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SHORT REPORT

No influence of *SLC6A3* 40 base VNTR polymorphism on the response to risperidone

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Abstract

Objectives. The SLC6A3 40 base variable number of tandem repeats (VNTR) polymorphism has been associated with several clinical phenotypes associated with dysregulation of dopamine transmission. However, there is only little evidence about a possible influence of such genetic variant on the response to antipsychotics. The aim of the present study is to investigate whether SLC6A3 40 base VNTR polymorphism could modulate response to risperidone in a sample of Korean schizophrenia subjects. *Methods.* One hundred and forty-two schizophrenia inpatients were treated with a flexible dose of risperidone. Efficacy was assessed at baseline and at discharge using the scores of the Clinical Global Impression-severity (CGI-S), Brief Psychiatric Rating Scale (BPRS) and Positive and Negative Symptom Score (PANSS). Multivariate analysis of covariance was used to test possible influences of SLC6A3 VNTR variants on clinical scores. *Results.* None of the genotypes and of the alleles under investigation was associated with clinical scores at discharge or with changes of clinical scores over time. In addition, we also failed to find any association between genotypes and allele frequency distribution in accordance with treatment response defined as a 20% (or 30%) or more reduction in the total PANSS scores from the baseline to the end of treatment. *Conclusion.* Our findings do not suggest a possible association between SLC6A3 40 base VNTR polymorphism and response to risperidone. However, because of several limitations including the investigation of a single drug, the flexible design of the present study and the absence of a complete coverage of features which could influence the response, further investigations could be required.

Key Words: *Risperidone, pharmacogenetics, schizophrenia, antipsychotics drugs*

Introduction

The dopamine transporter (DAT) is involved in the reuptake of dopamine in the presynaptic terminal and plays a key role in the regulation of dopamine neurotransmission. The gene coding for DAT, *SLC6A3* (solute carrier family 6, member 3), is located on chromosome 5p15.3 [1]. The most investigated polymorphism in *SLC6A3* is a 40 base variable number of tandem repeats (VNTR) located in the 3' untranslated region of the gene [1]. In the majority of samples, most common alleles of *SLC6A3* are the 9 and 10 repeat alleles, even though other variants including 3, 5, 7, 8 and 11 repeat alleles have been observed as well [2].

SLC6A3 40 base VNTR polymorphism has been associated with several clinical phenotypes associated with dysregulation of dopamine transmission. Several studies have suggested, in fact, an association between *SLC6A3* variants and ADHD [3], history of cocaine induced paranoia among cocaine dependent subjects [4], severity of alcohol withdrawal [5,6] and tobacco use [7,8]. On the other hand, the same polymorphism was not associated with cocaine dependence [4], methamphetamine abuse [9], polysubstance abuse [10], delusional disorder [11], Tourette's syndrome [12], alcoholism [13] and schizophrenia [14].

Even though *SLC6A3* 40 base VNTR does not seem to be associated with schizophrenia, it is noteworthy, however, that it could be related to a number of schizophrenia-related features. Guzey and colleagues recently observed that schizophrenia subjects treated with different antipsychotics carrying the 9-allele repeat of *SLC6A3* were more likely to have extrapyramidal symptoms in comparison to other

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subjects [15], even though this finding was not replicated in a following study [16]. In a further study, schizophrenia subjects carrying the 9-allele repeat of *SLC6A3* performed better on some domains of the Wisconsin Card Sorting Test (WCST) [17]. Also, a recent study showed that the combination of the 9/10 *SLC6A3* genotype and the dopamine receptor D3 Ser/Ser genotype had a protective effect against schizophrenia. Note, however, that replications of these findings are still lacking and that, accordingly, they should be interpreted with caution. Indeed, further neuroimaging studies failed to find any association between *SLC6A3* 40 base VNTR polymorphism and DAT binding in patients suffering from schizophrenia [16,18]. Additionally, two studies investigating the effects of the same genetic variant on the response to atypical antipsychotics in a Caucasian sample [19] and to risperidone in a Chinese sample of schizophrenia patients [20] did not observe any influence on drug response.

To the best of our knowledge, no study has yet focused on the influence of *SLC6A3* 40 base VNTR on the response to antipsychotics in Korean schizophrenia patients. As a consequence, the aim of the present study is to investigate whether *SLC6A3* 40 base VNTR could modulate risperidone response in a sample of Korean subjects suffering from schizophrenia.

