ORIGINAL ARTICLE

Association of the Glutamate Transporter Gene *SLC1A1* With Atypical Antipsychotics–Induced Obsessive-compulsive Symptoms

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Context: Several studies have indicated that atypical antipsychotics (AAP) induce obsessive-compulsive (OC) symptoms. Research exploring the mechanism of this phenomenon, however, has been extremely limited. Considering the indirect evidence of genetic control and difficulties in developing animal models and performing gene expression studies, genetic association studies could be an important approach to understanding the molecular mechanism of AAP-induced OC symptoms. The glutamate transporter gene *SLC1A1*, which was recently reported to be associated with obsessive-compulsive disorder (OCD), is a promising candidate gene for susceptibility to AAP-induced OC symptoms.

Objective: To determine whether polymorphisms in *SLC1A1* are associated with AAP-induced OC symptoms in patients with schizophrenia.

Design: A pharmacogenetic case-control association study.

Setting: Outpatient schizophrenia clinics.

Patients: Clinically stable patients with schizophrenia who were receiving AAP treatment (n=94; OC group). The OC group consisted of 40 patients with AAP-induced OC symptoms, and the non-OC group consisted of 54 patients who had received AAP for more than 24 months without developing OC symptoms.

Main Outcome Measures: Allele, genotype, and haplotype frequencies. The association was tested with a logistic regression model using age, sex, and medication type as covariates.

Results: Trends of association were observed in rs2228622 and rs3780412 (nominal $P=.01$; adjusted permutation $P=.07$) for the dominant model that was the inheritance model that best fit our data. In the haplotype-based analysis, the A/C/G haplotype at rs2228622-rs3780413-rs3780412 showed a significant association with AAP-induced OC symptoms; this association withstood multiple test correction (nominal $P=.01$; adjusted permutation $P=.04$; odds ratio, 3.955; 95% confidence interval, 1.366-11.452, for dominant model).

Conclusions: These results suggest that sequence variations in *SLC1A1* are associated with susceptibility to AAP-induced OC symptoms. This is the first published pharmacogenetic study on this phenomenon and provides preliminary evidence of the involvement of glutamatergic neurotransmission in the pathogenesis of AAP-induced OC symptoms.

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Since the introduction of atypical antipsychotics (AAP), both case reports[1-6] and clinical studies[7-9] have described the de novo onset of obsessive-compulsive (OC) symptoms during treatment with these drugs. In a previous investigation, we screened 209 clinically stable outpatients with schizophrenia who were receiving AAP and found that 12.4% showed AAP-induced OC symptoms.[10]

It is widely accepted that genetic factors play an important role in an individual's susceptibility to various AAP adverse effects,[11,12] and this assumption is the theoretical basis of pharmacogenetic studies. Nonetheless, heritability data for antipsychotic drug responses are extremely limited because of difficulties recruiting twin pairs who have received the same AAP treatment. We recently described clozapine-induced OC symptoms that emerged concordantly in a pair of monozygotic twins, which suggests the influence of genetic factors on this adverse effect.[13] Strong evidence of heritability has been shown by family and twin studies of obsessive-compulsive disorder (OCD).[14-17] Considering the indirect evidence of genetic control on AAP-induced OC symptoms and

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difficulties in developing animal models and performing gene expression studies, genetic association studies with candidate genes could play a pivotal role in exploring the molecular mechanism of AAP-induced OC symptoms.

Implications of the glutamatergic system in the development of OC symptoms were suggested by a few biological studies of patients with OCD, including magnetic resonance spectroscopy studies.[18-20] The glutamate transporter gene *SLC1A1* (OMIM 133550), which is located on chromosome 9p24, is regarded as a positional and functional candidate gene for vulnerability to OCD.[21] Recently, polymorphisms in the *SLC1A1* gene were reported to be associated with OCD by 3 independent studies.[22-24] *SLC1A1* codes for the glutamate transporter excitatory amino acid carrier 1 (EAAC1), which maintains extracellular glutamate concentrations by reuptake of synaptically released glutamate.[25] Excitatory amino acid carrier 1 is highly expressed in the cerebral cortex, striatum, and thalamus, regions that might be involved in the pathogenesis of OC symptoms. Regarding the relationship between EAAC1 and AAP, significant downregulation of EAAC1 by chronic treatment with clozapine was observed in the cingulate cortex of the rat brain.[26] Changes in transcript expression of excitatory amino acid transporter–interacting protein, which modulates subcellular localization and activity of the excitatory amino acid transporters, including EAAC1, were also described in clozapine-treated rats.[27] In animal studies that investigated the effects of AAP on the glutamatergic system, clozapine treatment enhanced K^+-stimulated glutamate release[28] and upregulated *N*-methyl-D-aspartate receptor binding[29] in the nucleus accumbens of rat brain. Increased expression of vesicular glutamate transporter mRNA[30] and decreased *N*-methyl-D-aspartate binding[31] by risperidone treatment were also reported. Considering the evidence of a biological relationship between the glutamatergic system and both OC symptoms and AAP, *SLC1A1* is a promising candidate gene for susceptibility to AAP-induced OC symptoms.

