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BRIEF REPORT

Tardive dyskinesia and DRD2, DRD3, DRD4, 5-HT2A variants in schizophrenia: an association study with repeated assessment

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Abstract

We performed an association study between four candidate genes, DRD2, DRD3, DRD4 and 5-HT2A for the presence of tardive dyskinesia (TD) on 84 patients with residual schizophrenia. The sample was evaluated again for the presence of TD after an interval of 3 years. The first group did not exhibit TD in either observation ($n=34$) while in the second group of patients exhibited TD in at least one of the observations ($n=20+18$). The clinical and socio-demographic characteristics were not significantly different between the two groups; the genetic analysis revealed a significant correlation between the C/C genotype of 5-HT2A and TD ($p=0.017$). An association trend was observed between the 'short' variant of DRD4 and TD ($p=0.022$). We did not observe any significant association for the DRD2 and DRD3 polymorphisms.

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Introduction

One of the most important side-effects in the long-term treatment of schizophrenia with typical antipsychotics is tardive dyskinesia (TD), an involuntary movement disorder of the oro-facial musculature that may also involve the trunk and extremities. In effect, up to one fifth of those patients can develop this potentially irreversible side-effect (Kane and Smith, 1982). The reasons for the development of TD in some patients and not in others are largely unknown, but risk factors are reported to be older age, female gender, occurrence of extrapyramidal symptoms in previous neuroleptic treatments, affective disorders diagnosis, dose and duration of antipsychotic therapy (Kane and Smith, 1982). Genetic factors have also been suggested to be implicated in TD development; although the pathophysiological mechanisms are not well understood, alterations of the dopaminergic or serotonergic neurotransmission have been postulated to play a relevant role.

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A genetically determined vulnerability has been suggested by animal studies (Tamminga et al., 1990). In family studies, moreover, a concordance for TD status among first-degree relatives was observed (Yassa and Ananth, 1981) and a family history of TD seems to represent a risk factor for TD, whereas a family history of schizophrenia does not. Regarding molecular determinants, it was hypothesized that a shift in the balance of dopamine D1/D2 receptor activity could cause a hypersensitivity of receptors; therefore, a chronic dopamine blockade may result in up-regulation of dopamine receptor responsiveness. The DRD2 dopaminergic receptor has been investigated: Chen et al. (1997) reported an increased frequency of the *Taq*I A2/A2 genotype of the D2 receptor gene in a subgroup of Japanese females with schizophrenia, although other studies did not confirm the result (Chong et al., 2003). The DRD3 gene has been suggested as a possible susceptibility factor for the development of TD (Steen et al., 1997). This finding was confirmed by other researchers, who found a strong association between TD and DRD3 Ser9Gly genotype (Segman et al., 1999), and by a recent meta-analysis (Lerer et al., 2002), while Rietschel et al. (2000) did not find any positive result. Although the dopamine D2 and D3 receptors have been traditionally

considered as being possibly implicated in the pathogenesis of TD, some authors suggested that other dopamine receptors are involved; however, there is only one study on the D4 receptor and TD, and no association was found (Segman et al., 2003). Regarding the serotonin system, some polymorphisms on the 5-HT2A receptor gene were investigated, and a correlation between C102C/G–1348G genotypes and TD was observed in patients with chronic schizophrenia, but no correlation with the His452Tyr polymorphism (Segman et al., 2001). A significant association between the 102C allele and patients with TD was also reported by Tan et al. (2001), while other authors failed to find any positive results (Basile et al., 2001).

The aim of this study was to investigate a possible association between the presence of TD and a set of candidate genes (DRD2, DRD3, DRD4, 5-HT2A) in a homogeneous Italian population affected by residual schizophrenia.

