schizophrenia.

With regard to the findings relevant to the relationship between *EAAT2* gene and cognitive function in schizophrenia patients, our results were consistent with previous investigations to a certain extent. Previous studies have shown that working

memory represents the core content of changes in schizophrenia, it has been proved that working memory can predict the severity of cognitive impairment and play a key role in the performance of other cognitive tasks in patients with schizophrenia [[iv]-[v]]. The results of prospective studies suggested that patients carrying the G allele of *EAAT2* SNP rs4354668 had significantly reduced gray matter volume and impaired working memory function [28]. Spangaro M et al. [[vi]] found that genotypes associated with low expression of EAAT2 were significantly associated with cognitive dysfunction such as executive function and working memory function. However, the relationship among the genes polymorphisms, expression of EAAT2 protein and the cognitive dysfunction in schizophrenia still remains to be further studied.

Our research has the following limitations: first, the sample size of this study is relatively small. Second, only one method (MCCB) was used to test the cognitive function of schizophrenic patients, so the impairment of cognitive function may not have been better explored. Third, we could not completely eliminate other possible factors, such as the previous medical treatment. Schizophrenia is a complex disease with unknown etiology; therefore further research on the interaction between genes and other genetic and environmental factors is needed.

Conclusions

In our study, we found that the polymorphisms of *EAAT1* rs2731880 may be weakly associated with schizophrenia in Chinese Han population. There is strong evidence that the polymorphism of *EAAT2* rs12294045 should be involved in the etiology of schizophrenia and CT genotype may increase the risk of the disease. Besides, CT genotype may be one of the risk factors for the dysfunction of verbal learning memory and working operational memory in patients.

Abbreviations

CNS: Central Nervous System

SNPs: Single nucleotide polymorphisms;

EAAT: Excitatory Amino Acid Transporter;

NMDA: N-methyl-D-aspartate;

MAF: Minor Allele Frequency;

PCR: Polymerase Chain Reaction;

DSM-IV: Fourth Edition of the Diagnostic and Statistical Manual for Mental Disorders;

PANSS: Positive And Negative Syndrome Scale;

MCCB: Matrics Consensus Cognitive Battery;

HWE: Hard-Weinberg Equilibrium;

LD: linkage disequilibrium;

BVMT-R: Brief Visuospatial Memory Test-Revised;

CPT-IP: Continuous Performance Test-Identical Pairs;

HVLT-R: Hopkins Verbal Learning Test-Revised;

LNS: Letter-Number Span Test;

MSCEIT: Mayer-Salovey-Caruso Emotional Intelligence Test;

NAB: Neuropsychological Assessment Battery;

TMT-A: Trail Making Test A;

WMS-III: Wechsler Memory Scale-Third Edition;

Declarations

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Author Contributions

Lanfen Liu and Lina Wang designed the research; Dongdong Qiao and Kaiyan Cui performed the experiments; Xiaojiao Bi, Chao Han, and Tantan Ma conducted sample collection and analyzed the data; Limin Yang, Mengmeng Sun and Lanfen Liu were responsible for clinical diagnosis; Lina Wang and Tantan Ma edited the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

Researchers interested in the study may contact corresponding author to obtain relevant data via email: liulf521@163.com.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. And the study was approved by the Ethics Committee of the Mental Health Center of Shandong Province, reference number: (2018) Ethics Review (R04).All participants signed the informed consent before participating in the study. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Coyle J T . Schizophrenia: Basic and Clinical[J]. Adv Neurobiol, 2017, 15:255-280.
2. Smeland OB, Frei O, Kauppi K, et al. Identification of genetic loci jointly influencing schizophrenia risk and the cognitive traits of verbal-numerical reasoning, reaction time, and general cognitive function. [J]. JAMA Psychiatry.2017,74(10):1065–75.
3. Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine.[J]. The Journal of neuroscience : the official journal of the Society for Neuroscience, 1998, 18(14):5545-5554.