This study aimed to determine whether polymorphisms in *SLC1A1* are associated with AAP-induced OC symptoms in patients with schizophrenia. Genotype and haplotype frequencies were investigated in the OC and non-OC groups for 10 single-nucleotide polymorphisms (SNPs) in the 3′ region of *SLC1A1* including SNPs that showed significant association with OCD in previous studies.[22-24] As AAP-induced OC symptoms are most frequently observed in clozapine-treated patients,[1,8,10] we performed a subgroup analysis for clozapine-induced OC symptoms. To the best of our knowledge, this is the first pharmacogenetic study investigating AAP-induced OC symptoms.

METHODS

SUBJECTS

Subjects consisted of an OC group (patients with schizophrenia with AAP-induced OC symptoms) and a non-OC group (patients with schizophrenia without OC symptoms). Subject recruitment was performed at the schizophrenia clinics of Samsung Medical Center, Seoul National University Hospital, and Asan Medical Center. Clinically stable patients with *DSM-IV*–diagnosed schizophrenia who were receiving AAP and were capable of providing reliable information regarding their symptoms were included in the screening of OC symptoms. At each center, 2 trained psychiatrists (of J.S.K., Y.H.J., M.L., M.H.J., J.-S.C., B.K., and K.S.H.) performed clinical interviews, and the assessment processes were standardized across sites. To screen for the presence of OC symptoms, direct interviews using the Korean version of the Structured Clinical Interview Schedule for *DSM-IV* Axis I Disorder[32] and assessment using the Korean version of the Yale-Brown Obsessive Compulsive Scale[33-35] were performed for each patient by 2 psychiatrists independently. In addition, collateral information from available family members of the patients and hospital records were used to identify whether OC symptoms emerged de novo on AAP. The final decision on AAP-induced OC symptoms was made by consensus between the 2 raters and required the following criteria: (1) OC symptoms developed de novo while receiving AAP, (2) all of the criteria for *DSM-IV*-diagnosed OCD except for criteria C (description of severity) and E (exclusion of drug-induced cases) were met, and (3) OC symptoms were not the direct result of delusions, and their contents were clearly different from residual psychotic symptoms. Forty patients were included in the OC group. The non-OC group included 54 patients matched by sex and medicine (type of AAP) who had been receiving AAP for more than 24 months and had not developed any OC symptoms. One hundred controls were also included in this study to obtain data on Hardy-Weinberg equilibrium and haplotype structures of SNP markers. All of the subjects (OC and non-OC patient groups and controls) were ethnically Korean, which was ascertained by asking the ethnicity of both parents.

This study was approved by the institutional review boards of Samsung Medical Center, Seoul National University Hospital, and Asan Medical Center, and written informed consent was obtained from all subjects.

SNP SELECTION AND GENOTYPING

Thirteen SNPs spanning 25 kB toward the 3′ end of the *SLC1A1* gene were selected for genotyping. Nine showed significant association with OCD in previous studies[22-24] (**Figure 1**). Four SNPs were additionally selected in this region considering minor allele frequencies (http://www.hapmap.org/, Release 22/Phase II, April 2007, on National Center for Biotechnology Information B36 Assembly) and intermarker distances between markers (http://genome.ucsc.edu/, March 2006 Assembly). Genomic DNA was extracted from peripheral blood using a Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin) following the manufacturer's protocol. Three SNPs (rs10974625, rs3780412, and rs301430) were genotyped by single-base primer extension assay using the ABI PRISM SNaPshot Multiplex kit (Applied Biosystems, Foster City, California), and the other SNPs were genotyped by sequencing reaction using ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems). In both analyses, electrophoresis was done using the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Genotypes were measured using Gene Scan analysis software (Applied Biosystems) for single-base primer extension assays, and Sequencher v4.1.4 software (Gene Codes, Ann Arbor, Michigan) for sequencing reactions. All markers were typed successfully in all subjects.

