Homozygosity for the Gly-9 variant of the dopamine D3 receptor and risk for tardive dyskinesia in schizophrenic patients

Roger Løvlie, Ann K. Daly, Richard Blennerhassett, Nicol Ferrier and Vidar M. Steen

1 Dr Einar Martens’ Research Group for Biological Psychiatry, Centre for Molecular Medicine, University of Bergen, Haukeland University Hospital, N-5021 Bergen, Norway
2 Department of Pharmacological Sciences, University of Newcastle upon Tyne, UK
3 Department of Psychiatry, University of Newcastle upon Tyne, UK

Abstract

This study was undertaken to re-examine whether homozygosity for the Gly-9 variant (allele 2) of the dopamine D3 receptor gene (DRD3) is associated with increased risk for tardive dyskinesia (TD) in schizophrenic patients. Seventy-one antipsychotic-treated subjects with schizophrenia from Newcastle upon Tyne, UK, were genotyped for the presence of allele 1 (Ser-9) and allele 2 (Gly-9) of the dopamine D3 receptor (DRD3) Ser-9-Gly polymorphism. Among 32 patients with TD, 7 subjects (22%) were homozygous for the Gly-9 variant (2–2 genotype), whereas 4 out of 39 patients (10%) without TD had this genotype. The non-significant tendency in this sample towards an over-representation of allele 2 and the 2–2 genotype among schizophrenic patients with TD is in line with our initial report as well as recent studies by others, indicating that the Gly-9 allele of DRD3 may be a susceptibility factor for the development of TD in neuroleptic-treated individuals with schizophrenia. There are, however, some recent non-supportive reports, and since the trend in our present study failed to reach statistical significance, further studies on larger samples and future meta-analysis may be necessary to establish the role of the DRD3 in the pathogenesis of TD.

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Introduction

Treatment with anti-psychotic drugs (neuroleptics) has a beneficial effect on many patients affected with schizophrenia and other psychiatric disorders. However, long-term anti-psychotic treatment is associated with the risk of extra-pyramidal side-effects, such as akathisia, parkinsonism and tardive dyskinesia (TD). Despite the fact that TD is a serious and common drug-related movement disorder, we still lack a clear understanding of the mechanisms underlying this side-effect. The most prominent risk factor for TD is ageing, but high neuroleptic exposure, psychiatric diagnosis, organic brain damage and coexistence of diabetes mellitus may also play a role in the development of TD (for further references and review, see Basile et al., 1999; Kane, 1995; Steen et al., 1997). Among other risk factors, genetic predisposition is also thought to confer susceptibility to TD (Müller et al., 1998; Yassa and Ananth, 1981; Youssef et al., 1989).

As an alternative approach to elucidate the pathophysiology of TD, we have used a candidate gene strategy in combination with association studies to identify putative genetic susceptibility factors for TD. In two independent investigations, we have shown that impaired drug metabolism due to inherited CYP2D6 mutations may be associated with increased risk for TD (Andreassen et al., 1997; Armstrong et al., 1997). More intriguingly, we have demonstrated that homozygosity for the Gly-9 variant (allele 2) of the Ser-9-Gly polymorphism in the dopamine D3 receptor gene (DRD3) may predict increased susceptibility for TD among neuroleptic-treated schizophrenic patients (Steen et al., 1997). This finding has recently been supported by two independent studies (Basile et al., 1999; Segman et al., 1999). In this study, we have determined the distribution of the dopamine D3 receptor (DRD3) genotypes in an independent sample of neuroleptic-treated schizophrenic
patients from Newcastle upon Tyne, UK, to re-examine the apparent association between TD and homozygosity for the Gly-9 variant of the DRD3, as reported in our initial study.

Subjects and methods
The patients studied have been described in detail elsewhere (Armstrong et al., 1997). In short, 75 subjects of European Caucasian origin, who met the DSM-III-R criteria for schizophrenia (American Psychiatric Association, 1987), were recruited from local hospitals in Newcastle upon Tyne, UK. All patients included in the study had been on moderate to high doses of typical antipsychotics, including depot, for more than 5 yr. Their mean age was 46 yr (s.d. 17 yr), and 27% were female.

The study was approved by the Newcastle Joint Ethics Committee, and written, informed consent was obtained from all participants. The cross-sectional prevalence of TD was assessed using the Abnormal Involuntary Movement Scale (AIMS) (US Department of Health, Education & Welfare, 1976). Any patient with a total AIMS score of four or more was assessed as currently suffering from TD, indicating the presence of moderate to severe TD (Armstrong et al., 1997; Morgenstern and Glazer, 1995).