4. Olney J W Farber N B. Glutamate receptor dysfunction and schizophrenia.[J]. Arch Gen Psychiatry, 1995, 52: 998-1007.
5. Fairman W A, Amara S G. Functional diversity of excitatory amino acid transporters: ion channel and transport modes.[J]. American Journal of Physiology, 1999, 277(4 Pt 2):F481-486.
6. Suzuki M, Nelson A D, Eickstaedt J B, et al. Glutamate enhances proliferation and neurogenesis in human neural progenitor cell cultures derived from the fetal cortex[J]. European Journal of Neuroscience, 2006, 24(3):645-653.
7. Lucas D R, Newhouse J P. The toxic effect of sodium L-glutamate on the inner layers of the retina.[J]. Ama Arch Ophthalmol, 1957, 58(2):193-201.
8. Choi D W. Glutamate neurotoxicity and diseases of the nervous system.[J]. Neuron, 1988, 1(8):623-634.
9. Sheldon A L, Robinson M B. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention.[J].eurochemistry International, 2007, 51(6-7):333-355.
10. Lee M, Cheng M M, Lin C Y, et al. Decreased EAAT2 protein expression in the essential tremor cerebellar cortex[J]. Acta Neuropathol Commun, 2014, 2(1):1-11.
11. Lin C L, Kong Q, Cuny G D, et al. Glutamate transporter EAAT2: a new target for the treatment of neurodegenerative diseases[J]. Future Medicinal Chemistry, 2012, 4(13):1689-1700.
12. Rao J S, Kellom M, Reese E A, et al. Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients[J]. Journal of Affective Disorders, 2017, 220(1-2):156.
13. Plitman E, Nakajima S , Camilo F S, et al. Glutamate-mediated excitotoxicity in schizophrenia: A review[J]. European Neuropsychopharmacology, 2014, 24(10):1591-1605.
14. Marcaggi P, Attwell D. Role of glial amino acid transporters in synaptic transmission and brain energetics.[J]. Glia, 2004, 47(3):217-25.
15. Shan D, Lucas E K, Drummond J B, et al. Abnormal expression of glutamate transporters in temporal lobe areas in elderly patients with schizophrenia[J]. Schizophrenia Research, 2013, 144(1-3):1-8.
16. Parkin G M , Gibbons A , Udawela M , et al. Excitatory amino acid transporter (EAAT)1 and EAAT2 mRNA levels are altered in the prefrontal cortex of subjects with schizophrenia[J]. Journal of Psychiatric Research, 2020, 123.
17. Walsh T, Mcclellan J M, Mccarthy S E, et al. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia.[J]. Science, 2008, 320(5875):539-543.
18. Ohnuma T, Augood S J, Arai H, et al. Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia.[J]. Brain Research Molecular Brain Research, 1998, 56(1-2):207.
19. Ohnuma T, Tessler S, Arai H, et al. Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus.[J]. Molecular Brain Research, 2000, 85(1):24-31.
20. Egan M F, Straub R E, Goldberg T E, et al. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia.[J]. Proceedings of the National Academy of Sciences of the United States of America, 2004, 101(34):12604-9.
21. Robinson M B. The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype.[J]. Neurochemistry International, 1998, 33(6):479-491.
22. Deng X, Shibata H, Ninomiya H, et al. Association study of polymorphisms in the excitatory amino acid transporter 2 gene (SLC1A2) with schizophrenia.[J]. BMC Psychiatry, 2004, 4(1):21.
23. Javitt D C, Zukin S R. Recent advances in the phencyclidine model of schizophrenia.[J]. American Journal of Psychiatry, 1991, 148(10):1301.
24. Mohn A R, Gainetdinov R R, Caron M G, et al. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia.[J]. Cell, 1999, 98(4):427-436.
25. Olney J W, Newcomer J W, Farber N B. NMDA receptor hypofunction model of schizophrenia.[J]. Journal of Psychiatric Research, 1999, 33(6):523-533.

