Association between a polymorphism in the promoter region of the dopamine D2 receptor gene and schizophrenia in Japanese subjects: replication and evaluation for antipsychotic-related features

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Abstract

To replicate a previously found negative association between the Del allele of the −141C Ins/Del polymorphism in the 5′-promoter region of the dopamine D2 receptor gene (DRD2) and schizophrenia in Japanese subjects and to examine whether this polymorphism is related to the features of antipsychotic drug treatment, we genotyped 94 control subjects and 234 schizophrenic patients. The schizophrenic patients had a significantly lower frequency of the Del allele (p < 0.05). We found a non-significant trend towards a higher frequency of the Del allele in schizophrenic patients susceptible to neuroleptic-induced extrapyramidal symptoms. The daily dosage of haloperidol, the steady-state concentration of serum haloperidol per daily dosage, and the recent 1-yr cumulative neuroleptic dosage were lower in patients with the Del/Del genotype than in the other patients. These findings support the view that the polymorphism is associated with schizophrenia in Japanese subjects and provide hints for further attempts to establish the relationship between this polymorphism and the features of antipsychotic drug treatment.

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Key words: Dopamine D2 receptor gene, schizophrenia, promoter region, extrapyramidal symptoms, tardive dyskinesia.

Introduction

Most antipsychotic drugs used to treat schizophrenia block postsynaptic dopamine D2 receptors. Therefore, changes in the function of this receptor, some of which may result from genetic variation, may be related to the susceptibility of an individual to the development of schizophrenia and neuroleptic-induced extrapyramidal symptoms (EPS). One polymorphism (Ser311Cys) and two rare variants (Val96Ala and Pro310Ser) of the dopamine D2 receptor gene (DRD2) that cause alterations in the amino-acid sequence of this receptor have been identified (Gejman et al., 1994; Itokawa et al., 1993). The Ser311Cys polymorphism and the Pro310Ser variant significantly alter signal transduction (Cravchik et al., 1996). However, their effects on the phenotype are either unclear or inconsistent.

Recently, the −141C Ins/Del polymorphism was found in the promoter region of DRD2, and the frequency of the Del allele was observed to be significantly lower in schizophrenic patients (Arinami et al., 1997). Reporter constructs containing the deletion variant (Del allele) drive lower transcription activity than the Ins allele, which was suggested to be associated with schizophrenia. Subsequent studies have revealed that allele frequency of this polymorphism shows wide racial differences (Gelernter et al., 1998) and inconsistent results in terms of the association of this polymorphism with schizophrenia (Furlong et al., 1998; Jonsson et al., 1998; Kaiser et al., 1998; Li et al., 1998; Ohara et al., 1998; Stober et al., 1998).

We examined the relationships between the −141C Ins/Del polymorphism in the promoter region of DRD2 and schizophrenia and the features of antipsychotic drug treatment in a Japanese sample independent of the previous study by Arinami et al. (1997).
Subjects and methods

Ethical considerations

This research was approved by the Ethics Committee of the Kohnodai Area, National Centre of Neurology and Psychiatry, Chiba, Japan. Written informed consent was obtained from all participating subjects.

Subjects

The patients were recruited from several psychiatric facilities located in and around the Tokyo area (Inada et al., 1995, 1996). All patients satisfied the criteria for a DSM-III-R diagnosis of schizophrenia (American Psychiatric Association, 1987) and had been receiving neuroleptic therapy for more than 6 months. Volunteer control subjects were mostly medical staff with no history of psychosis or substance abuse, or of receiving neuroleptic medication. All subjects were Japanese.

Status of EPS

As many of the schizophrenic patients as possible were systematically and clinically evaluated for neuroleptic-induced EPS using the Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS) (Inada, 1996), starting in 1994. The DIEPSS is a 9-item scale consisting of 8 individual items (gait, bradykinesia, salivorrhoea, muscle rigidity, tremor, akathisia, dystonia, and dyskinesia) and 1 global item (overall severity of EPS), each rated from 0 (none, normal) to 4 (severe). When the global item was rated 3 or higher within 3 months of the initial neuroleptic therapy, the patient’s condition was defined as ‘acute EPS present’. For patients whose initial neuroleptic therapy had started before our systematic evaluation of their DIEPSS, we accepted that acute EPS had been present within 3 months of the initial neuroleptic therapy if the clinical records clearly described EPS and a subsequent reduction in the neuroleptic dosage or a subsequent addition of anti-parkinsonian drugs. Patients who showed no signs of acute EPS despite receiving neuroleptic therapy for more than 3 months were regarded as not having acute EPS.