To identify the Ser-9 (allele 1) and Gly-9 (allele 2) variants of the Ser-9-Gly polymorphism of the human DRD3 gene, the 5’-end of the gene containing the codon 9 polymorphism was amplified from genomic DNA by polymerase chain reaction (PCR), followed by MscI restriction enzyme digestion of the resulting PCR products, with minor modifications in comparison to the method previously described by others (Lannfelt et al., 1992). The genotyping was performed blind to the clinical status of the patients.

Since four DNA samples failed to amplify by PCR despite repeated attempts, a total of 71 patients were included in the genetic study and statistical analyses.

Exact statistical and power analyses were performed using StatXact for Windows, v. 4.0.1 (Cytel Software Corp., MA, USA). The Mann-Whitney U test and Kruskal-Wallis test included in the SPSS statistical package v. 8.0 (SPSS Inc., IL, USA) were used to compare the difference in age between patient groups. The utility programs of J. Ott (ftp://linkage.rocke-feller.edu/software/utilities) were used to test for Hardy–Weinberg equilibrium of the genotype distributions.

Results
In the cross-sectional evaluation of movement disorders among the 71 schizophrenic patients included, 32 (45%) of the subjects had been assessed as having TD (Armstrong et al., 1997). The patients with TD were significantly older (mean age 53 yr, s.d. 18 yr) than the subjects without TD (mean age 41 yr, s.d. 12 yr; \( p = 0.005 \) by Mann-Whitney U test).

DNA samples from the patients were subjected to DRD3 genotyping, and the DRD3 allele frequencies and genotype distribution are shown in Table 1. Allele 2 was non-significantly more frequent among subjects with TD, compared to the patients without TD [44 vs. 33%; \( p = 0.23 \); OR 1.56 (95% CI 0.74–3.26) by two-sided Fisher exact probability test]. The distribution of the 1–1, 1–2, and 2–2 genotypes in the total sample, in the TD group and in the non-TD group was in accordance with a Hardy–Weinberg equilibrium (data not shown). Mean age was not significantly different when the patients were grouped according to their genotype (1–1, 1–2, or 2–2), neither in the total sample (Kruskal–Wallis test, \( p = 0.77 \)) nor in the non-TD group (Kruskal–Wallis test, \( p = 0.40 \))

Table 1. The cross-sectional prevalence of TD and the frequency of DRD3 alleles and genotypes

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Allele frequency (%)</th>
<th>Genotype distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allele 1</td>
<td>Allele 2</td>
</tr>
<tr>
<td>TD status</td>
<td>n</td>
<td>Freq. (%)</td>
</tr>
<tr>
<td>TD</td>
<td>32</td>
<td>56</td>
</tr>
<tr>
<td>No TD</td>
<td>39</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>62</td>
</tr>
</tbody>
</table>

* TD vs. no TD, \( p = 0.005 \), by two-tailed Mann–Whitney U test.
† TD vs. no TD, \( p = 0.23 \), OR 1.56 (95% CI 0.74–3.26) by two-sided Fisher exact probability test.
‡ TD vs. no TD, \( p = 0.20 \), OR 2.45 (95% CI 0.55–12.53) by two-sided Fisher exact probability test.
overall genotype distribution was not significantly different in the TD vs. non-TD group (Fisher–Freyman–Halton test, \( p = 0.37 \)).

Our initial finding (Steen et al., 1997) showed an apparent association between the 2–2 genotype and TD, in agreement with a recessive model of inheritance. We therefore grouped our data into the 1–1 and 1–2 genotypes vs. the 2–2 genotype, and found that homozygosity for the Gly-9 variant (2–2 genotype) was non-significantly more frequent in the TD group, compared to the patients without this movement disorder \( [22 \text{ vs. } 10\% ; \ p = 0.20; \ OR \ 2.45 \ (95\% \ CI \ 0.55–12.53) \] by two-sided Fisher exact probability test). With respect to the genotype, 7 out of 11 (64%) patients homozygous for the Gly-9 allele were assessed as suffering from TD, whereas 14 out of 32 (44%) and 11 out of 28 (39%) schizophrenic patients with the 1–2 and 1–1 genotypes, respectively, had TD.

