POLYMORPHISMS IN GLYCINE TRANSPORTER WITH SCHIZOPHRENIA

Shih-Jen Tsai[1,2], Chih-Ya Cheng[3,4], Chen-Jee Hong[1,2], Ding-Lieh Liao[5], Ying-Jay Liou[3,6,7]

[1]Department of Psychiatry, Taipei Veterans General Hospital, Taipei;
[2]Division of Psychiatry, [3]Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan;
[4]Ching Kuo Institute of Management and Health, Keelung, Taiwan
[5]Department of Psychiatry, Executive Yuan Department of Health, Pali Psychiatric Hospital, Taipei, Taiwan
[6]Department of Psychiatry, Yuli Veterans Hospital, Hualien, Taiwan;
[7]Division of Mental Health and Substance Abuse Research, National Health Research Institutes, Taiwan

GLICIN TRANSZPORTER POLIMORFIZMUS SZKIZOFRÉNIÁBAN

A glicin mint agonista, a glutamáttal együtt hat a N-methyl-D-aspartat (NMDA) receptorokon. Az agyi glicin elérhetőségét a glicin visszavételt közvetítő glicin transzporterek (GlyT1 vagy SLC6A9) határozzák meg. Mivel a szkizofrénia patofiziológiájában az NMDA receptorok csökkent működése észlelhető, ez a vizsgálatsorozat a GlyT1 genetikus variációit a szkizofrénia iránti fogékonysággal veti össze. Négy GlyT1 polimorfizmust vizsgáltak, 249 szkizofrén és 210 normál személyből álló mintában.

Egy polimorfizmus (rs16831541) a kínai populációban nem volt informatív, a többi hármat (rs1766967, rs2248632, rs2248253) chi-négyzet próbával és haplotip analízissel elemezték. Sem az egyes markerek, sem a haplotip analízis nem fedett fel kapcsolatot a GlyT1 locus variációi és a szkizofrénia között, ennek alapján valószínűtlen, hogy a vizsgált GlyT1 polimorfizmusok jelentős szerepet játszanának a kínai populáció szkizofrénia iránti fogékonyságában. A GlyT1 variánsok további vizsgálata javasolt, a szkizofrénia, a pszichotikus tünetek és a terápiás válasz vonatkozásában.

KULCSSZAVAK: asszociációs vizsgálat, polimorfizmus, glicin transzporter, szkizofrénia, haplotip

SUMMARY

Glycine acts as an obligatory co-agonist with glutamate on N-methyl-D-aspartate (NMDA) receptors. Brain glycine availability is determined by glycine transporters (GlyT1 or SLC6A9), which mediate glycine reuptake into nerve terminals. Since hypofunction of NMDA receptors has been implicated in the pathophysiology of schizophrenia, this study tests the hypothesis that GlyT1 genetic variants confer susceptibility to schizophrenia. Four GlyT1 polymorphisms were studied in a sample population of 249 people with schizophrenia and 210 normal controls.

One polymorphism (rs16831541) was not informative in our Chinese population while the other three polymorphisms (rs1766967, rs2248632 and rs2248253) were analysed with chi-square tests and haplotype analysis. Significant linkage disequilibrium was obtained among the three polymorphisms. Neither single marker nor haplotype analysis revealed an association between variants at the GlyT1 locus and schizophrenia, suggesting that it is unlikely that the GlyT1 polymorphisms investigated play a substantial role in conferring susceptibility to schizophrenia in the Chinese population. Further studies with other GlyT1 variants, relating either to schizophrenia, psychotic symptoms or to therapeutic response in schizophrenia, are suggested.

KEYWORDS: association study, polymorphism, glycine transporter, schizophrenia, haplotype

Introduction

Schizophrenia is a complex psychiatric disorder and has heritability of around 80%. Although the dopamine hypothesis of schizophrenia is the most popular neurochemical disease model at present, increasing attention has been paid to defective glutamatergic neurotransmission in the psychiatric manifestations of schizophrenia. The glutamate hypothesis of schizophrenia stems from the finding that phencyclidine (PCP) induces psychotic-like behaviour in rodents, possibly by blocking N-methyl-D-aspartate (NMDA) glutamate receptors and thereby causing increased glutamate release (Luby et al., 1959). Furthermore, drugs such as PCP and ketamine, which both antagonize glutamatergic signalling through NMDA receptors, induce psychosis in normal individuals and exacerbate psychotic symptoms in schizophrenia (Lieberman et al., 1987; Krystal et al., 1994). In the NMDA receptor hypofunction model of schizophrenia, stimulation of NMDA receptors may improve the symptoms of schizophrenia. In the mammalian brain, NMDA receptor activation requires both binding of glutamate to its recognition site and occupancy of the glycine modulatory site to which glycine and/or D-serine bind (Johnson and Ascher, 1987). Therefore, a compound potentiating NMDA neurotransmission by acting at the glycine modulatory site could possess antipsychotic activity. Recently a number of clinical trials of full or partial glycine site-agonists, including glycine, D-serine and D-cycloserine, have shown effectiveness in schizophrenia (for review see Coyle et al., 2002).

