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DRD2 C957T polymorphism interacts with the COMT Val158Met polymorphism in human working memory ability

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Abstract

The C957T polymorphism in the dopamine D2 receptor (DRD2) gene and the Val158Met polymorphism in the Catechol-*O*-Methyl-Transferase (COMT) gene affect dopamine transmission and have been found to be associated with schizophrenia. Since DRD2 in mice and the COMT gene in humans modulate working memory, we examined the relationship and possible interaction of both polymorphisms to working memory performance in 188 healthy adults. Subjects having the DRD2 C/C allele showed the poorest performance in a word serial position test. Moreover, the effect of the C957T genotype was strengthened when interaction with the COMT Val158Met polymorphism was included in the analysis. We propose that an interaction of the DRD2 C957T and COMT Val158Met may be involved in the generation of some working memory deficits in schizophrenia.

Keywords: Working memory; Dopamine D2 receptor; C957T polymorphism; Catechol-*O*-Methyl-Transferase; Val158Met polymorphism; Schizophrenia

1. Introduction

Genes affecting dopamine transmission have been implicated in schizophrenia. However, no single gene accounts for all of the risk for the disease. Schizophrenia is likely to result from the interaction of two or more genes (Gogos and Gerber, 2006). One approach to understanding the contribution of risk genes to the pathogenesis of schizophrenia is to study endophenotypes, biobehavioral elements of the disorder that may be more strongly affected by individual gene variants in patients or normal populations (Gottesman and Gould, 2003). In this study we examined the effects of two schizophrenia risk genes involved in dopamine transmission, the dopamine D2 receptor (DRD2) gene and Catechol-*O*-Methyl Transferase (COMT) gene, on

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working memory. Working memory deficits are among the most pervasive cognitive symptoms of schizophrenia (Goldman-Rakic, 1994), which also include decreased verbal memory and attention (Cornblatt and Keilp, 1994; Saykin et al., 1991). These cognitive deficits are also observed, in a milder form, in unaffected first-degree relatives of patients, suggesting a genetic contribution to these deficits (Cannon et al., 1994; Cornblatt and Keilp, 1994; Park et al., 1995).

Although dopamine D2 receptors are believed to be involved in the positive symptoms of schizophrenia, D2 receptors also may play a role in the cognitive symptoms. We recently generated transgenic mice that over-express D2 receptors selectively in the striatum. These mice show behavioral deficits in working memory tasks without affecting spatial reference memory (Kellendonk et al., 2006). A synonymous SNP (C957T) in the gene encoding the dopamine D2 receptor (DRD2) has been reported to increase striatal DRD2 binding in humans (Hirvonen et al., 2004) and be associated with schizophrenia (Lawford et al., 2005; Hanninen et al., 2006). C/C individuals have the highest striatal binding potential and the highest schizophrenia risk. To determine if increased D2 receptor binding affects working memory in humans as it does in mice, we genotyped the C957T polymorphism in healthy individuals who were tested in four working memory tests differing in their task demands. We further examined the interaction of this polymorphism with the COMT Val158Met polymorphism, which has been shown to be associated with schizophrenia, and to affect dopamine transmission and working memory (Egan et al., 2001; Bruder et al., 2005; Tunbridge et al., 2006).

2. Methods

2.1. Subjects

We attempted to genotype the 217 healthy Caucasian individuals in our previous study (Bruder et al., 2005) for the C957T SNP and successfully determined unambiguous genotypes for 188 samples. Briefly, these volunteers were between the ages of 18–55 with no current or past psychiatric or neurological disorders, or substance abuse. Written informed consent was obtained from all participants after detailed description of the study, which was approved by the New York State Psychiatric Institute–Columbia University Department of Psychiatry Institutional Review Board. The subjects also completed self-rating scales for assessing psychopathology, including the Beck Depression Inventory (BDI-II; Beck et al., 1961) and the Hopkins Symptom Checklist (SCL-90; Derogatis et al., 1974), and they had no excessive scores. Subjects were tested on a battery of working memory tests, including a 6-item version of the Word Serial Position Test (WSPT) that requires retention of the serial position of 6 words over a period of 9 s, an n-back test, the WAIS-III Letter–Number sequencing subtest and the Spatial Delayed-Response test. To control for general intellectual ability, the WAIS-III Vocabulary subtest was also conducted. The details of the cognitive tasks are described elsewhere (Bruder et al., 2005).