Methods

Sample description

One hundred and forty-two schizophrenia inpatients were recruited in the Department of Psychiatry of the Catholic University of Korea College of Medicine, Seoul, Republic of korea. Patients were eligible for inclusion if they had documented clinical diagnosis of schizophrenia according to the DSM-IV criteria assessed by Mini-International Neuropsychiatric Interview (MINI) [21]. Exclusion criteria were comorbidities with other Axis I psychiatric disorders, neurological disorders or severe unstable medical conditions, risk for suicide including either having active clinically significant suicidal ideation or recently attempted suicide, a significant history of intolerance to multiple antipsychotic treatments, current treatment with a long-acting antipsychotic and a history of antipsychotic malignant syndrome. All patients (18–65 years old) provided written informed consent before participating in the study. All patients were treated with risperidone and were followed prospectively from admission to discharge. Dosage of risperidone was flexibly adjusted during the course of the study and it was recorded at discharge. Demographical and clinical features were recorded as well.

Efficacy evaluation

Efficacy was assessed at baseline and at discharge using the CGI-S severity scale [22], the Brief Psychiatric Rating Scale [23] and Positive and negative symptoms scale (PANSS) [24]. PANSS subscales' scores including positive, negative and general subscales were recorded as well. All clinical and safety assessments were completed by trained investigators blind with respect to the genotypes of the patients.

DNA analysis

Genomic DNA was extracted from blood by standard methods and quantified. SLC6A3 VNTR polymorphisms were genotypized as described elsewhere [20].

Statistical analysis

Multivariate analysis of covariance (MANCOVA) was used to test possible influences of *SLC6A3* 40 base VNTR on clinical scores. On account of the paucity of genotypes other than the 9/10 and the 10/10 VNTR, subjects carrying such variants were grouped together in the sub-sample "other". With the aim of reducing possible sources of variance, we included sex and age as covariates in the MANCOVA model. Further parameters which were significantly correlated to clinical scores were introduced in our statistical model as described below. Statistical analyses were performed using "Statistica" package [25]. Haploview 3.2 was employed to test for the Hardy–Weinberg equilibrium (HWE) [26]. All P values were two-tailed. Statistical significance was set at the 0.05 level. With these parameters we had a sufficient power (0.80) to detect a small medium effect size of 0.2, allowing to detect, for example, a difference of 1.8 scores on the PANSS severity at discharge between subjects carrying the 10/10 and the 9/10 genotype [27].

Results

Socio-demographic features of the sample

Socio demographical features of the global sample and of the sample displayed according to genotype are showed in Table I. There were no significant associations *SLC6A3* variants and clinical-demographic scores at baseline. However, we observed a significant correlation between PANSS positive scores at baseline and days of admission ($\beta=0.18$; $P=0.03$) as well as final dose of risperidone ($\beta=0.21$; $P=0.009$). As a consequence, we introduced such variables along with sex and age as covariates in the analysis of the PANSS positive scores.

Table I. Clinical and demographic features at baseline of the global sample and of the sample divided according to genotypes.

Baseline sample characteristics	Overall sample (142)	*SLC6A3* 10-10 genotype (116)	*SLC6A3* 9-10 genotype (16)	*SLC6A3* miscellaneous genotypes (10)
Gender				
Males (%)	67 (47%)	56 (48%)	7 (44%)	4 (40%)
Females (%)	75 (53%)	60 (52%)	9 (56%)	6 (60%)
Familiarity				
Positive	31 (21.83%)	25 (21.55%)	2 (12.50%)	4 (40%)
Negative	79 (55.63%)	63 (54.32%)	11 (68.75%)	5 (50%)
Missing	32 (22.54%)	28 (24.13%)	3 (18.75%)	1 (10%)
Age (mean±sd)	30.51±9.56	30.50±9.48	30.68±6.81	30.40±10.84
Age at onset	25.59±7.42	25.56±7.73	25.93±5.61	25.40±6.76
Duration of illness	4.92±6.40	4.94±6.81	4.75±3.35	5.00±5.43
Days of hospitalisation	37.98±13.08	36.78±12.85	38.61±12.27	38.20±17.35
Final dose	5.72±1.91	5.65±1.87	6.12±2.24	6.00±1.88
Total PANSS	89.71±11.60	90.49±11.89	87.43±10.00	84.30±9.15
PANSS positive	24.38±4.48	24.44±4.66	24.56±3.74	23.30±3.56
PANSS negative	20.45±4.52	20.67±4.66	19.31±3.45	19.70±4.29
PANSS general	44.88±6.67	45.37±6.83	43.56±5.16	41.30±5.75
BPRS	34.42±8.21	34.48±7.72	36.31±11.73	30.70±6.48
CGI	5.33±0.74	5.37±0.73	5.18±0.75	5.00±0.81