26. Steinpreis R E. The behavioral and neurochemical effects of phencyclidine in humans and animals: some implications for modeling psychosis[J]. Behavioural Brain Research, 1996, 74(1-2):45-55.
27. Bustillo J R, Chen H, Gasparovic C, et al. Glutamate as a marker of cognitive function in schizophrenia: a proton spectroscopic imaging study at 4 tesla.[J].Biological Psychiatry, 2011, 69(1):19.
28. Aoyama N, Théberge J, Drost D J, et al. Grey matter and social functioning correlates of glutamatergic metabolite loss in schizophrenia.[J]. British Journal of Psychiatry the Journal of Mental Science, 2018, 198(6):448-456.
29. Spangaro M, Bosia M, Zanoletti A, et al. Exploring effects of EAAT polymorphisms on cognitive functions in schizophrenia.[J]. Pharmacogenomics, 2014, 15(7):925-932.
30. Poletti S, Radaelli D, Bosia M, et al. Effect of glutamate transporter EAAT2 gene variants and gray matter deficits on working memory in schizophrenia.[J]. European Psychiatry, 2014, 29(4):219-225.
31. Smith R E , Haroutunian V, Davis K L , et al. Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia[J]. Am J Psychiatry, 2001, 158(9):1393-1399.
32. Karlsson R M, Tanaka K, Saksida L M, et al. Assessment of Glutamate Transporter GLAST (EAAT1)-Deficient Mice for Phenotypes Relevant to the Negativeand Executive/Cognitive Symptoms of Schizophrenia[J]. Neuropsychopharmacology, 2009, 34(6):1578-1589.
33. Merk W, Kucia K, Mędrala T, Kowalczyk M, Owczarek A, Kowalski J. Association study of the excitatory amino acid transporter 2 (EAAT2) and glycine transporter 1 (GlyT1) gene polymorphism with schizophrenia in a Polish population. Neuropsychiatr Dis Treat. 2019;15:989-1000.
34. Perlstein W M, Carter C S, Noll D C, et al. Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia.[J]. Am J Psychiatry, 2001, 158(7):1105-1113.
35. Silver H, Feldman P, Bilker W, et al. Working Memory Deficit as a Core Neuropsychological Dysfunction in Schizophrenia. [J]. American Journal of Psychiatry, 2003, 160(10):1809-1816.
36. Spangaro M, Bosia M, Zanoletti A, et al. Cognitive dysfunction and glutamate reuptake: effect of EAAT2 polymorphism in schizophrenia.[J]. Neuroscience Letters, 2012, 522(2):151-155.

Tables

Table 1 List of primer pairs for multiple PCR

Primer	Sequence (5'–3')	Length (bp)
rs2269272-F	TCCTTAGAATGAGGGAAAC	258
rs2269272-R	CAGCGTCTTTGACTGGATA	
rs12360706-F	GGGAAGTAACTCTTATGGA	281
rs12360706-R	AACTGACTGTTAGCCTTGT	
rs3088168-F	TATAGATGCTCTGTGCTACGTGACT	282
rs3088168-R	AAGGGTAAAGCCTACAATA	
rs12294045-F	CATGCCCTCAAAGATCTAAGGTAAA	300
rs12294045-R	CAGTTACAGCAGGCCAGAA	
rs10836387-F	CTGCGTGAGTTGCTGATTC	246
rs10836387-R	GTTGTCTTCTATTGCCTGA	
rs2731880-F	TTTGTAAATGCTCCTCCTGC	455
rs2731880-R	CAAACATTGAGCAACCACTG	
rs2269272-YF	ATAAGAGAAATGGTAGAAGATGAATCAGTATGAAGACACTGT	42
rs12360706-YF	CCTCAGAGATGTGCTGGACCAACTTCCTTGGCTAGT	36
rs3088168-YF	TGAAAGGAGTTGAAGAAGCCACATTTTCAAGGAAAAATTAGCCTGTCCACCATA	54
rs12294045-YF	ACTTGGGTTTCTCAAAGGGCAAGAATGAGAAAGAGAAGAATTAAAGTCTACTTAGTTGGTTTTCTC	66
rs10836387-YF	AATCTGTAGGGAGAAGCTGAGCTGCACTGGATGACTGTTATGCTCCCA	48