STATISTICAL ANALYSIS

Differences in demographic and clinical variables between OC and non-OC groups were tested using a t test or χ^2 test. We evaluated the Hardy-Weinberg equilibrium of 13 SNPs using genotype data from controls. Because linkage disequilibrium

(LD) analysis showed that 3 pairs of SNPs were in perfect linkage equilibrium, 1 from each pair (rs7871691, rs7042333, and rs301435) was excluded from the analysis. Ten SNPs were used in the final statistical analysis (**Table 1**; Figure 1).

The allelic and genotypic association between SNPs and AAP-induced OC symptoms was initially tested using Pearson χ^2 test or Fisher exact test (depending on the sample sizes of individual cells). Our main analysis used to test for association was logistic regression, which controls for possible confounding effects. In this analysis, sex, age, and medicine (type of antipsychotic drug) were used as model covariates. For the genetic model, we considered 4 types—additive, codominant, dominant, and recessive—based on the minor allele of each SNP. The inheritance model with the least Akaike Information Criterion[36] was accepted as the best-fitting model.

Haploview v4.0 (http://www.broadinstitute.org/haploview/haploview)[37] was used to estimate pairwise LD of SNP markers. The default confidence interval algorithm of the Haploview program identified 1 haplotype block consisting of SNP7 (rs301430) and SNP8 (rs301979) from the OC and non-OC patient group data (**Figure 2**). Another haplotype block consisting of SNP2 (rs2228622), SNP3 (rs3780413), and SNP4 (rs3780412) was added in the analysis, considering strong pairwise LD between markers ($D' \geq 0.88$) and results of single-marker analysis. In each of the 3 subject groups (OC, non-OC, and control), SNPs within the same haplotype block showed strong pairwise LD ($D' \geq 0.79$ for SNP2/SNP3/SNP4 block and $D' \geq 0.84$ for SNP7/SNP8 block) with a low LD gap between these 2 haplotype blocks. Haplotypes of individual subjects were estimated using SAS/Genetics haplotype procedure (SAS Institute Inc, Cary, North Carolina). Logistic regression was applied to test the association between haplotypes and AAP-induced OC symptoms. To use this regression model with haplotype data, ambiguity of haplotypes was additionally adjusted using each subject's probability of having a particular haplotype pair. The most frequent haplotype was automatically selected as the reference category, and rare haplotypes (haplotype frequency ≤ 0.05) were pooled together in a group.

Considering multiple comparisons, we performed 10 000 permutations and obtained an adjusted *P* value for each analysis. We adopted the step-down minP multiple testing procedure proposed by Westfall and Young[38] and Ge et al.[39]

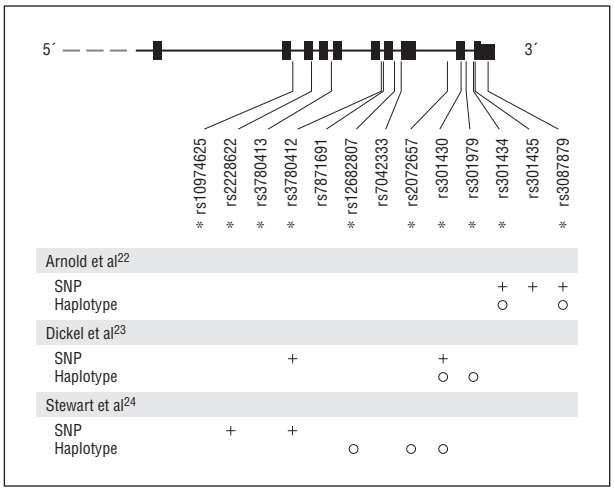

Figure 1. *SLC1A1* organization and single-nucleotide polymorphism (SNP) locations (from the University of California at Santa Cruz Genome Browser, March 2006 Assembly). The horizontal line represents the genomic sequence and vertical bars represent exons. Plus signs and circles denote SNPs significantly associated with obsessive-compulsive disorder in single-marker and haplotype analyses of previous studies,[22-24] respectively, with asterisks representing SNPs included in the current association study of atypical antipsychotics–induced obsessive-compulsive symptoms.

