Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Common variants in *SLC1A2* and schizophrenia: Association and cognitive function in patients with schizophrenia and healthy individuals

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ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form 30 September 2015
Accepted 6 October 2015
Available online xxxx

Keywords:
Schizophrenia susceptibility
SLC1A2 gene
Glutamate uptake
Perseverative errors
Cognitive performance

ABSTRACT

SLC1A2 is reported to be responsible for the majority of glutamate uptake, which has a crucial role in neural development and synaptic plasticity, and a disturbance in glutamatergic transmission has been suggested to be involved in the pathophysiology of schizophrenia (SCZ) and cognition. To evaluate the relationship of common variants within *SLC1A2* with SCZ and cognition in Han Chinese, 28 tag SNPs were genotyped in the discovery stage, which included 1117 cases and 2289 controls; significantly associated markers were genotyped in the replication stage with 2128 cases and 3865 controls. The rs4354668 SNP was identified to be significantly associated with SCZ in both datasets, and a similar pattern was also observed in the two-stage study on conducting imputation and haplotype association analyses. In addition, significant associations between the rs4354668 SNP and cognition were observed when processing the perseverative error of the Wisconsin Card Sorting Test in patients and controls. Our results provide supportive evidence for an effect of *SLC1A2* on the etiology of SCZ, suggesting that genetic variation (rs4354668 and its haplotypes) in *SLC1A2* may be involved in impaired executive function, which adds to the current body of knowledge regarding the risk of SCZ and the impairment of cognitive performance.

1. Introduction

Glutamate, which is considered to be the major mediator of excitatory neurotransmission in the central nervous system (CNS), plays a crucial role in neural development, synaptic plasticity, learning and memory under physiological conditions (Fairman and Amara, 1999; Spangaro et al., 2012; Suzuki et al., 2006). Studies have suggested that abnormalities and dysfunction in the uptake and metabolism of glutamate may be involved in some neuropsychiatric disorders: defective glutamate transport may also play a critical role in the neurotoxicity associated with ischemia, epilepsy, neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) (Catalano et al., 2002). In contrast, an excessive uptake, leading to a relative glutamatergic hypofunction, is suspected to be involved in psychiatric disorders including schizophrenia (SCZ) (Gegelashvili and Schousboe, 1997). Because a disturbance in glutamatergic transmission has been suggested to contribute to the development of SCZ pathophysiology, its role in this complicated disease has been increasingly studied (Docherty et al., 2015; Gaisler-Salomon et al., 2009; Myles-Worsley et al., 2013). This SCZ etiology hypothesis is primarily based on disturbances in brain glutamatergic pathways and the impairment in signaling at glutamate receptors, such as that caused by phencyclidine, which induces schizophrenic-like symptoms (Halberstadt, 1995; Poels et al., 2014). Thus, glutamate receptors, receptor-related genes and other molecules that are involved in glutamatergic neurotransmission have been studied as candidate risk factor genes for SCZ.

Excitatory amino acid transporters (EAATs) potentially affect glutamatergic neurotransmission by excluding excessive glutamate from the synaptic cleft. The EAATs family is comprised of five proteins (EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5) that are high-affinity glutamate transporters and two proteins (ASCT1 and AAAT) that function as amino acid transporters. Among them, EAAT2 is encoded by the *SLC1A2* gene, which is located on chromosome 11 and consists of 11 exons spanning over 165 kb in humans. It is normally predominantly expressed in astrocytes but can be detected in oligodendrocytes and in neurons, as well (Lauriat and McInnes, 2007), and is responsible for

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http://dx.doi.org/10.1016/j.schres.2015.10.012
0920-9964/

greater than 90% of cerebral glutamate uptake (Haugeto et al., 1996). *SLC1A2* mRNA was found to be significantly increased in the thalamus of SCZ brains, suggesting that excessive glutamate uptake may occur in SCZ (Smith et al., 2001), and significantly decreased in the prefrontal cortex and parahippocampal gyrus in postmortem SCZ brains, implying that downregulation of *SLC1A2* may compensate for hypoglutamatergic neurotransmission (Ohnuma et al., 1998; Ohnuma et al., 2005; Ohnuma et al., 2000). Therefore, *SLC1A2* has emerged as a candidate gene for SCZ.

Given its central role in glutamatergic neurotransmission, several studies have investigated the possible implications of *SLC1A2*'s association with SCZ. Previously, an association between single nucleotide polymorphisms (SNPs) in *SLC1A2* and Japanese patients with SCZ was reported in a population of two hundreds individuals (Deng et al., 2004). Another more recent study reinvestigated this association in a moderate sample size of Japanese individuals and failed to show any significant association with SCZ (Nagai et al., 2009). In a recently published large scale Psychiatric Genomics Consortium (PGC) GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) with 150,000 samples, including some Asian cohorts with thousands of individuals, researchers identified 108 genes independently associated with SCZ. Although *SLC1A2* was not identified in this study, the true signals may have been discarded, considering the stringent *P*-value used in the GWASs to correct for the multiple comparison problem. Moreover, some recent reviews have supported the notion that GWASs have not yet explained most of the underlying genetic risks for SCZ and the high genetic heterogeneity that exists in the pathogenesis of this disease (McClellan et al., 2007; Riancho, 2012). Based on the important role of the *SLC1A2* gene in SCZ and these controversial results, it is necessary to explore the possible association between genetic loci and SCZ in additional large-scale populations. In addition, the associations between the genetic variants in the *SLC1A2* gene and cognitive dysfunction were investigated in SCZ patients in some previous studies, but they were analyzed in small European populations (Poletti et al., 2014; Spangaro et al., 2012; Spangaro et al., 2014). Despite evidence of a strongly significant association in European patients, the underlying biological mechanisms are largely unknown and the genetic loci contributing to cognition remain to be elucidated in additional genetically independent populations with larger samples. Although various cognitive deficits (working memory, executive function, verbal fluency and episodic memory) have been reported in schizophrenia (Barch, 2005), perseverative error processing appears to be not only a marker of frontal lobe dysfunction in SCZ but might also serve as a vulnerability marker for schizophrenia (Baune et al., 2010). A recent study among college students has offered some supportive evidence for this, which reported that those students rating high on the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991) showed more perseverative errors compared with subjects scoring average on the SPQ (Wilson et al., 2008).