In a dominant model, the 1–2 and 2–2 genotypes were non-significantly more frequent than the 1–1 genotype in the group of TD patients vs. the non-TD patients \( [60 \text{ vs. } 56\% ; \ p = 0.47; \ OR \ 1.48 \ (95\% \ CI \ 0.51–4.36) \] by two-sided Fisher exact probability test), but, this difference was completely due to the over-representation of the 2–2 genotype among the TD subjects.

Discussion

We have previously demonstrated a significantly higher frequency of homozygosity for the Gly-9 variant of the DRD3 gene among antipsychotic-treated schizophrenic patients with TD, compared to patients without TD (Steen et al., 1997). In this study of an independent sample of schizophrenic patients from Newcastle upon Tyne, UK, we observed a similar, but non-significant over-representation of allele 2 and the 2–2 genotype in the subgroup of TD patients. Two recent studies have supported a relationship between allele 2 of DRD3 and increased risk of TD. Segman and co-workers have demonstrated that the genotypes containing allele 2 were significantly associated with the presence of TD in antipsychotic-treated patients with schizophrenia, mainly due to an over-representation of the heterozygous 1–2 genotype in the TD group (Segman et al., 1999). A similar relationship between the Gly-9 variant of DRD3 and typical neuroleptic-induced TD has been reported by Basile et al. (1999). They also found that patients homozygous for the Gly-9 allele (2–2 genotype) had significantly higher AIMs scores compared to subjects with the 1–2 and 1–1 genotypes, while Segman and co-workers reported significantly higher AIMs scores in patients carrying both the 1–2 and 2–2 genotypes, compared to the 1–1 genotype. An association between early dyskinesia and the 2–2 genotype in neuroleptic-treated schizophrenic patients has recently been suggested (Rohrmeier et al., 1998).

In accordance with most other studies, we found that the mean age of the patients with TD was significantly higher when compared to the patients without TD. There is, however, no significant difference in the mean age of the patients in the three genotype groups. These data indicate that the trend towards an over-representation of the 2–2 genotype among patients with TD is not due to a spurious accumulation of older patients among those being homozygous for the Gly-9 variant.

However, the lack of statistical significance in our present study indicates that the possible association between the DRD3 polymorphism and TD remains to be finally established. Indeed, two groups have recently failed to detect an association between TD and the DRD3 Gly-9 polymorphism (Aschauer et al., 1998; Rietschel et al., 1997). On the other hand, it should be noted that the risk of a false negative result in our present study is high, since the power of the sample to detect a difference of the same magnitude as in our initial cross-sectional study is approx. 60% at a significance level of \( p = 0.05 \). It is therefore possible that the lack of a significant replication in the sample of schizophrenic patients from Newcastle could be due to a type II error on the basis of the low number of subjects. Unfortunately, we have not been able to overcome this problem by increasing the size of the sample. It should also be noted that population stratification is a major concern in unmatched case/control studies. In this study, we have only included schizophrenic patients of Caucasian ethnicity from the Newcastle region. This area in the north-east of England tends to have a stable population with ethnic ancestry mainly of the group from northern England, Scotland, Ireland and the Scandinavian countries, and it is therefore less likely that an ethnic stratification has influenced our results.

There are several possible explanations for the possible association between the DRD3 allele 2 and risk for TD. Firstly, the Gly-9 variant of the DRD3 may display altered binding affinity or functional response to dopamine and antipsychotic drugs (Kane, 1995; Lundstrom and Turpin, 1996). Second, patients homozygous for allele 2 of the DRD3 may constitute a subgroup of schizophrenic subjects, with an especially high risk for TD. Several studies have demonstrated an association between both negative symptoms and cognitive impairment, and increased susceptibility for TD (Liddle et al., 1993; Waddington et al., 1990; Waddington and Youssef, 1996). We have recently suggested that the conflicting reports regarding the apparent link between schizophrenia per se and the DRD3 may be explained by
a selection bias in the sampling of patients, since schizophrenic subjects with TD (as well as negative symptoms and cognitive impairment) may need more frequent and prolonged psychiatric care, leading to an increased chance of being included in research projects (Steen et al., 1997). It is also important to note that abnormal movements and TD may be present at a high frequency even in never-treated schizophrenic patients (McCreadie et al., 1996; Owens et al., 1982). Therefore, to further explore the role of the DRD3 in TD development and schizophrenia, the distribution of DRD3 genotypes should be determined in both treated and never-treated schizophrenic patients with and without TD. Future studies on large samples and subsequent meta-analysis seems to be necessary to elucidate the role of the DRD3 in the pathogenesis of TD.

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