Methods

Sample

A sample of 84 subjects with DSM-IV diagnosis of residual schizophrenia were recruited in a chronic in-patient psychiatric institute in Milan (male/female = 40/48; age = 54.33 ± 10.5 yr; onset = 25.15 ± 6 yr). The clinical information was obtained through clinical records. The sample was previously published in a clinical descriptive study (Cavallaro et al., 1993) and it was included in a multicentre analysis for DRD3 gene variants (Lerer et al., 2002). All the patients gave informed consent concerning the collection of data, the analysis of blood samples and the genotyping of DNA. To better characterize the TD phenotype all patients underwent a clinical examination at T1 and 3 years later at T2. TD was diagnosed according to the criteria of Schooler and Kane (1982), while the TD assessment was obtained with the Rockland–Simpson Scale (Simpson et al., 1979). This enabled us to consider the more stable phenotypes in the final analysis. In fact, at the first observation (T1) 30 patients exhibited TD, whereas 54 did not. At the second observation (T2), only 18 of the previous 30 subjects still showed signs of TD, whereas 12 no longer did, for whom a diagnosis of Transitory TD was used. Moreover, of the 54 patients that had not developed TD at the first observation (T1), 34 did not present symptoms of TD at T2, whereas 20 did. The first comparison of clinical and genetic data took into account four patients without lifetime diagnosis of TD at both observations and 38 patients with a diagnosis of Transitory/Persistent TD after 3 years. A second evaluation considered the group of 34 patients that never exhibited TD together with the 18 patients that presented it at both observations. None of the patients suffered from cerebral illness or experienced a surgical intervention during the period of observation.

DNA analysis

Genomic DNA was extracted from leucocytes by NaCl (Lahiri et al., 1991). Polymerase chain reaction (PCR) was performed with the following primers – for DRD2: 5′-ACC AGC TGA CTC TCC CCG ACC GGT-3′ and 5′-GGA AGG ACA TGG CAG GGA ATG GGA C-3′; for DRD3: 5′-GCT CTA TCT CCA ACT CTC ACA-3′ and 5′-AAG TCT ACT CAC CTC CAG GTA-3′; for DRD4: 5′-GCG ACT ACG TGG TCT ACT CG-3′ and 5′-AGG ACC CTC ATG GCC TTG-3′; for 5-HT2A (T102C): 5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG TCT GCT ACA AGT TCT GGC TT-3′ and 5′-CTG CAG CTT TTT CTC TAG GG-3′. For each polymorphism analysis 100 ng of genomic DNA was diluted to 5 ml and heated to 99 °C for 3 min. Then a reaction mix containing 0.025 U/ml *Taq* polymerase (Applied Biosystems, Monza, Italy), 1 × PCR buffer (PerkinElmer, Monza, Italy), 0.2 mM of each primer, 200 mM of dATP, 200 mM of dCTP, 200 mM of dTTP, and 200 mM of dGTP was added in a total volume of 10 ml. PCR was performed with the following annealing temperature: 60 °C for DRD2 and 5-HT2A, 56 °C for DRD3 and 54 °C for DRD4. This profile was followed by a step at 72 °C for 4 min. The PCR products were digested by the *Hpa*II, *Sau*961 or *Msc*I restriction enzymes (New England Biolabs, England, UK) respectively for the DRD2 (Arinami et al., 1994), DRD3 (Lannfelt et al., 1992), DRD4 (Van Tol et al., 1991) and 5-HT2A polymorphisms (Arranz et al., 1995). The digested products for the DRD2, DRD3 and 5-HT2A polymorphisms and the PCR product for the DRD4 polymorphism were separated on a high-resolution agarose gel and visualized by ethidium bromide.

Statistical analysis

Chi-square was used to compare frequencies, logistic regression was used to include possible confounders and when analysing multiple polymorphisms simultaneously. Both genotype and allele frequencies were analysed because of the absence of unequivocal evidence towards dominance or recessivity. Given the small cell number for some gene variants, we reported the empirical p value obtained through Monte Carlo simulation with 10000 replicates (Sham and Curtis,

Table 1. Tardive dyskinesia (TD) and 5-HT2A/DRD4 associations (only significant results are presented)

	No TD	Yes TD	
5-HT2A genotypes			
T/T	8 (24%)	10 (26%)	$\chi^2 = 7.96$, d.f. = 2, $p = 0.017$
T/C	23 (68%)	15 (39%)	
C/C	3 (9%)	13 (34%)	
DRD4 genotypes			
l/l	4 (12.5%)	0 (0%)	$\chi^2 = 5.3$, d.f. = 2, $p = 0.065$
l/s	8 (25%)	7 (20%)	
s/s	20 (62.5%)	28 (80%)	
DRD4 alleles			
Long allele	16 (25%)	7 (10%)	$\chi^2 = 5.3$, d.f. = 1, $p = 0.022$
Short allele	48 (75%)	63 (70%)	

1995). The scores obtained using the Rockland–Simpson Scale were analysed by ANOVA across the genotypes within subjects showing TD in any observation time. For the subjects with a diagnosis of TD at both evaluations, we used the average Rockland–Simpson Scale score. Given the number of analyses performed we applied a Bonferroni correction and we considered a significance level of 0.013 (00.5/4 genes). For categorical analyses, the power of our sample to detect differences between groups was calculated considering an α-value of 5% two-tailed. With these parameters we had a high power (0.80) to detect a small-medium effect size ($w = 0.23$) that corresponded to a difference of approx. 21% between two alleles (Cohen et al., 1999).