Thus, we focused on the association between common variants in *SLC1A2* and SCZ by performing a large-scale case–control association study using two-stage independent samples. Furthermore, to better increase the understanding of the interaction between the *SLC1A2* gene and perseverative error under pathological and non-pathological conditions, our secondary aim was to evaluate the relationship between the *SLC1A2* gene and perseverative error processing in SCZ patients and healthy individuals.

2. Materials and methods

2.1. Subjects

Two independent datasets were included in our study, and we implemented a two-stage experimental strategy: 1) a discovery stage in which we genotyped a relatively larger set of markers in a smaller subject subset of 1117 patients with SCZ (536 males and 581 females; age: 18–51; mean age: 36.50 ± 9.23 years) and 2289 healthy controls (1107 males and 1182 females; age: 18–47; mean age: 36.42 ± 6.78 years); 2) a replication stage in which we only genotyped a smaller set of markers in the same linkage disequilibrium (LD) block with a certain marker that passed the screening *P* value threshold (0.05) in a relatively larger subject subset of 2128 patients with SCZ (1030 males and 1098 females; age: 18–57; mean age: 35.38 ± 10.39 years) and 3865 healthy controls (1863 males and 2002 females; age: 18–57; mean age: 35.53 ± 11.38 years). All patients were recruited from the inpatient and outpatient clinical services of a psychiatric unit at the Xi'an Mental Health Center and were diagnosed by at least two experienced psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for SCZ (American Psychiatric Association, 1994). A total of 2128 patients and 2794 controls in the replication set completed the cognitive assessments, and relevant useful data were obtained. All unrelated healthy controls were selected from a combination of local volunteers and blood transfusion donors; those with a personal family history of mental illness in the previous three generations and with current or past evidence of psychoses were ruled out from the present study. All subjects enrolled from the city of Xi'an in Shaanxi Province were of Han descent, and we excluded anyone not born locally or whose immediate family members from the previous three generations were not born locally. This research 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. All participants completed written informed consent forms.

2.2. Cognitive assessment

Cognitive performances were assessed using the Wisconsin Card Sorting Test (WCST). The WCST mainly assesses executive function, including cognitive flexibility in response to feedback, and requires participants to match a target card to one of four alternatives. After each match, the participants receive correct or incorrect feedback in which they are required to understand the underlying principle governing the matching rule (e.g., color, number or shape of items that appear on the cards). After 10 consecutive correct matches, the rule shifts without any announcement, and the participants are required to adjust their strategy accordingly.

2.3. SNP selection and genotyping

We first searched for all SNPs from the CHB database of HapMap with minor allele frequencies (MAF) ≥ 0.01 in the ±5 kb of the *SLC1A2* gene region, and 131 SNPs were found. The 131 SNPs were then pair-wise tagged with $r^2 \geq 0.5$, and 26 tag SNPs were obtained (rs7130385, rs3812779, rs7936950, rs1570216, rs10836364, rs3763889, rs2281634, rs3794092, rs3794087, rs2273688, rs7102331, rs6484784, rs4756222, rs7116961, rs1885346, rs10836387, rs4756226, rs11827417, rs10768132, rs16927465, rs1923291, rs2421897, rs1923294, rs4354668, rs16927467 and rs7122389). As a first screen of common SNPs within the *SLC1A2* gene in Han Chinese, an MAF ≥ 0.01 with pair-wise tagging and $r^2 \geq 0.5$ was used as the cut-off when choosing tagSNPs, as these parameters allowed us to obtain enough informative tagSNPs at a lower cost from the most common SNPs covering the region of the *SLC1A2* gene (Table S1). In addition, 2 SNPs (rs7110985 and rs4755404) previously investigated in the Japanese population (Deng et al., 2004) were incorporated in our study. Therefore, those 28 SNPs completely covering the region of the *SLC1A2* gene were included in our further analyses.

DNA was extracted from whole blood according to the standard protocol of the DNA Isolation Kit for Mammalian Blood (Tiangen Biotech Co., Ltd., Beijing, China). The DNA was stored at −80 °C for genotyping. The genotyping was conducted for all of the SNPs using the Sequenom MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA, USA) on the genomic DNA isolated from the peripheral leukocytes. The final data were

Please cite this article as: Zhang, B., et al., Common variants in *SLC1A2* and schizophrenia: Association and cognitive function in patients with schizophrenia and healthy individuals..., Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.10.012