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Dopamine D_3 receptor variant and tardive dyskinesia

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Abstract In the search for genetic factors contributing to tardive dyskinesia, dopamine receptor genes are considered major candidates. The dopamine D_3 receptor is of primary interest as dopamine D_3 receptor knock-out mice show locomotor hyperactivation resembling extrapyramidal side-effects of neuroleptic treatment. Furthermore, Steen and colleagues (1997) recently reported an association between tardive dyskinesia and a dopamine D3 receptor gene variant.

In the present study we tried to replicate this finding. We investigated 157 patients with schizophrenia or schizoaffective disorder receiving long-term neuroleptic medication who never or persistently displayed tardive dyskinesia. As advanced age is a main risk factor for tardive dyskinesia, we also compared older patients with a long duration of schizophrenia not displaying tardive dyskinesia to younger patients with a shorter duration of the illness displaying tardive dyskinesia. However, we found no evidence that the dopamine D_3 receptor gene is likely to confer susceptibility to the development of tardive dyskinesia.

Key words Pharmacogenetics · Genetics · Risk factor · Choreoathetotic movements

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Introduction

Neuroleptic-induced tardive dyskinesia (TD) continues to be a serious problem in the psychopharmacology of schizophrenia. While it is one of the most serious adverse effects of neuroleptic treatment in psychotic disorders, it is common with an overall mean prevalence rate of 24% (for review, see Yassa and Yeste 1992), is frequently chronic and has no definitive treatment.

The underlying pathomechanism of TD still remains unknown, and while some patients develop TD, others do not. Known risk factors such as age, gender, ethnicity, psychiatric diagnosis, smoking, diabetes mellitus, 'organic' brain dysfunction, and neuroleptic treatment variables such as type of drug and lifetime dosage predict only a minor part of variance in the incidence of TD (for review, see Casey 1995; Jeste and Caligiuri 1993). Evidence for the contribution of genetic factors to the development of TD comes from animal studies and clinical observations. Rats treated chronically with neuroleptics develop vacuous chewing movements (VCMs), similar in some respects to TD in man (Clow 1980; Iversen et al. 1980; Gunne et al. 1982; Waddington et al. 1982; Mithani et al. 1987). VCMs vary in severity and frequency between different rat strains, suggesting that a genetic mechanism might underlie vulnerability (Belmaker et al. 1981; Tamminga et al. 1990; Rosengarten et al. 1994). This observation is consistent with clinical reports in which affected members of the same family show concordance for development or absence of TD (Yassa and Ananth 1981; Youssef et al. 1989; Weinhold et al. 1981; Waddington and Youssef 1988).

In the search for genetic vulnerability factors the dopamine receptor genes are considered major candidates, as all antipsychotics binding to dopamine receptors are potentially able to induce TD. The D_3 receptor is of major interest for research into psychopharmacology and TD in light of the fact that most of the antipsychotic drugs used in clinical practice display affinity for this receptor. Postmortem studies have found elevated levels of D_3 receptor

in the basal ganglia and ventral forebrain of chronic schizophrenic patients who received no antipsychotic drugs in contrast to those with antipsychotic medication (Gurevich et al. 1997). Furthermore, mice lacking the D_3 receptor show locomotor hyperactivation resembling extrapyramidal side-effects of neuroleptic treatment (Accili et al. 1996).

The gene for the human dopamine D_3 receptor has been cloned (Sokoloff et al. 1990) and is localized on chromosome 3q13.3 (Le Coniat et al. 1991). Lannfelt et al. (1992) identified a common G to A transition 25 bp downstream from the start codon resulting in the substitution of a glycine for a serine at position 9 in the extracellular N-terminal part of the receptor. The Ser9Gly variant is the only known variant of the human D_3 receptor which affects the structure of the protein (Lannfelt et al. 1992; Asherson et al. 1996; Griffon et al. 1996). The observation of functional differences between the two receptor variants (Lundstrom and Turpin 1996) together with the common occurrence of the mutation in the population makes it an interesting candidate to study for a possible involvement in determining individual development of TD. This hypothesis was previously tested by Steen and colleagues (1997) in a sample of 100 Scottish patients who were evaluated for the presence of TD. The Gly9 variant was found to increase risk for TD with an odds ratio of 6.5 in patients carrying the variant on both chromosomes.