Table 2 The age and gender distributions of subjects in case and control group

Group	Case（n=233）	Control（n=342）	c^2/t	*R*
Gender[n(%)]				
Male	110（47.21）	160（46.78）	0.041	0.084
Female	123（52.79）	182（53.22）		
Age（C±S）	32.94±10.77	31.30±10.13	1.858	0.064

Table3 Comparison of allele frequencies of 6 SNPs loci between the two groups

SNP	Allele	Case	Control	c^2	*P*
rs2269272	C	321（0.689）	474（0.693）	0.022	0.881
	T	145（0.311）	210（0.307）		
rs2731880	C	274（0.588）	443（0.648）	4.205	0.040*
	T	192（0.412）	241（0.352）		
rs3088168	C	189（0.406）	313（0.458）	3.050	0.081
	T	277（0.594）	371（0.542）		
rs10836387	G	160（0.343）	252（0.368）	0.758	0.384
	A	306（0.657）	432（0.632）		
rs12294045	C	330（0.708）	535（0.782）	8.144	0.004*
	T	136（0.292）	149（0.218）		
rs12360706	G	376（0.807）	559（0.876）	0.197	0.657
	A	90（0.193）	125（0.124）		

Notes: * $P < 0.05$

Table4 Comparison of genotype frequencies of 6 SNPs loci between two groups

SNP	Genotype	Case	Control	χ^2	*P*
rs2269272	CC	113（0.485）	164（0.480）		
	TT	25（0.107）	32（0.094）	0.394	0.821
	CT	95（0.408）	146（0.426）		
rs2731880	CC	82（0.352）	145（0.424）		
	TT	41（0.176）	44（0.129）	4.106	0.128
	CT	110（0.472）	153（0.447）		
rs3088168	CC	37（0.159）	67（0.196）		
	TT	81（0.348）	96（0.281）	3.313	0.191
	CT	115（0.493）	179（0.523）		
rs10836387	GG	32（0.137）	41（0.120）		
	AA	105（0.451）	131（0.383）	4.043	0.132
	GA	96（0.412）	170（0.497）		
rs12294045	CC	117（0.502）	211（0.617）		
	TT	20（0.086）	18（0.053）	8.054	0.018*
	CT	96（0.412）	113（0.330）		
rs12360706	GG	150（0.644）	227（0.664）		
	AA	7（0.030）	10（0.029）	0.249	0.883
	GA	76（0.326）	105（0.307）		

Notes: * $P < 0.05$

Table 5 Comparison of clinical features and symptom severity in patients with different genotypes of rs12294045