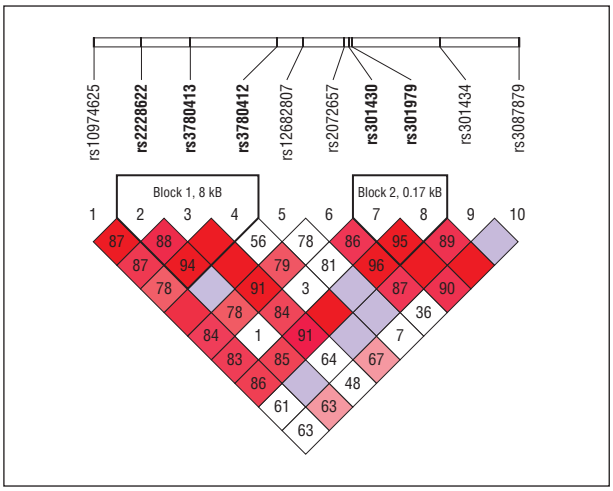

Figure 2. Linkage disequilibrium (LD) structure of the single-nucleotide polymorphisms and haplotype blocks analyzed in the current study. The number, in each LD indicates the value of D′ (D′=1 not shown) calculated from patient (both obsessive-compulsive [OC] and non-OC groups) data. Block 2 is determined using the default confidence interval algorithm of Haploview 4.0. Block 1 is an additional block including SNPs that show significant association with atypical antipsychotics–induced OC symptoms in single-marker analysis, and having high intermarker LD ($D' \geq 0.88$).

Table 1. Characteristics of SNP Markers on *SLC1A1*

SNP No.	rs No.	Location	Gene/Region	Statistical Tests for HWE			Allele	MAF
				χ^2	*df*	*P* Value		
1	rs10974625	4551 596	Intron 3	0.560	1	.45	G>A	0.424
2	rs2228622	4554 432	Exon 4(syn)	0.205	1	.65	G>A	0.318
3	rs3780413	4557 353	Intron 5	0.956	1	.33	C>G	0.268
4	rs3780412	4562 480	Intron 7	0.572	1	.45	A>G	0.308
5	rs12682807	4564 022	Intron 8	0.189	1	.66	A>C	0.162
6	rs2072657	4566 451	Intron 9	1.785	1	.18	T>G	0.273
7	rs301430	4566 680	Exon 10(syn)	0.237	1	.63	C>T	0.424
8	rs301979	4566 851	Intron 10	3.253	1	.07	C>G	0.258
9	rs301434	4572 082	Intron 10	1.115	1	.29	A>G	0.096
10	rs3087879	4576 808	3′-UTR	0.190	1	.66	G>C	0.157

Abbreviations: HWE, Hardy-Weinberg equilibrium in controls; MAF, minor allele frequency in controls; SNP, single-nucleotide polymorphism; syn, synonymous SNP; UTR, untranslated region.

Table 2. Demographic and Clinical Characteristics of Subjects

Variables	Group: OC[a] (n=40)	Group: Non-OC[b] (n=54)	Statistic	*P* Value
Mean (SD) age, y	31.1 (7.1)	34.6 (8.3)	$t = -2.120$	.04
Sex, No. (%)				
Male	23 (57.5)	37 (68.5)	$\chi^2 = 1.208$	.27
Female	17 (42.5)	17 (31.5)		
Subtype of schizophrenia, No. (%)				
Paranoid	9 (22.5)	11 (20.4)	$\chi^2 = 0.843$	>.99
Disorganized	0 (0.0)	1 (1.8)		
Undifferentiated	3 (7.5)	4 (7.4)		
Residual	28 (70.0)	38 (70.4)		
Mean (SD) duration of illness, y	10.7 (5.9)	12.1 (6.5)	$t = -1.076$	.28
Mean (SD) duration of medication use, mo	28.8 (26.9)	60.1 (30.9)	$t = 4.969$	<.001
Type of antipsychotics, No. (%)				
Clozapine	32 (80.0)	43 (79.6)		
Olanzapine	5 (12.5)	5 (9.3)	$\chi^2 = 0.399$	.93
Risperidone	3 (7.5)	6 (11.1)		
Mean (SD) maximum daily dose of antipsychotics, mg				
Clozapine	380.5 (140.5)	362.2 (142.9)	$t = -0.551$	.58
Olanzapine	19.0 (5.5)	15.0 (5.0)	$t = -1.206$	.26
Risperidone	3.0 (1.4)	4.8 (1.8)	$t = 1.250$	.27

[a] Patients with schizophrenia with atypical antipsychotics–induced obsessive-compulsive (OC) symptoms.
[b] Patients with schizophrenia who received atypical antipsychotics for more than 24 months without developing OC symptoms.

