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RESEARCH NOTE

Lack of association between the –839C/T polymorphism in the *SLC6A3* gene promoter and schizophrenia in the Iranian population

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Introduction

Schizophrenia is a severely debilitating psychiatric disorder that affects about 1% of the world's population with similar prevalence throughout different geographic areas (Thaker and Carpenter 2001). Family, twin and adoption studies have shown high heritability for schizophrenia (McGuffin *et al*. 2003). The dopamine hypothesis of schizophrenia is based on the observation that dopamine agonists such as amphetamine and cocaine cause psychotic-like symptoms and, moreover, traditional antipsychotic drugs act by blocking dopamine receptors in the brain (Seeman and Lee 1975). Dysfunction of central dopaminergic neurotransmission has been suggested to play an important role in the etiology of certain neuropsychiatric disorders, such as schizophrenia (Jellinger 1991; Chen *et al*. 2003). The human dopamine transporter is organized as a tetramer in the plasma membrane. The *SLC6A3* is a central regulator of the time course and synaptic concentration of released dopamine by rapid reuptake of dopamine into presynaptic terminals and mediating synaptic re-accumulation of dopamine (Amara and Kuhar 1993). The *SLC6A3* gene is located at the chromosomal region 5p15.33 and consists of 15 exons (Vandenbergh *et al*. 1992, OMIM126455). The 5′-flanking region has many characteristics, previously thought typical of house-keeping promoters (Donovan *et al*. 1995; Kawarai *et al*. 1997; Kouzemenko *et al*. 1997). The *SLC6A3 (hDAT1)* gene is expressed exclusively in the central nervous system, primarily in midbrain dopaminergic neurons of the substantia nigra and ventral tegmental regions (Bannon *et al*. 2001). In order to show the importance of the *SLC6A3* gene as a functional candidate gene, several association studies were performed between *SLC6A3* and developing schizophrenia. In these studies, polymorphisms in the 3′-flanking region of the *SLC6A3* gene was mostly investigated, but all reports failed to establish a significant association (Georgieva *et al*. 2002; Hauser *et al*. 2002) or linkage (Byerley *et al*. 1993; Perisco *et al*. 1995) with schizophrenia. A systematic mutation analysis of the 1.6-kb area of the 5′-untranslated region (5′-UTR) revealed five diallelic polymorphisms: T-67A, G-660C, C-839T, C-1169G and T-1476G (Rubie *et al*. 2001). Moreover, a family-based study demonstrated potential association between these markers and schizophrenia (Stober *et al*. 2005). Recently, a case–control study was performed in an Iranian population that showed an association between the –67A/T SNP and schizophrenia (Khodayari *et al*. 2004). Therefore, because of the importance of the 5′-flanking region of the *SLC6A3* gene in the etiology of schizophrenia (Stober *et al*. 2005), we first conducted the potential association study between schizophrenia and the –839 C/T SNP in an Iranian ethnic group.

Materials and methods

Subject

For the case–control study, 200 unrelated patients with a mean age of 43.34 years (s.d. = 11.353) were recruited from the hospitals of south and southwest Iran. Of these, 117 were men (63 from south and 54 from southwest) and 83 were women (48 from south and 35 from southwest). The diagnosis of schizophrenia was based on DSM-IV criteria (Thaker and Carpenter 2001). The control group consisted of 200 healthy blood donors with a mean age of 39.43 years (s.d. = 11.103), which were matched for gender and ethnicity to the patient cohort. Informed consent was obtained from all participants.

Genotyping

From each subject 5 mL whole blood was drawn. Genomic DNA was extracted using standard salting out method. The

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–839 C/T polymorphism in the *SLC6A3* gene was screened using PCR-RFLP method. Primer sequences used have been previously reported (Rubie *et al*. 2001). The PCR with the condition: 3 min at 94°C, 30 s at 94°C, 40 s at 52°C, 30 s at 72°C for 35 cycles and 5 min at 72°C was carried out in 25-μL volume containing 10-ng genomic DNA, 10 mM tris-HCl (pH 8.3), 50 Mm KCl, 2 Mm $MgCl_2$, 200 μM dNTP, 50 pmol of each primer and 0.25 unit of *Taq* DNA polymerase (Roche, Mannheim, Germany). Five μL of the PCR product were digested overnight at 37°C with 1 unit of the *Msp*I restriction enzyme (Fermentase, Heldinki, Finland) and the fragments were resolved on the 12% PAA gel. Bands were visualized by silver staining. To control the digestion reactions, a number of the PCR products were then randomly selected for sequencing. The sequence reactions were analysed on the automated sequencer (ABI, model 377, London, England).