In brain, the availability of glycine is chiefly determined by activity of the glycine transporter (GlyT1) (Kim et al., 1994), which mediates glycine reuptake into nerve terminals and adjacent glial cells. Thus GlyT1 inhibitors may increase glycine levels and be of therapeutic value in schizophrenia. This notion is supported both by preclinical and clinical studies. In a recent animal study, it was found that ALX5407, a GlyT1 inhibitor, displayed similar effects to the atypical antipsychotic clozapine, in both prepulse inhibition and latent inhibition mouse model of psychosis (Lipina et al., 2005). In another animal study, Williams et al. (2004) revealed that antipsychotics such as clozapine potently block GlyT1-mediated uptake and raise the possibility that an inhibitory interaction between some antipsychotics and GlyT1 might mediate the antipsychotic mechanism. Furthermore, it was demonstrated that reduced expression of GlyT1 enhances hippocampal NMDA receptor function and protects against amphetamine-induced disruption of sensory gating in animal models of psychosis, suggesting that drugs that inhibit GlyT1 might have antipsychotic effects (Tsai et al., 2004b). In a clinical trial, Tsai et al. (2004a) demonstrated that N-methylglycine (sarcosine), an endogenous GlyT1 antagonist, had beneficial effects on schizophrenic patients treated with antipsychotics.

The human GlyT1 (OMIM: Solute carrier family 6 [neurotransmitter transporter, glycine]; SLC6A9) is located on chromosome lp33 (Jones et al., 1995), is about 19.8 kb in length and includes 13 exons. Based on the NMDA hypofunction hypothesis of schizophrenia and the above preclinical and clinical findings, GlyT1 may play a role in the pathogenesis of schizophrenia. To investigate the pathological role of the GlyT1 gene and schizophrenia, the association between the disorder and four polymorphisms of the GlyT1 gene were examined in a case-control study. In addition, linkage disequilibrium measurement among the GlyT1 polymorphisms and haplotype analysis between patient and control groups were conducted to assess the association between markers within the GlyT1 gene and schizophrenia.

Subjects and methods

Human subjects

The patient group consisted of 249 chronic psychiatric inpatients (mean age 42.5±10.3 years; male/female: 129/120) diagnosed with schizophrenic disorders, according to the relevant criteria from the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV). The diagnoses were re-evaluated by a senior psychiatrist who was blind to patient genotype.

A total of 210 normal comparison subjects (mean age 41.2±11.9 years; male/female: 113/97) who had been interviewed by a psychiatric staff member to rule out major psychiatric disorders were enrolled as controls. The entire sample, which consisted of Taiwanese ethnic Han Chinese, had been used in our previous study (Hong et al., 2004). The study was approved by the Ethics Committee of the Taipei Veterans General Hospital. Written, informed consents were obtained from all subjects.

Polymorphisms in the GlyT1 Region

We selected the polymorphisms in the GlyT1 region from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Depending on the relative importance of polymorphisms potentially associated with functional effects and the minority allele frequency, four GlyT1 genetic polymorphism including rs1766967 (intron 3), rs16831541 (intron 3), rs2248632 (intron 10) and rs2248253 (3' untranslated region), which cover 12.4 kb in the GlyT1 gene, were selected for this study. The exact location of the four studied polymorphisms were obtained from the Genewindow website (http://genewindow.nci.nih.gov/Welcome).

Genotyping

For determination of these four GlyT1 polymorphisms, peripheral venous blood was withdrawn from the study subjects after informed consent had been obtained. Genomic DNA was isolated by using the PUREGENE DNA purification system (Gentra Systems). GlyT1 polymorphism genotyping was performed by using high-throughput MALDI-TOF mass spectrometry. Briefly, primers and probes were designed with Spectro-DESIGNER software (Sequenom, San Diego). Multiplex polymerase chain reaction was performed, and unincorporated dNTPs were dephosphorylated with shrimp alkaline phosphatase (Hoffman-LaRoche, Basel) followed by primer extension. The purified primer extension reaction was spotted onto a 384-element silicon chip (SpectroCHIP, Sequenom) and analyzed in the Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer (Sequenom). The resulting spectra were processed with SpectroTYPER (Sequenom).

Statistical Analysis

Categorical data were analysed using the chi-square test, or Fisher's exact test if necessary. Differences for continuous variables were evaluated using the Student's t-test. Data are presented as mean±standard deviation (SD).

The software SNP Alyze® V3.2 (Dynacom Co., Ltd. Kanagawa, Japan) was used to evaluate the status of pairwise LD for the studied polymorphisms, to infer the haplotype frequency and to determine whether haplotype frequency varied between groups. The significance level of these analyses obtained from the SNP Alyze® V3.2 was set as p value <0.05 after 100.000 permutation tests.

Results

The age and sex distributions for the schizophrenic and control populations were similar (p=0.185 and p=0.708, respectively). The rs3180325 marker was monomorphic and was excluded from further analysis. The genotype and allele distributions for the other three GlyT1 polymorphisms for the schizophrenic patients and control subjects are presented in Table 1.