Tardive dyskinesia (TD) was assessed with the Japanese version of the Abnormal Involuntary Movement Scale (AIMS) starting in 1987, and was diagnosed according to the criteria of Schooler and Kane (1982). The inclusion criteria used to subclassify patients with or without TD were those reported by Inada et al. (1997). Briefly, patients who had been suffering from TD with at least one item persistently rated 3 for more than 1 yr, or patients who had been suffering from persistent TD for more than 1 yr (having TD develop within 5 yr of first neuroleptic exposure) were classified as having TD. Patients who had never developed TD despite receiving neuroleptics for more than 10 yr were regarded as not having TD.

In rating EPS and TD, suspected cases were videotaped for later evaluation. When information about the presence of acute EPS in the initial stage of neuroleptic therapy for the first episode of schizophrenia was incomplete, the patient was excluded from the acute EPS study. When the TD status could not be obtained, the patient was omitted from the TD study.

Neuroleptic dosage

Information about the neuroleptic therapy that schizophrenic patients had been receiving was obtained from their clinical records. Recent 1-yr cumulative neuroleptic dosage was calculated for the patients who had been receiving neuroleptic therapy for more than 1 yr. The steady-state daily neuroleptic dosage was calculated only for patients who had been receiving a steady dose of neuroleptics as maintenance therapy for more than 1 yr. The chlorpromazine equivalent administered to each patient was calculated from a table developed specifically for Japanese patients (Inagaki et al., 1998).

Haloperidol (HPD) concentration

Serum HPD levels were routinely measured at approx. 2-month intervals for patients who had been receiving HPD. Only patients receiving a steady dose as maintenance therapy for more than 3 months, without receiving any other butyrophenone derivative (bromperidol, timiperone, pipamperone, moperone, or spiperone) were selected for the HPD concentration study. An average value was adopted when two or more steady-state serum HPD concentrations were available.

Genetic analysis

Genomic DNA was extracted from leucocytes by standard techniques. The target segment of the 5’-flanking region of DRD2 was amplified with Native Pfu Polymerase (Stratagene, La Jolla, CA, USA) by PCR at 98 °C for 1 min followed by 35 cycles at 98 °C for 20 s and 74 °C for 5 min for primers of 5’-ACT GGC GAG CAC GGT ACG GTG AGG ACC C and 5’-TTC GGC GGT CAG GCT CCC GTG TCG G (Arinami et al., 1997). Following digestion of the amplified fragments with BstN1, the fragments were fractionated on 2% agarose gel, stained with ethidium bromide, and examined under UV light. The second author (T. A.) did the genotyping, blind to the demographic and psychiatric variables data.
**Statistics**

Allele and genotype frequencies were compared by using the $\chi^2$ test for $2 \times 2$ and $2 \times 3$ contingency tables. Because a main purpose of the study was replication of a previously found negative association between schizophrenia and the Del allele (Arinami et al., 1997), differences in the allele and genotype frequencies between schizophrenics and controls were tested for significance at the 5% level by using 1-tailed $\chi^2$ statistics when the Del allele was less frequent in schizophrenics than controls. The association between neuroleptic-induced EPS (the status of TD and acute EPS) and polymorphism was also assessed by the $\chi^2$ test. Comparisons of the daily dosage of HPD, steady-state serum HPD levels, steady-state daily neuroleptic dosage, and recent 1-yr cumulative neuroleptic dosage between Del/Del and other genotypic subgroups, or between Ins/Ins and other genotypic subgroups were done using the Mann–Whitney $U$ test ($p$ values of $< 0.1$ or $< 0.05$ are denoted in the tables). For the six psychopharmacological variables shown in Tables 2 and 3, the significance level was set at $p = 0.0042$ following Bonferroni’s multiple test correction. The SPSS for Windows 95 (release 7.5, SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

**Results**

A total of 234 schizophrenic patients (130 men and 104 women) and 94 control subjects (47 men and 47 women) participated. Their mean ages were 54 yr (range 20–81 yr) for schizophrenic patients and 46 yr (range 33–64 yr) for control subjects.

Allele and genotype frequencies of the polymorphism in control subjects and schizophrenic patients are shown in Table 1. The genotype distribution in each subgroup was not significantly different from that expected according to Hardy–Weinberg equilibrium. The frequency of the Del allele was significantly lower in schizophrenic patients ($\chi^2 = 3.59$, d.f. = 1, $p = 0.039$, one-tailed; relative risk = 0.68). A significantly higher frequency of the Ins/Ins genotype (vs. Ins/Del + Del/Del; $\chi^2 = 4.44$, d.f. = 1, $p = 0.018$, one-tailed) was observed in schizophrenic patients.