2.2. Genotyping

A saliva swab was obtained from each subject and DNA was extracted with BuccalAmp® DNA extraction kit from Epicentre. A 138-bp fragment in DRD2 gene was amplified by PCR from genomic DNA, which includes the C957T polymorphism (SNP rs6277). The forward primer was designed not to include a common SNP (rs6275) 18 bases upstream of C957T. The genotypes were determined with Fluorescent Polymorphism (FP, Perkin Elmer). An FP primer adjacent to the SNP on the complementary strand (TCTGGTTTGGCGGGGCTGTC) was used to distinguish C from T. Details of the COMT genotyping are given elsewhere (Bruder et al., 2005).

2.3. Statistical analyses

All raw working memory test accuracy scores were adjusted for demographic influences (age, sex, and education) using step-wise regression. Demographically-adjusted scores for each test were then compared among genotypes using one-way ANOVA, and $p<0.05$ was considered significant. A chi-square test for independence was used for initial assessment of the interaction between polymorphisms. When the polymorphisms appeared not to be independent, an ANOVA was conducted by including both polymorphisms and their interaction in the linear model to further explore the effect of interaction.

3. Results

For the DRD2 C957T polymorphism, there was no deviation from Hardy–Weinberg equilibrium based on the chi-square statistic. Because postnatal developmental changes occur in prefrontal dopaminergic function and COMT activity, we determined the mean ages of the individuals in each DRD2 and COMT genotype group and found no significant group differences (see Supplementary

Table 1). We found no relation between genotype and general intellectual ability, but did find an association between the DRD2 genotypes and performance on one working memory task, i.e., the WSPT (word serial position test). Subjects with the C/C genotype performed poorer on the WSPT when compared to those with at least 1 T allele (T/T and C/T; $F=4.22$, $df=1$, $p=0.04$). We observed no significant differences between TT and CT genotype groups. Fig. 1 shows the accuracy scores of these two genotype groups at the 6 serial positions in WSPT. When accuracy at the 6 serial positions in the WSPT was analyzed individually, only accuracy at the 5th position differed significantly between groups ($F=8.03$, $df=1$, $p=0.005$). A table reporting statistical analyses for all tasks broken down by DRD2 and COMT genotypes is provided in the supplementary section.

As previously reported (Bruder et al., 2005), the COMT Val158Met polymorphism was also genotyped in our sample. A chi-square test suggested possible interaction between the COMT Val158Met and DRD2 C957T polymorphisms (chi-square=19.80, $df=4$, $p<0.001$). Since these two genes are not linked, while both are involved in dopamine neurotransmission that is essential to working memory ability, we investigated the effect of their interaction on test performance. When COMT Val158Met was included in the analysis model, the relation of the DRD2 C957T genotype to overall performance on the WSPT ($F=6.39$, $df=1$, $p=0.01$) and accuracy at the 5th position ($F=11.16$, $df=1$, $p=0.001$) were strengthened. To further explore the interaction, association between the WSPT performance and DRD2 C957T genotype was tested in subgroups defined by COMT Val158Met genotypes. Table 1 lists the number of individuals and the average WSPT accuracy for the subgroups. A significant association between DRD2 C957T genotype and the WSPT performance score was found only in the COMT Met/Met group ($F=4.98$, $df=1$, $p=0.03$). This effect was not significant for the other COMT genotypes, but Val/Val genotype almost achieved significance (Val/Val group: $F=3.858$, $df=1$, $p=0.06$. Val/Met group: $F=0.049$, $df=1$, $p=0.83$).