Results

The sample was in Hardy–Weinberg equilibrium (HWE) for all markers [DRD2 (HWE = 0.093, d.f. = 1, $p = 0.76$); DRD3 (HWE = 0.92, d.f. = 1, $p = 0.34$); DRD4 (HWE = 3, d.f. = 1, $p = 0.08$); 5-HT2A (HWE = 0.23, d.f. = 1, $p = 0.63$)].

The analysis of the main clinical and socio-demographic characteristics of the two groups ($n = 34$ vs. 38) did not reveal any significant difference.

For genetic analysis, we compared the group of patients never displaying TD ($n = 34$) with the group of Transitory/Persistent TD ($n = 20 + 18$). Only the 5-HT2A variants were associated with Transient or Persistent TD at any time in the direction of a higher frequency of C/C genotypes in subjects affected by TD ($p = 0.017$) (Table 1). When included in a logistic regression model including sex, age, duration of illness and 5-HT2A variants, none of the clinical variables was significantly associated with TD, while the 5-HT2A genotype effect remained significant. This effect was not observed when considering alleles ($p = 0.18$). The mean Rockland–Simpson Scale score was not different among genotypes ($F = 1.57$, d.f. = 2, 35, $p = 0.22$), however, only a trend for lower scores in T/T-carrying subjects was observed. We also found a trend towards a positive association with the DRD4 polymorphism ($p = 0.065$ and 0.022 when considering genotype and allele respectively) (Table 1). For the DRD2 and DRD3 polymorphisms we were unable to detect any significant associations. All dopaminergic polymorphisms were not associated with symptomatology scores (data not shown). Following previous studies in the literature, we tried to replicate the results on interaction between DRD3 and 5-HT2A and between DRD4 and 5-HT2A genes. However, we did not observe any significant interaction.

In a further analysis we compared the group of patients that did not show stable TD ($n = 34$) with individuals with Persistent TD ($n = 18$). However, because of the reduction of sample size, the results went in the same direction as the previous ones but without any statistical significance.

Discussion

The exclusive feature of our study was the accurate selection of the sample through a clinical double assessment over a period of 3 years. This enabled us to subdivide the patients in two groups: the group of individuals that never exhibited TD at either observation ($n = 34$) and the group of patients exhibiting TD in at least one of the observations ($n = 18 + 20$). In a second phase, in order to obtain a further restriction of the phenotype, we compared the group of patients never exhibiting TD ($n = 34$) with the group of patients with Persistent TD at both observations ($n = 18$). In our sample we could not observe any association of TD with female sex. In genotype analysis we found a significant association between the presence of TD and an excess of the 5-HT2A C/C genotype, a silent mutation in the linkage disequilibrium with the G-1438A polymorphism of the promoter (Ohara et al., 1999). However the association was not confirmed considering the C allele only. The analysis of the symptomatology score did not show positive associations, except for a trend vs. lower scores in T/T-carrying subjects. Larger samples are required to further investigate the possible influence of those genes on TD. The subjects with TD also showed a

lower frequency of short DRD4 alleles, although not significant; this result could support the hypothesis that variants of D4 may explain some of the inter-individual variation seen in patient response to anti-psychotics (Cohen et al., 1999).

In our sample we observed that 40% ($n=12$) of patients, exhibiting TD symptoms at the first observation (T1), did not present symptoms at the second observation (T2); this phenomenon should be investigated in larger prospective studies. A relevant limit of this study is the absence of a cumulative neuroleptic dose; however, all the subjects were treated with stable pharmacological doses during the observation period. A further limitation is linked to multiple testing; with the use of Bonferroni correction our results would not survive statistical significance. Some of the genes analysed, however, have been found associated with TD by other investigators, thus reducing the need of corrections. Another limitation is linked to ethnic origin that is frequently a cause of stratification bias, but our sample was composed of subjects mainly collected in the North of Italy with Italian antecedents for at least two generations and Italy is characterized by a substantial genetic homogeneity (Gasparini et al., 1997). Our results support the hypothesis of an involvement of 5-HT2A and DRD4 polymorphisms in TD but further studies are necessary to confirm our observation.

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None.

Statement of Interest

None.

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