Sufficient data were obtained for 139 patients to subclassify their TD status: 31 had TD (mean age 62 yr; range 22–81 yr) and 108 did not (mean age 55 yr; range 28–78 yr). Sixty-three patients were subclassified for their status of acute EPS: 40 present (mean age 57 yr; range 21–76 yr) and 23 absent (mean age 56 yr; range 28–78 yr). Table 2 shows genotype and allele frequencies of the polymorphism in terms of TD and acute EPS status. The frequency of the Del allele was 1.5 times higher in patients with TD than in those without (23 vs. 15%). A similar ratio was observed for patients with acute EPS (24 vs. 15%). However, these differences did not reach statistical significance. The genotype distribution tended to differ between patients with and without TD ($\chi^2 = 4.65$, d.f. = 2, $p = 0.098$).

Table 3 shows the features of neuroleptic treatment for the three subgroups of $-141C$ Ins/Del polymorphism. Of the 234 schizophrenic patients, 167 satisfied the inclusion criteria of the HPD concentration study. The genotype distribution for these patients was 109 wild-type homozygotes (Ins/Ins group), 53 heterozygotes (Ins/Del group), and 5 mutant-type homozygotes (Del/Del group). The daily dosages of HPD tended to be lower for patients in the Del/Del group (7.2 mg/d) than for patients in the Ins/Del + Ins/Ins group (15.3 mg/d) ($p = 0.08$, $z = -1.77$, Mann–Whitney $U$ test). Consistent with this finding, the steady-state serum concentration of HPD per daily dosage for patients in the Del/Del group (8.5 ng/ml) was approx. 57% of that in patients in the Ins/Del + Ins/Ins group (15.0 ng/ml), although this difference did not reach statistical significance ($p = 0.13$, $z = -1.51$, Mann–Whitney $U$ test). Likewise, the recent 1-yr cumulative neuroleptic dosages of patients in the Del/Del group were approx. 26% lower than those of patients in the Ins/Del + Ins/Ins group.

**Table 1.** Genotype and allele frequencies of the $-141C$ Ins/Del polymorphism in the DRD2 gene in controls and schizophrenic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Del/Del</td>
<td>Ins/Del</td>
</tr>
<tr>
<td>Controls ($n = 94$)</td>
<td>3.2%</td>
<td>42.0%</td>
</tr>
<tr>
<td>Schizophrenia ($n = 234$)</td>
<td>2.6%</td>
<td>30.8%</td>
</tr>
</tbody>
</table>

In the case-control comparison of this replication study, one-tailed $\chi^2$ tests for $2 \times 2$ and $2 \times 3$ contingency tables were performed.

* $p < 0.05$, when compared to the controls.
**Table 2.** Genotype and allele frequencies of the −141C Ins/Del polymorphism in the DRD2 gene and the status of TD and acute EPS in schizophrenic patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Del/Del</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td></td>
</tr>
<tr>
<td>Status of TD*</td>
<td></td>
</tr>
<tr>
<td>Patients with TD (n = 31)</td>
<td>3.2%**</td>
</tr>
<tr>
<td>Patients without TD (n = 108)</td>
<td>0.0%</td>
</tr>
<tr>
<td>Status of acute EPS</td>
<td></td>
</tr>
<tr>
<td>Acute EPS present (n = 40)</td>
<td>7.5%</td>
</tr>
<tr>
<td>Acute EPS absent (n = 23)</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

For comparison of the status of TD and acute EPS, two-tailed $\chi^2$ tests for 2×2 and 2×3 contingency tables were performed.

* $p < 0.1$, genotype distribution between the patients with and without TD.