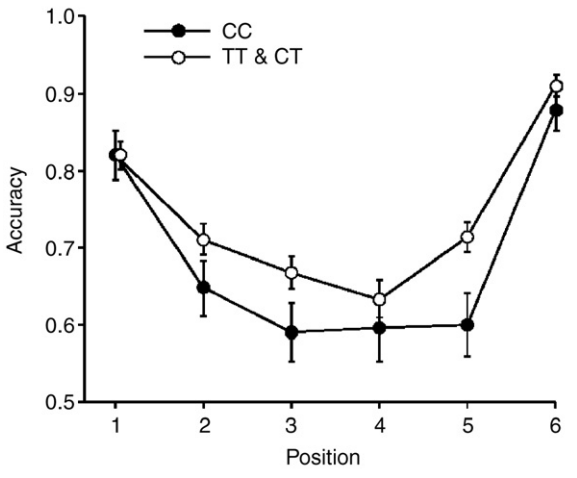

Fig. 1. Adjusted mean accuracy scores at 6 positions in WSPT for DRD2 C/C group and combined T/T and C/T group (error bars = +/− 1 standard error).

Table 1
Interaction of COMT Val158Met and DRD2 C957T polymorphisms

D2 C957T	Val/Val	Val/Met	Met/Met	Total
C/C	**0.59** (15)	**0.69** (21)	**0.61** (10)	**0.64** (46)
C/T	**0.68** (27)	**0.68** (57)	**0.72** (23)	**0.69** (107)
T/T	**0.69** (15)	**0.71** (21)	**0.76** (8)	**0.71** (35)
Total	**0.66** (57)	**0.69** (90)	**0.70** (41)	**0.68** (188)

Adjusted accuracy scores for WSPT (averaged over serial position) in bold face, and the number of subjects in each group in parentheses.

4. Discussion

The C957T polymorphism in the human DRD2 gene modulates the D2 binding potential in the striatum and has been associated with schizophrenia (Hirvonen et al., 2004; Lawford et al., 2005). In mice, excess of striatal D2 receptor impairs working memory (Kellendonk et al., 2006). To test whether the C957T polymorphism affects working memory in humans, we tested a sample of healthy adults on four working memory tasks and genotyped them for the C957T polymorphism. We found that the allele with the highest striatal binding potential (C/C) showed the poorest performance in the WSPT and that the T/T allele, which has been reported to be protective for schizophrenia, was associated with the best performance in WSPT. This suggests that the DRD2 C957T polymorphism is related to human working memory on a test requiring maintenance of phonological and serial order information. In this regard, studies have found evidence of a specific verbal memory deficit in a subgroup of schizophrenia patients tested on the WSPT (Wexler et al., 1998; Bruder et al., 2004). The DRD2 C957T polymorphism effect was, however, seen in only one of the four tasks indicating a rather mild influence of this polymorphism on working memory or a highly specific effect on a particular aspect of working memory.

We and others have recently shown that another polymorphism that has been associated with schizophrenia, the COMT Val158Met polymorphism, affects cognitive functions, including working memory (Egan

et al., 2001; Bruder et al., 2005; Tunbridge et al., 2006). COMT metabolizes dopamine and therefore, like the D2 receptor, plays a critical role in dopamine signaling. When subjects were divided into subgroups on the basis of COMT Val 158 Met genotypes, a significant association of the DRD2 polymorphism to WSPT performance was found only for the Met/Met genotype. Those carrying the Val/Val allele showed a similar trend but it was not statistically significant. There is no genetic linkage between the COMT and the DRD2 polymorphisms because these two genes reside on different chromosomes (DRD2 on 11q23 and COMT on 22q11). The genetic interaction indicated in our study may therefore reflect a functional relation between these two genes, which may arise from their function in dopaminergic transmission.

Our finding regarding the interaction should be treated with caution because of the relatively small sizes of the subgroups. It does, however, illustrate the potential benefit of studying interactions between functional polymorphisms known to be involved in the same biological pathway, especially when studying multigenic disorders such as schizophrenia.

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Appendix A. Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2006.10.001.

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