Identical association tests were applied for a subgroup of patients receiving clozapine (32 in the OC group and 43 in the non-OC group).

Statistical analyses were performed using SAS version 9.1 (SAS Institute Inc). *P* values of less than .05 were considered statistically significant.

RESULTS

Demographic and clinical characteristics of the 2 patient groups are presented in **Table 2**. Patients in the OC group were younger than those in the non-OC group. There was no difference in duration of illness, clinical subtype, type of AAP, and mean maximum daily dose of each drug that has ever been reached between the OC and non-OC groups. The mean (SD) score of Yale-Brown Obsessive Compulsive Scale for the OC group was 14.89 (6.65).

For all 10 SNPs used in the analysis, no significant deviation from Hardy-Weinberg equilibrium was observed in the controls (Table 1). **Table 3** shows results of allelic and genotypic association analyses using a simple χ^2 (or Fisher exact) test without controlling for possible confounding effects of demographic or clinical variables. Single nucleotide polymorphisms 2 (rs2228622) and 4 (rs3780412) showed an association with AAP-induced OC symptoms. However, no marker reached the level of significance after applying multiple test correction in the permutation tests.

In the logistic regression that controlled for confounding effects of age, sex, and medicine (**Table 4**), trends of association were observed in SNP2 (nominal $P=.01$; adjusted permutation $P=.07$) for dominant and codominant models (nominal $P=.007$; adjusted permutation $P=.06$) and SNP4 for dominant models (nominal $P=.02$; adjusted permutation $P=.09$). As represented in the Akaike Information Criterion values, the dominant model was accepted as the best inheritance model fitting our data for both SNP2 and SNP4. Other SNPs did not show any trend of association with AAP-induced OC symptoms.

In the haplotype-based logistic regression analysis, an association with nominal $P<.05$ was observed for the A/C/G haplotype of the SNP2/SNP3/SNP4 block in additive, codominant, and dominant models (nominal $P=.04$, $P=.006$, and $P=.01$, respectively) (**Table 5**). The dominant model showed the lowest Akaike Information Criterion value and was accepted as the best inheritance model. The other haplotype block (SNP7/SNP8) did not show any association with AAP-induced OC symptoms. In permutation tests adjusting for multiple comparisons, the association between A/C/G haplotype of SNP2/SNP3/SNP4 and AAP-induced OC symptoms remained significant for dominant and codominant models.

In the subgroup of patients receiving clozapine treatment, allelic and genotypic association was observed in SNP2 ($\chi^2=4.906$, $P=.03$ for allelic association; $\chi^2=7.074$, $P=.02$ for genotypic association) and SNP4 ($\chi^2=5.900$, $P=.02$ for allelic association; $\chi^2=6.214$, $P=.04$ for genotypic association). However, no marker reached statistical significance after applying multiple test correction. Regression analysis showed trends of association in SNP2 for dominant (nominal $P=.01$; adjusted permutation $P=.07$) and codominant (nominal $P=.01$; adjusted permutation $P=.07$) models (Table 4). In the haplotype-based analysis, association of the A/C/G haplotype of SNP2/SNP3/SNP4 block and clozapine-induced OC symptoms was observed in the additive, codominant, and dominant models (nominal $P=.02$, $P=.008$, and $P=.02$, respectively) (Table 5). After adjusting for multiple

Table 3. Genotypic and Allelic Association Analysis

SNP by Group	Genotype Count			Genotypic Association		Allelic Association	
				P Value[a]	Adjusted *P* Value[b]	*P* Value[c]	Adjusted *P* Value[b]
SNP1							
rs10974625	GG	AG	AA				
OC group[d]	8	28	4				
Non-OC group[e]	12	29	13				
SNP2							
rs2228622	GG	AG	AA	.02[f]	.15	.04[f]	.19
OC group	16	21	3				
Non-OC group	36	14	4				
SNP3							
rs3780413	CC	CG	GG	.29	.77	.48	.96
OC group	23	14	3				
Non-OC group	24	28	2				
SNP4							
rs3780412	AA	AG	GG	.03[f]	.18	.02[f]	.09
OC group	15	20	5				
Non-OC group	35	15	4				
SNP5							
rs12682807	AA	AC	CC	.91	.97	.75	.99
OC group	27	12	1				
Non-OC group	38	15	1				
SNP6							
rs2072657	TT	GT	GG	.70	.97	.81	.99
OC group	19	17	4				
Non-OC group	25	26	3				
SNP7							
rs301430	CC	CT	TT	.56	.97	.42	.95
OC group	12	21	7				
Non-OC group	18	31	5				
SNP8							
rs301979	CC	CG	GG	.08	.32	.96	>.99
OC group	22	15	3				
Non-OC group	26	28	0				
SNP9							
rs301434	AA	AG	GG	.78	.97	.67	.99
OC group	34	6	0				
Non-OC group	44	10	0				
SNP10							
rs3087879	GG	CG	CC	.62	.97	.69	.99
OC group	28	12	0				
Non-OC group	41	12	1				