** $p < 0.1$, when compared to the patients without TD.

**Table 3.** Therapeutic features of the antipsychotic drug treatment among 3 subgroups showing −141C Ins/Del polymorphism in the DRD2 gene

<table>
<thead>
<tr>
<th>−141C Ins/Del polymorphism of DRD2</th>
<th>Del/Del</th>
<th>Ins/Del</th>
<th>Ins/Ins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily dosage of haloperidol (mg/d)</td>
<td>7.2 $\pm$ 1.6*</td>
<td>14.8 $\pm$ 8.7</td>
<td>15.5 $\pm$ 12.5</td>
</tr>
<tr>
<td>(n = 167)</td>
<td>(n = 5)</td>
<td>(n = 53)</td>
<td>(n = 109)</td>
</tr>
<tr>
<td>Serum haloperidol level (ng/ml)</td>
<td>8.5 $\pm$ 3.8</td>
<td>15.2 $\pm$ 8.8</td>
<td>14.9 $\pm$ 10.3</td>
</tr>
<tr>
<td>(n = 167)</td>
<td>(n = 5)</td>
<td>(n = 53)</td>
<td>(n = 109)</td>
</tr>
<tr>
<td>Steady state daily neuroleptic dosage (mg/d)</td>
<td>788 $\pm$ 477</td>
<td>1102 $\pm$ 1060</td>
<td>1091 $\pm$ 1942</td>
</tr>
<tr>
<td>(n = 93)</td>
<td>(n = 2)</td>
<td>(n = 31)</td>
<td>(n = 60)</td>
</tr>
<tr>
<td>Recent 1-yr cumulative neuroleptic dosage (g)</td>
<td>239 $\pm$ 110</td>
<td>346 $\pm$ 330</td>
<td>324 $\pm$ 547</td>
</tr>
<tr>
<td>(n = 191)</td>
<td>(n = 4)</td>
<td>(n = 57)</td>
<td>(n = 130)</td>
</tr>
</tbody>
</table>

Data are expressed as mean $\pm$ s.d. Neuroleptic dosage was calculated as chlorpromazine equivalent dose using the table of Inagaki et al. (1998). Mann–Whitney U tests were performed for comparison between the Del/Del vs. other genotypic subgroups or between Ins/Ins vs. other genotypic subgroups.

* $p < 0.1$, when compared to the patients with other genotypes.

**Discussion**

These results replicate the previous findings by Arinami et al. (1997) and Ohara et al. (1998) that this polymorphism may affect susceptibility to schizophrenia in Japanese subjects. These three Japanese studies found similar odds ratios for the Del allele association with schizophrenia: 0.68 (this study), 0.59 (Arinami et al., 1997), and 0.60 (Ohara et al., 1998). Meta-analysis of this data found a significant negative association between the Del allele and schizophrenia with an odds ratio of 0.64 (95% CI 0.52–0.79, $p = 0.00004$) in 664 patients and 527 controls. In contrast, this association was not observed in German (Stober et al., 1998) or Chinese subjects (Li et al., 1998), which suggests that the association might be ethnically bound. However, because a significant negative association was indicated in a Swedish population (Jonsson et al., 1998), further studies are needed to resolve the issue. Although genetic linkage studies have not shown suggestive or significant linkage between schizophrenia and DRD2 (Kalsi et al., 1995; Su et al., 1993), they did not exclude weak linkage (Shaw et al., 1998).

Our findings that the daily dosage of HPD and the recent 1-yr cumulative neuroleptic dosage were lower for schizophrenic patients with the Del/Del genotype might indicate that these patients require lower doses of antipsychotic drugs as maintenance therapy than patients with other genotypes. If reduced transcriptional activity of the Del allele in cultured cells correlates with in vivo transcriptional activity, the D2 receptor density or a
compensatory increase in it after blocking by antipsychotics would be lower in Del/Del individuals than in those with other genotypes. An alternative interpretation for the reduced antipsychotic dosage administered to these patients as maintenance therapy may simply be that the antipsychotic drug dosage cannot be increased owing to the development of neuroleptic-induced EPS in them.

The risk of neuroleptic-induced EPS and TD appears to be higher for schizophrenic patients with the Del allele. This could be interpreted as meaning that the presence of the Del allele impairs their ability to efficiently compensate for the blockade of dopamine D2 receptors by antipsychotic drugs. Indeed, it has been shown in positron emission tomography studies that akathisia appears when dopamine D2 receptor occupancy is at maximum during therapy with raclopride (Farde, 1992) or HPD (Nordstrom et al., 1992); that is, when the number of available dopamine D2 receptor sites is at minimum.

Our findings related to the antipsychotic drug therapy showed only non-significant trends. The inflation of a false-positive result associated with multiple testing should be taken into account. Therefore, the association of this polymorphism with individual differences in response to antipsychotic therapy implied in this study is just a hint to guide future replication attempts.

In summary, we replicated a previously found negative association between the Del allele of the DRD2 — 141C Ins/Del polymorphism and schizophrenia in Japanese subjects. The polymorphism might be related to individual differences in antipsychotic dosage and the development of EPS or TD.

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