For the GlyT1 polymorphisms investigated, genotype distributions did not deviate significantly

Table 1.
Genotype distribution and allele frequencies for the GlyT1 genetic polymorphisms for schizophrenia patients (n=249) and control subjects (n=210)

	Genotype, n (%)				Allele, n (%)		
rs2248632	A/A	A/G	G/G	p*	A	G	p*
Schizophrenia	6 (2.4)	61 (24.5)	182 (73.1)	0.946	73 (14.7)	425 (85.3)	1.000
Controls	6 (2.9)	50 (23.8)	154 (73.3)		62 (14.8)	358 (85.2)	
rs2248253	A/A	A/G	G/G	p*	A	G	p*
Schizophrenia	6 (2.4)	53 (21.5)	190 (76.3)	0.999	65 (13.1)	433 (86.9)	1.000
Controls	5 (2.4)	45 (21.4)	160 (76.2)		55 (13.1)	365 (86.9)	
rs1766967	A/A	A/G	G/G	p*	A	G	p*
Schizophrenia	6(2.4)	63(25.3)	180(72.3)	0.927	75 (15.1)	423 (84.9)	0.715
Controls	6 (2.9)	55 (26.2)	149 (71.0)		67 (16.0)	353 (84.0)	

Compared with control group.

from the expected values at the Hardy- Weinberg equilibrium for either cases or controls (Table 1). For the three GlyT1 polymorphisms tested, we found no significant differences in the frequency of the genotype distribution between schizophrenia and controls (Table 1).

The three markers (rs1766967, rs2248632 and rs2248253) were found to be in strong LD to each other both in the case and the control groups (Table 2). The results between groups are presented in Table 3. Global case-control haplotype analysis showed that there was no significant difference in haplotype distribution between the groups (P=0.473). Individual haplotype analysis showed that the frequencies of all haplotypes are similar in the control and case groups (Table 3).

Discussion

In the present study, the association of four polymorphisms in the GlyT1 locus and schizophrenia was investigated. To the best of our knowledge, this is the first study to investigate the role of GlyT1 in the pathogenesis of schizophrenia. No significant difference was observed between the controls and patients in allelic frequencies or genotypic distributions of the GlyT1 polymorphisms. Permutation tests showed no significant difference in estimated haplotype frequencies of the GlyT1 gene between the controls and patients. Thus, the present study provides no positive evidence of association between the GlyT1 locus and schizophrenia in a Chinese population. Although the current results do not provide evidence supporting the proposed relationship between schizophrenia and the GlyT1 polymorphisms investigated, an association with other GlyT1 polymorphisms, particularly functional ones, in the GlyT1 gene cannot be completely excluded. Another possible explanation for our negative findings could stem from the clinical heterogeneity of

Table 2.
Pairwise linkage disequilibrium (D' below diagonal) and statistical significance (r^2 and p value above diagonal) for the three GlyT1 polymorphisms in schizophrenia and normal controls

Schizophrenia			
	rs2248632	rs2248253	rs1766967
rs2248632	—	0.830 (p<0.001)	0.968 (p<0.001)
rs2248253	0.981	—	0.815 (p<0.001)
rs1766967	1.000	0.981	—
Normal control			
	rs2248632	rs2248253	rs1766967
rs2248632	—	0.796 (p<0.001)	0.877 (p<0.001)
rs2248253	0.956	—	0.690 (p<0.001)
rs1766967	0.980	0.932	—

Table 3.
Inferred haplotype frequency in GlyT1 gene and haplotype comparison between schizophrenia and control group

	Schizophrenia	Normal	Permutation p value
GGG	0.847	0.833	0.536
AAA	0.128	0.123	0.770
AGA	0.018	0.021	0.635
GGA	0.004	0.014	0.074
Global		0.473	

The order of the markers (left to right) is rs2248632, rs2248253 and rs1766967. Only haplotypes with frequency >1% are listed in this table.

schizophrenia, because schizophrenia probably comprises a group of disorders with heterogeneous aetiologies. Thus, there is still a possibility that associations occur in subsets of schizophrenic patients or that genetic variation in GlyT1 has a very small effect. In both circumstances, a larger sample size would be required to establish whether such associations exist.

Though the current study cannot find association between GlyT1 genetic variants and schizophrenia, others have shown that sarcosine, a GlyT1 inhibitor, improved negative symptoms and cognition in schizophrenic subjects receiving antipsychotics (Tsai et al., 2004a). Furthermore, GlyT1 may be involved in the therapeutic mecha-

nisms of antipsychotics such as clozapine (Williams et al., 2004). From these findings, it appears appropriate to recommend investigations to determine the relationship between GlyT1 genetic variants and the symptomatology or the antipsychotic-treatment response in schizophrenia.

Corresponding author:
Dr. Shih-Jen Tsai,
Taipei Veterans General Hospital, No. 201,
Shih-Pai Road, Sec. 2, 11217, Taipei, Taiwan
Tel: +886-2-2875 7027, ext. 276
Fax: +886-2-2872 5643
E-mail: cjhong@vghtpe.gov.tw

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