Abbreviations: OC, obsessive-compulsive; SNP, single-nucleotide polymorphism.
[a] Fisher exact tests, case vs control (2×3).
[b] A total of 10 000 permutations and step-down minP multiple testing procedure proposed by Westfall and Young[38] and Ge et al.[39]
[c] Fisher exact tests, case vs control (2×2).
[d] Patients with schizophrenia with atypical antipsychotics–induced OC symptoms.
[e] Patients with schizophrenia who received atypical antipsychotics for more than 24 months without developing OC symptoms.
[f] $P < .05$.

comparisons in the permutation test, significant association remained for the codominant model.

COMMENT

Several lines of evidence indicate that AAP induces or aggravates OC symptoms.[1-9] To the best of our knowledge, however, there has been no pharmacogenetic study that explored candidate genes involved in this adverse drug reaction. In the present study, we investigated the relation between AAP-induced OC symptoms and 10 SNPs in the *SLC1A1* gene that was recently reported to be associated with OCDs in 3 independent studies.[22-24]

Obsessive-compulsive symptoms can be observed in patients in various stages of schizophrenia,[40,41] ie, before the onset of illness, in the active psychotic phase, and after stabilization by pharmacotherapy. To limit the phenotype of the current study to drug-associated OC symptoms, we applied strict inclusion criteria, ie, de novo onset of OC symptoms during AAP treatment and OC symptoms of an egodystonic nature, reported by clinically stable patients, that could be clearly differentiated from delusions or other residual psychotic symptoms. According to a summary of 55 reported cases,[1] the duration of AAP medication at the onset of AAP-induced OC symptoms ranged from a few weeks to 15 months. Therefore, we included patients who had been

Table 4. Association Analysis of AAP-Induced OC Symptoms With SNP2 and SNP4 on *SLC1A1* Using Logistic Regression Model Adjusted for Age, Sex, and Type of Medicine

SNP Model	χ^2	*P* Value	Adjusted *P* Value[a]	OR (95% CI)	AIC	Global Test[b] χ^2	Global Test[b] *P* Value	Global Test[b] Adjusted *P* Value[a]
All Subjects								
SNP2								
Additive	3.985	.05[c]	.26	2.076 (1.013-4.253)	129.198			
Codominant								
2 vs 0	0.353	.55	.99	1.683 (0.302-9.382)	127.765	7.226	.03[c]	.11
1 vs 0	7.226	.007[c]	.06	3.772 (1.433-9.932)				
Dominant	6.504	.01[c]	.07	3.275 (1.316-8.151)	126.570			
Recessive	0.000	>.99	>.99	0.998 (0.191-5.220)	133.400			
SNP4								
Additive	4.672	.03[c]	.19	2.146 (1.074-4.287)	128.463			
Codominant								
2 vs 0	1.825	.18	.81	2.919 (0.617-13.807)	129.213	5.947	.05	.21
1 vs 0	5.440	.02[c]	.16	3.102 (1.198-8.032)				
Dominant	5.942	.02[c]	.09	3.066 (1.245-7.547)	127.219			
Recessive	0.548	.46	.70	1.750 (0.398-7.701)	132.846			
Subgroup With Clozapine Treatment								
SNP2								
Additive	4.661	.03[c]	.18	2.516 (1.089-5.812)	102.353			
Codominant								
2 vs 0	0.518	.47	.97	2.193 (0.259-8.593)	102.374	6.607	.04[c]	.15
1 vs 0	6.589	.01[c]	.07	3.780 (1.369-10.433)				
Dominant	6.395	.01[c]	.07	3.553 (1.330-9.490)	100.621			
Recessive	0.023	.88	.92	1.172 (0.147-9.322)	107.321			
SNP4								
Additive	4.517	.03[c]	.18	2.371 (1.069-5.256)	102.525			
Codominant								
2 vs 0	2.103	.15	.70	4.048 (0.612-26.777)	104.200	4.954	.08	.34
1 vs 0	4.097	.04[c]	.30	1.110 (0.403-3.055)				
Dominant	4.841	.03[c]	.16	2.993 (1.127-7.949)	102.338			
Recessive	0.887	.35	.48	2.390 (0.390-14.668)	106.413			