Items	Classification	CC（117）	TT（20）	CT（96）	χ^2/F	*P*
Gender	Male（110）	53（0.48）	9（0.08）	48（0.44）	0.510	0.775
	Female（123）	64（0.52）	11（0.09）	48（0.39）		
Age of onset		24.06±7.77	26.70±7.14	23.99±6.90	1.205	0.302
Onset form	Acute（22）	10（0.46）	4（0.18）	8（0.36）	5.560	0.235
	Subacute（28）	14（0.50）	0（0）	14（0.50）		
	Chronic（183）	93（0.51）	16（0.09）	74（0.40）		
Duration		97.94±96.04	109.00±105.4	99.56±96.45	0.111	0.895
Family history	Positive（162）	73（0.45）	14（0.09）	75（0.46）	6.162	0.046*
	Negative（71）	44（0.62）	6（0.08）	21（0.30）		
Interpersonal relationship	Good（15）	8（0.53）	2（0.13）	5（0.34）	3.794	0.435
	General（123）	67（0.54）	11（0.09）	45（0.37）		
	Poor（95）	42（0.44）	7（0.08）	46（0.48）		
Premorbid character	Extro（37）	20（0.54）	2（0.05）	15（0.41）	0.734	0.947
	Neutral（14）	7（0.50）	1（0.07）	6（0.43）		
	Intro（182）	90（0.49）	17（0.09）	75（0.41）		
Marital status	Unmarried（116）	59（0.51）	8（0.07）	49（0.42）	11.423	0.179
	Married（75）	39（0.52）	6（0.08）	30（0.40）		
	Living apart（10）Divorced（28）	5（0.50）	3（0.30）	2（0.20）		
		14（0.50）	2（0.07）	12（0.43）		
	Loss of spouse（4）	0（0）	1（0.25）	3（0.75）		
State of occupation	Full-time（64）	32（0.50）	5（0.08）	27（0.42）	5.027	0.889
	Part-time（38）	19（0.50）	3（0.08）	16（0.42）		
	Jobless（93）	43（0.46）	9（0.10）	41（0.44）		
	Unemployment（9）	7（0.78）	0（0）	2（0.22）		
	Retired（28）	15（0.54）	3（0.10）	10（0.36）		
	Other works（1）	1（1）	0（0）	0（0）		
PANSS	Positive score	27.74±4.17	28.05±4.05	28.52±5.40	*1.299*	0.541
PANSS	Negative score	23.72±5.45	24.10±4.52	23.80±5.11	0.046	0.955
PANSS	psychopathology	47.74±7.02	49.80±7.75	48.44±7.12	0.798	0.452
PANSS	Total score	99.20±11.46	101.95±10.43	100.76±11.35	0.801	0.450

Notes: * $P < 0.05$; *Italics* indicates Kruskal-Wallis test for non-parametric test, and the statistic is χ^2.

Table 6 Comparison of cognitive test scores in patients with different genotypes of rs12294045 locus

Subtests		rs12294045		*F*	*R*	*R*1	*R*2	*R*3
	CT（n=96）	CC（n=116）	TT（n=20）					
TMT-A	45.23±7.86	46.28±11.97	47.95±7.54	0.571	0.566	0.573	0.300	0.467
Symbol Coding	42.00±10.56	40.98±10.10	44.60±11.07	1.186	0.307	0.410	0.328	0.143
Category Fluency	47.75±11.32	47.35±11.25	48.55±10.88	0.153	0.858	0.693	0.806	0.634
CPT-IP	41.84±11.11	42.05±10.75	45.25±13.08	0.803	0.449	0.937	0.221	0.231
LNS	43.76±10.97	46.96±11.32	50.75±9.89	4.22	0.016#	0.038#	0.011#	0.161
WMS-III	44.34±12.03	44.68±12.93	47.10±12.63	0.393	0.675	0.886	0.382	0.420
HVLT-R	42.22±12.18	44.94±10.88	48.80±11.08	3.048	0.049#	0.117	0.023#	0.156
BVMT-R	43.89±11.79	43.16±11.16	44.30±13.07	0.116	0.891	0.706	0.867	0.699
NAB-MAZES	43.02±8.99	42.94±11.05	42.65±10.70	0.026	0.974	0.853	0.851	0.933
MSCEIT	47.03±12.30	48.09±11.91	51.75±14.55	1.196	0.304	0.563	0.124	0.219

Note: #*R*<0.05. *R*1represents the comparison between CT genotype patients and CC genotype patients; *R*2 represents the comparison between CT genotype patients and TT genotype patients; *R*3 represents the comparison between CC genotype patients and TT genotype patients.(TMT-A: Trail Making Test A; CPT-IP: Continuous Performance Test-Identical Pairs; LNS: Letter-Number Span Test; WMS-III: Wechsler Memory Scale-Third Edition; HVLT-R: Hopkins Verbal Learning Test-Revised; BVMT-R: Brief Visuospatial Memory Test-Revised; NAB: Neuropsychological Assessment Battery; MSCEIT: Mayer-Salovey-Caruso Emotional Intelligence Test).