In the present study we tried to replicate the finding of Steen and colleagues (1997) in a large sample of German schizophrenic and schizoaffective patients receiving long-term neuroleptic medication. We compared genotype frequencies between patients with and without TD. To avoid confounding affects of heterogeneity we also analyzed subgroups formed with respect to duration of illness. Furthermore, we compared older patients with a long duration of schizophrenia not displaying tardive dyskinesia to younger patients with a shorter duration of the illness displaying tardive dyskinesia. If genetic factors have an influence on the development of TD, one expects those patients to be carrier of a susceptibility gene for TD who are young and display TD already after a short duration of the disorder. Those patients, on the other hand, who are older and do not develop TD, even after a long duration of schizophrenia, are less likely to carry this vulnerability gene.

Subjects and Methods

Patients

Two hundred and sixty-six unrelated German patients receiving long-term neuroleptic treatment were included in the study. Written informed consent was obtained from all patients. All patients had been interviewed by experienced psychiatrists using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) (Endicott and Spitzer 1978). Lifetime 'best estimate' diagnoses according to DSM-IV criteria (American Psychiatric Association 1994) were based on multiple sources of information including personal structured interviews (SADS-L), medical records, and family history. TD were rated at least twice during a three-month period by the means of the Tardive Dyskinesia Rating Scale (Simpson et al. 1979). Presence or absence of TD was defined according to RDC criteria (Schooler and Kane 1982).

Patients with transient or developed TD and those with a diagnosis other than schizophrenia or schizoaffective disorder were excluded from the analysis. One hundred and fifty-seven out of the 266 patients fulfilled these inclusion criteria and were included in the analysis. Lifetime neuroleptic dose was assessed in chlorpromazine equivalents. Assessment of lifetime neuroleptic dose was done retrospectively based on medical records and patients report. In case of uncertainty, it was coded as 'unknown'.

All psychiatric assessments were carried out prior to genetic analysis.

DNA Analysis

DNA was extracted from blood samples using standard methods. Genotyping was performed as previously described (Rietschel et al. 1993). All genotypes were independently scored by two investigators.

Statistical analysis

The χ^2-goodness-of-fit test was used to test for deviations from Hardy-Weinberg equilibrium. To compare allele and genotype frequencies between patients with persistent TD and patients without TD, Fisher's exact test was used. The t-test for independent samples was used to compare the difference in age at onset, age at interview, lifetime neuroleptic dose, and the Mann-Whitney test for differences in duration of schizophrenia between the groups.

Results

One hundred and fifty-seven patients (75 males, 82 females) with a duration of illness of at least five years were included in the primary analysis. One hundred and nineteen patients had the DSM-IV diagnosis of schizophrenia and 38 that of schizoaffective disorder. Seventy-nine patients showed persistent TD and 78 patients did not show TD. Patients with TD (TD+) and patients without TD (TD–) did not differ with respect to sex and diagnosis, and lifetime neuroleptic dose. As there was uncertainty of lifetime neuroleptic dose in some patients, comparison of lifetime chlorpromazine equivalents is based on data of 67 TD+ and 66 TD– patients. Although there was a tendency for TD+ patients to be older at interview (TD+: 43.85 years, SD 8.73; TD–: 42.24 years, SD 7.92) and to have a longer duration of disease (TD+: 19.14 years, SD 7.56; TD–: 17.55 years, SD 7.53) than TD– patients, these differences proved to be nonsignificant.

Genotype counts are shown in Table 1. The genotype distribution did not differ significantly from that expected according to Hardy-Weinberg equilibrium in TD+ patients ($\chi^2 = 2.62$, df = 1, p = 0.15) and in TD– patients ($\chi^2 = 0.34$, df = 1, p = 0.561). No significant differences of allele frequencies (p = 0.618) and of the Gly/Gly genotypes (p = 0.328) were found between the two groups.

In the following we formed subgroups of patients including only those with a duration of the psychiatric illness of 10, 15, and 20 years. Results are shown in Table 1. Furthermore, we compared two extremegroups: A) TD–

Table 1 Allele frequencies and genotype counts in TD+ and TD- patients subtyped with respect to duration of the psychiatric illness

Duration in years (mean ± SD)	n	Frequency Gly-allele	Genotype					
			Ser/Ser	Ser/Gly	Gly/Gly	odds ratio[a]	95% CI[a]	P[a]
>5 (18.35 ± 7.56)								
TD+	79	0.27	39	37	3			
TD–	78	0.30	37	35	6	0.474	0.114–1.965	0.328
>10 (20.56 ± 9.10)								
TD+	75	0.26	39	33	3			
TD–	67	0.31	31	31	5	0.517	0.119–2.250	0.476
>15 (23.34 ± 7.74)								
TD+	58	0.28	28	27	3			
TD–	48	0.31	22	22	4	0.6	0.128–2.823	0.699
>20 (27.63 ± 7.32)								
TD+	33	0.26	18	13	2			
TD–	27	0.30	12	14	1	1.677	0.144–19.563	1.0

[a] TD+ versus TD– by two-tailed Fisher's exact test for Gly/Gly genotype frequencies.