Abbreviations: AAP, atypical antipsychotics; AIC, Akaike's information criterion[36]; CI, confidence intervals; OC, obsessive-compulsive; OR, odds ratio; SNP, single-nucleotide polymorphism; 0, major homozygous; 1, heterozygous; 2, minor homozygous.

[a] 10 000 permutations and step-down minP multiple testing procedure proposed by Westfall and Young[38] and Ge et al.[39]

[b] Global test for codominant model.

[c] $P < .05$.

receiving AAP for more than 24 months without development of any OC symptoms in the non-OC group. Because AAP-induced OC symptoms showed preponderance in men and were most frequently observed in clozapine-treated patients,[1,8,10] we tried to match sex and type of AAP between the OC and non-OC groups. We also controlled for these variables in the logistic regression model used in the analysis. In addition, we analyzed patients receiving clozapine separately.

Trends of association were detected between AAP-induced OC symptoms and SNP2 (rs2228622) and SNP4 (rs3780412). These 2 SNPs are tightly linked within the same haplotype block (SNP2/SNP3/SNP4) of *SLC1A1*, and the A/C/G haplotype of this block showed significant association with AAP-induced OC symptoms, which withstood multiple test correction. The haplotype association indicated that the odds of AAP-induced OC symptoms are 3.96 times higher in patients who have the A/C/G haplotype than in patients who have the reference haplotype (G/C/A, most frequent haplotype; frequency, 0.44) (odds ratio, 3.955; 95% confidence interval, 1.366-11.452). The same nature of association was observed in the separate analysis of the subgroup of patients receiving clozapine treatment.

Interestingly, the associated SNPs (SNP2 and SNP4) in the present study are identical to the SNPs that showed significant association with OCD in the investigation of Stewart et al,[24] and one of them (SNP4) was also associated with OCD in another study.[23] Single-nucleotide polymorphism 2 is a synonymous SNP located in exon 4, and SNP4 is an intronic SNP. The possible functional effects of these SNPs have not yet been investigated. If the polymorphisms do not directly confer susceptibility to AAP-induced OC symptoms, they might be in LD with nearby functional SNPs. Genotyping of additional polymorphisms and extensive sequencing in this region are warranted to clarify the causative variants. Given that clozapine showed the strongest potential to induce OC symptoms of the AAP and was reported to downregulate EAAC1 in the cingulate cortex of rat brain,[26] and given the indirect evidence that other antipsychotic drugs also affect the glutamatergic system,[29,42,43] the etiologic connection between the *SLC1A1* gene and molecular mechanisms of AAP-induced OC symptoms needs to be more intensively pursued.

Table 5. Association Analysis of AAP-Induced OC Symptoms With Haplotype (SNP2/SNP3/SNP4) Block on *SLC1A1* Using Logistic Regression Model Adjusted for Age, Sex, and Type of Medicine