Genetic analysis

Hardy–Weinberg equilibrium (HWE) for the *SLC6A3* 40 base VNTR was 0.98. The frequencies of the *SLC6A3* VNTR polymorphism were 116 (82%) for the 10/10 genotype, 16 (11%) for the 9/10 genotype and 10 (7%) for other genotypes. These frequencies were not significantly different from those reported in previous studies in Asian populations (Chinese [20] and Korean [28]) with respect to *SLC6A3* 40 base VNTR. None of the genotype and of the alleles under investigation was significantly associated to clinical scores at discharge (all P values >0.05). Similarly, repeated-measures ANOVA did not show any influence of *SLC6A3* 40 base VNTR polymorphisms on clinical scores over time from admission to discharge (all P values >0.05).

Discussion

The aim of the present study was to investigate whether *SLC6A3* 40 base VNTR polymorphism could modulate response to risperidone in a sample of Korean schizophrenia subjects. We did not observe any influence of such polymorphism on drug response. Notably, our results are in accordance with previous findings in both Caucasian [19] and Chinese [20] populations of schizophrenia patients. Additionally, they are similar to those reported in a further association study between the *SLC6A3* 40 base VNTR and response to risperidone in delirium patients which failed to find any association as well [29]. As a consequence, current findings do not support any evidence for an association between *SLC6A3* 40 base VNTR polymorphism and response to common antipsychotics in psychotic disorders.

Additionally, data about whether the *SLC6A3* 40 base VNTR is involved in the function of *SLC6A3* are conflicting [18,30–32]. Overall these findings, along with those suggesting no evidence for an associations between *SLC6A3* 40 base VNTR polymorphisms and schizophrenia [14] raise important questions about the role of *SLC6A3* in schizophrenia. As previously reported, there is some evidence suggesting a possible modulator effect of such variant on cocaine induced paranoia among cocaine dependent subjects [4], appearance of extrapyramidal symptoms [15] and performances on the WCST in schizophrenia patients [17]. As a consequence, it could be preliminary suggested that *SLC6A3* 40 base VNTR could influence some psychosis-related traits but not psychosis and response to antipsychotics themselves. Other assumption may include that current response criteria to detect statistical significance relating to the effect of *SLC6A3* 40 base VNTR on risperidone should not be proper. The current psychometric rating scale is not sensitive to detect difference occurred in pharmacogenetic studies. The included subjects were not previously drug naive but had chronic clinical course, which might also affect the limited response to risperidone resulting in negative impact on the *SLC6A3* 40 base VNTR pharmacogentic result.

The present work is affected by several limitations that should be taken into account in the interpretation of our findings. First of all, we had only a sufficient power to detect small-medium effect size so that smaller effects exerted by the polymorphism under investigation could not be noticed. Second, our findings observed in Korean schizophrenia subjects treated with risperidone could not be generalized to other populations

of subjects treated with different antipsychotics. Further limitations of the present study include the different duration of patients' admission, the flexible dosage of risperidone and the incomplete coverage of further factors that could influence the response such as the appearance of adverse events and social support.

In conclusion, our results do not suggest any influence of *SLC6A3* 40 base VNTR polymorphism on the response to risperidone in Korean schizophrenia patients. Further studies could be needed in order to extend our findings in other populations of patients treated with different antipsychotics.

Key points

- The SLC6A3 40 base variable number of tandem repeats (VNTR) polymorphism has been associated with several clinical phenotypes associated with dysregulation of dopamine transmission.
- None of the genotypes and of the alleles under investigation was associated with clinical scores at discharge or with changes of clinical scores over time. In addition, we also failed to find any association between genotypes and allele frequency distribution in accordance with treatment response defined as a 20% (or 30%) or more reduction in the total PANSS scores from the baseline to the end of treatment.
- Further researches are needed to confirm the role of SLC6A3 40 VNTR for antipsychotic response.

Statement of interest

None.

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