Statistical analysis

Statistical differences in the genotype and allele frequencies between the patients and control group in south and southwest provinces have been evaluated using the chi-square test. The association between genotype and development of schizophrenia was examined using odds ratio (ORs) and 95% confidence intervals (c.i.). Chi-square test, ORs, and their c.i. were calculated using the SPSS13 statistical package (SPSS, Chicago, USA). According to the difference between the genotypic frequencies in the two regions, Mantel–Haenszel weighted odd ratio and chi-square were performed to analyse the entire samples. EpiInfo 6 statistical software (Epilinfo, Atlanta, USA) was used to carry out the Mantel–Haenszel tests.

Results and discussion

The study of the socio-demographic features of the cohort revealed that the subjects in the case group were significantly lower than those in the control group in terms of marital status and educational level ($P < 0.05$). Samples from south and southwest regions were analysed separately, since differences in allelic and genotypic frequencies were observed between the regions (table 1).

The patients from southwest Iran did not show association for the *T* allele and increasing risk of schizophrenia in the case of heterozygosity (*CT* genotype: OR = 0.746; 95%c.i. = 0.362–1.610; $P = 0.479$) and homozygosity (*TT* genotype: OR = 1.154; 95%c.i. = 0.555–2.400; $P = 0.702$). In contrast, samples from south region revealed a weak association between the *CC* genotype and schizophrenia (*CT* genotype: OR = 0.603; 95%c.i. = 0.316-1.154; $P = 0.127$ and *TT* genotype: OR = 0.451; 95%c.i. = 0.215–0.945; $P = 0.035$). Further, the entire samples were analysed using Mantel–Haenzsel test to increase the statistical power. This test revealed a lack of association between the –839C/T SNP and developing schizophrenia (*CT* genotype: OR = 0.67; 95%c.i. = 0.40–1.12; P = 0.134 and *TT* genotype: OR = 0.72; 95%c.i. = 0.42–1.25; $P = 0.2690$).

Table 1. Allelic frequencies and genotypic distribution of the –839C/T polymorphism in the *SLC6A3* gene.

	Cases	Controls
Allelic frequencies		
Southwest		
G	0.472	0.443
C	0.528	0.557
South		
C	0.554	0.451
T	0.446	0.549
Genotypic distributions		
Southwest		
CC	26	25
TC	27	34
TT	36	30
South		
CC	36	23
TC	51	54
TT	24	34

However, regarding the reported characteristics, *SLC6A3* gene seems to be an attractive candidate for association studies (Greenwood and Kelsoe 2003; Kapur 2003). To date, the regulatory effect of the five discovered polymorphisms in the promoter region on the *SLC6A3* gene expression is poorly investigated. However, computational analysis of these SNPs predicted that –839C/T polymorphism can create a binding site for a leader binging protein 1 (LBP1 binding site) which in turn acts as a repressor (Rubie *et al*. 2001).

Considering the biological importance of the DAT1 promoter region, especially the –839C/T SNP, the present case–control study was performed for the first time in an Iranian population, which did not show any association with schizophrenia. Stober *et al*. (2005) reported previously that this polymorphism did not expose a risk factor for schizophrenia, but in combination with the four other polymorphisms in the 5′-flanking region, the –839C/T polymorphism may influence the risk for developing schizophrenia. However, according to our result, the single –839C/T SNP do not represent an effective factor for increasing the risk of schizophrenia, at least in the south and southwest Iranian population. Further association studies with the other four SNPs on the promoter region of the *DAT1* gene might be beneficial.

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