Model by Parameter	χ^2	*P* Value	Adjusted *P* Value[a]	OR (95% CI)	AIC	Global Test[b] χ^2	Global Test[b] *P* Value	Global Test[b] Adjusted *P* Value[a]
All Subjects								
Additive								
A/C/G	4.347	.04[c]	.13	2.415 (1.054-5.530)	132.163			
G/G/A	0.431	.51	.82	1.352 (0.550-3.323)				
Rare group[d]	1.226	.27	.58	2.583 (0.482-13.848)				
Codominant								
A/C/G					133.755	.454	.02[c]	.03[c]
2 vs 0	0.696	.40	.86	2.219 (0.341-14.436)				
1 vs 0	7.425	.006[c]	.02[c]	4.676 (1.542-14.178)				
G/G/A					1.417	.49	.86	
2 vs 0	1.409	.24	.67	4.407 (0.381-51.002)				
1 vs 0	0.084	.77	>.99	1.171 (0.401-3.416)				
Rare group[d]					1.715	.42	.76	
2 vs 0	0.013	.91	.97	2.947 (0.000-381 267 581.411)				
1 vs 0	1.707	.19	.64	3.217 (0.557-18.561)				
Dominant								
A/C/G	6.427	.01[c]	.04[c]	3.955 (1.366-11.452)	129.580			
G/G/A	0.316	.57	.96	1.348 (0.476-3.817)				
Rare group[d]	1.517	.22	.59	2.967 (0.526-16.745)				
Recessive								
A/C/G	0.001	.98	>.99	1.021 (0.194-5.368)	136.993			
G/G/A	0.409	.52	.84	2.059 (0.225-18.826)				
Rare group	0.001	.98	>.99	1.239 (0.000-161 000 817.407)				
Subgroup With Clozapine Treatment								
Additive								
A/C/G	5.105	.02[c]	.08	3.114 (1.162-8.340)	105.276			
G/G/A	0.603	.44	.74	1.498 (0.540-4.151)				
Rare group[d]	1.291	.26	.64	2.389 (0.532-10.730)				
Codominant								
A/C/G								
2 vs 0	0.790	.37	.87	2.866 (0.281-29.214)	105.216	7.076	.03[c]	.03[c]
1 vs 0	7.071	.008[c]	.02[c]	5.030 (1.529-16.548)				
G/G/A								
2 vs 0	3.041	.08	.22	12.973 (0.728-231.246)		3.372	.18	.20
1 vs 0	0.000	>.99	>.99	0.996 (0.305-3.254)				
Rare group[d]								
2 vs 0	0.705	.40	.88	105.278 (0.002-5 526 117.238)		0.803	.67	.99
1 vs 0	0.115	.74	>.99	1.445 (0.171-12.196)				
Dominant								
A/C/G	5.328	.02[c]	.07	3.721 (1.219-11.354)	104.758			
G/G/A	0.020	.89	.99	1.085 (0.355-3.316)				
Rare group[d]	0.611	.43	.91	2.086 (0.330-13.184)				
Recessive								
A/C/G	0.053	.82	.89	1.278 (0.158-10.363)	108.142			
G/G/A	1.673	.20	.07	5.620 (0.411-76.893)				
Rare group[d]	0.456	.50	.64	41.575 (0.001-2 074 295.218)				

Abbreviations: AAP, atypical antipsychotics; AIC, Akaike's information criterion[36]; CI, confidence intervals; OC, obsessive-compulsive; OR, odds ratio; SNP, single-nucleotide polymorphism; 0, major homozygous; 1, heterozygous; 2, minor homozygous.

[a] 10 000 permutations and step-down minP multiple testing procedure proposed by Westfall and Young[38] and Ge et al.[39]

[b] Global test for codominant model.

[c] $P < .05$.

[d] Rare group, pooling of rare haplotypes (frequency ≤0.05); reference haplotype, G/C/A (most frequent haplotype, frequency = 0.44).

The present study has certain methodological limitations. First, the possibility that patients in the non-OC group (defined as lacking OC symptoms after 24 months of treatment) would later develop OC symptoms could not be ruled out because there has been no systematic evaluation of the duration of medication before the onset of AAP. Delayed onset of phenotypes might be an inevitable confounding factor in genetic studies. Second, considering the small sample size and adoption of multiple test corrections, this study might be vulnerable to type II error. Replication studies in independent samples are required. Third, we investigated only 1 gene implicated in OCD. There might be a large number of functional candidate genes for AAP-induced OC symptoms,

eg, genes involved in the dopaminergic and serotonergic systems that are involved in the mechanism of action of AAPs. Association studies for those genes and evaluation of gene-gene interactions are needed in future studies. Because AAPs are used for the treatment of psychiatric disorders other than schizophrenia, association studies for a broader range of diagnoses are also warranted. Fourth, though all subjects in the OC and non-OC groups were ethnically Korean, population differences between the 2 groups have not been checked at the genetic level. Therefore, the possibility of a false-positive result originating from undetected population stratification could not be completely excluded.

Within the above discussed limitations, the findings in the current study suggest that *SLC1A1* is involved in susceptibility to developing OC symptoms in patients with schizophrenia who are receiving AAP treatment. It is noteworthy that this is the first published pharmacogenetic study on this condition, and it provides preliminary evidence of the involvement of glutamatergic neurotransmission in the pathogenesis of AAP-induced OC symptoms.

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