Table 2 Extreme groups: genotype counts in TD+ higher age patients versus TD– lower age patients

Age in years (mean ± SD)	n	Genotype					
		Ser/Ser	Ser/Gly	Gly/Gly	odds ratio[a]	95% CI[a]	P[a]
Extreme group A							
TD+							
<35 (32.9 ± 1.8)	13	5	8	0			
TD–							
>50 (53.3 ± 3.2)	13	5	6	2	*		0.480
Extreme group B							
TD+							
<40 (36.1 ± 2.8)	37	16	19	2			
TD–							
>45 (50.1 ± 3.9)	28	13	12	3	0.476	0.074–3.063	0.644

[a] TD+ versus TD– by two-tailed Fisher's exact test for Gly/Gly genotype frequencies. * odds ratio could not be computed due to a zero cell

patients older than 50 years were compared to TD+ patients younger than 35 years, and B) TD– patients older than 45 years were compared to TD+ patients younger than 40 years (Table 2). As expected, duration of disorder was significantly ($p < 0.001$) longer in TD– patients (A: 25.23 years, SD 9.23; B: 21.71 years, SD 9.21) than in the TD+ patients (A: 13.92 years, SD 2.90; B: 15.78 years, SD 4.23). No association with the Gly/Gly genotype and TD could be found in any of the subgroups (Tables 1 and 2). Similiar negative results were observed for comparison of genotypes Gly/Ser and Ser/Ser as well as for comparison of allele frequencies (data not shown).

Discussion

Since the original report on neuroleptic drug induced TD by Schönecker in 1957, the underlying pathophysiological mechanisms of this iatrogenic condition still remain unknown.

Family studies (Yassa and Ananth 1981; Youssef et al. 1989; Weinhold et al. 1981; Waddington and Youssef 1988) together with the results of animal studies (Belmaker et al. 1981; Tamminga et al. 1990; Rosengarten et al. 1994) are in favour of genetic factors contributing to the development of TD.

The aim of genetic association studies is to identify these influences on a molecular level. In complex clinical phenotypes which result from the interaction of numerous small gene effects, the advantage of the association strategy is its power to detect genes of small effects (Nöthen et al. 1993; Risch and Merikangas 1996). Positive results, however, have to be regarded preliminary until they are replicated in independent samples. In the present study we could not replicate the finding of Steen and colleagues (1997) in 157 German patients who were included in a longitudinal evaluation of TD. As mean lengths of psychiatric illness in the patient sample of Steen and colleagues (1997) were longer (TD+: 25 years, SD 15; TD–: 21 years, SD 14) than in our sample (TD+: 19 years, SD 8; TD–: 18 years, SD 8), we also formed subgroups of patients including only those with a minimal duration of the psychiatric illness of 10, 15, and 20 years, respectively. However, no effect was found in any of these subgroups. As aging is considered a major risk factor for TD (Smith and Baldessarini 1980, Jeste and Wyatt 1982, Kane et al. 1992, Woerner et al. 1988) we formed extremegroups with respect to age of patients: TD– patients with higher age and a long duration of illness were compared to young TD+ patients with short duration of illness. Patients who do not display TD despite their high age-associated risk for TD are likely to carry less genetic risk factors for TD

than those patients displaying TD already in young age. Although the number of patients belonging to extremegroups is relatively small, the power to detect effects, if they are present, is probably high. In our sample however, we could not detect any association.

Our inability to detect association of the dopamine D_3 receptor gene with TD does not appear to be secondary to insufficient power. We had a power of 95% for detecting an association with the Gly9/Gly9 genotype of the magnitude reported by Steen's group. However, our inability to replicate the association could be related to several other factors. A possibility is the D_3 gene may be associated with the development of TD only in conjunction with other (as yet undefined) population-specific genetic or environmental factors. It remains also possible that the Ser9Gly polymorphism is not the true pathogenic variant and that a population-specific mutation conveying an effect on the development of TD is in close linkage disequilibrium with the Gly9 variant in the Scottish but not in Germans, although no data exist to support or refute this hypothesis. Phenotypic differences might be encountered between the two samples. These differences may arise on account of differences in the total neuroleptic amount, duration of illness, and age of patients. However, we tried to circumvent a potential error induced by these influences by forming subgroups of patients with respect to duration of illness and by comparing extremegroups. Finally, the possibility has to be considered that the effect observed by Steen et al. (1997) was a chance finding.

In conclusion, our study, given our sample size, should have detected the reported effect if this would substantially contribute to vulnerability for developing tardive dyskinesia in the German population.

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