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Association study of tardive dyskinesia and twelve DRD2 polymorphisms in schizophrenia patients

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Abstract

Tardive dyskinesia (TD) is a side-effect of chronic antipsychotic medication. Abnormalities in dopaminergic activity in the nigrostriatal system have been most often suggested to be involved because the agents which cause TD share in common potent antagonism of dopamine D_2 receptors (DRD2), that notably is not balanced by effects such as more potent serotonin $(5\text{-HT})_{2A}$ antagonism. Thus, a number of studies have focused on the association of dopamine system gene polymorphisms and TD. The most consistent findings have been found with the Ser9Gly polymorphism of the *DRD3* gene. Although DRD2 has long been hypothesized to be the main target for antipsychotics, only a few polymorphisms in DRD2 have been investigated for their potential involvement in the aetiology of TD. In the present study, we investigated 12 polymorphisms spanning the *DRD2* gene and their association with TD in our European Caucasian ($n=202$) and African–American ($n=30$) samples. Genotype frequencies for a functional polymorphism, C957T (Duan et al., 2003; Hirvonen et al., 2004), and the adjacent C939T polymorphism were found to be significantly associated with TD ($p=0.013$ and $p=0.022$ respectively). DRD2 genotypes were not significantly associated with TD severity as measured by AIMS (Abnormal Involuntary Movement Scale) with the exception of a trend for C939T ($p=0.071$). Both TD and total AIMS scores were found to be significantly associated with two-marker haplotypes containing C939T and C957T ($p=0.021$ and $p=0.0087$ respectively). Preliminary results indicated that C957T was also associated with TD in our African–American sample ($p=0.047$). Taken together, the present study suggests that DRD2 may be involved in TD in the Caucasian population, although further studies are warranted.

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Introduction

Tardive dyskinesia (TD) is a potentially irreversible motor side-effect that develops in schizophrenia patients treated chronically with typical antipsychotic drugs. It is characterized by involuntary choreoathetotic movements mostly in the orofacial regions, with more severe cases involving the trunk as well as the upper and lower limbs. Its reported prevalence varies from 16% to 43%, with an annual incidence rate of around 5% (reviewed in Tarsy and Baldessarini, 2006). Patients with this condition often struggle with the immediate difficulties of motor function, but also with adhering to treatment, discrimination, and poorer quality of life (Gerlach, 2002; Marsalek, 2000),

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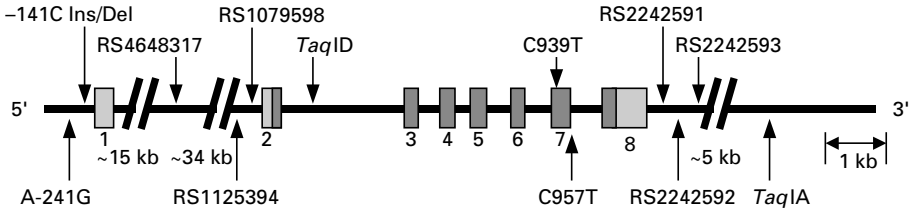

Figure 1. Schematic diagram of the *DRD2* gene with its exons and introns. The positions of the 12 polymorphisms used for the present study are indicated within the gene.

so predicting which patients are vulnerable to TD remains a high priority for psychiatrists in treatment selection.

The aetiology of TD is complex and remains unclear. Age, gender, and ethnicity are all suggested risk factors for TD. More specifically, Woerner and co-workers found that patients aged >50 yr were 3–5 times more likely to develop TD than younger patients in other studies (Kane et al., 1988; Morgenstern and Glazer, 1993; Woerner et al., 1998). A review of 13 studies found females to be more likely to develop TD with an odds ratio of 1.69 (Smith and Dunn, 1979), although other studies reported the opposite findings (Morgenstern et al., 1987; van Os et al., 1997). The difference was attributed to possible selection bias for more severe cases in the female gender group in earlier studies. Jeste and co-workers reported a yearly TD incidence of over 45% for African–Americans compared to 27% in Caucasians (Jeste et al., 2000), but socioeconomic factors could have contributed to their findings. Environmental risk factors such as smoking as well as alcohol and recreational drug use could further increase the risk for TD (Bailey et al., 1997; Menza et al., 1991; Oliveira et al., 1990). Concordance for the presence or absence of TD among first-degree relatives provided evidence for a genetic component of TD (Müller et al., 2001). A number of mechanisms leading to TD have been hypothesized, including GABA insufficiency (Casey, 2000), free radical-mediated neuronal injury (Andreasson and Jorgensen, 2000), and structural abnormalities in brain regions involved in motor function such as the caudate nucleus (Chakos et al., 1994; Corson et al., 1999). The best known hypothesis postulates TD as being caused by hypersensitivity of dopamine receptors induced by dopamine receptor-blocking medication including antipsychotics (Abilio et al., 2003; Gerlach and Casey, 1988; Klawans et al., 1980; Tarsy and Baldessarini, 1977). This hypothesis is based on several observations. Persistent dyskinesia was induced by neuroleptic treatment in a non-human primate model of TD with significantly decreased dopamine turnover in the caudate and substantia nigra (Gunne et al., 1984). The dopamine D_2 receptor is most densely expressed in the basal ganglia, an area of the central nervous system that regulates movements (Hall et al., 1994). Moreover, clinical studies (Beasley et al., 1999; Jeste et al., 1999a,b; Kane et al., 1993) and rodent vacuous chewing TD models (Gao et al., 1998; Johansson et al., 1986) have revealed that compared to atypical antipsychotics, TD is more likely to develop after treatment with typical antipsychotics that generally have higher affinities for D_2 given by in-vitro competition assays (Schwartz et al., 2000). Some antipsychotic drugs that have high affinities for D_2 have a lower risk of causing TD, e.g. olanzapine, risperidone and ziprasidone. This appears to be due to these drugs also being more potent 5-HT_{2A} antagonists (Meltzer et al., 2003). Thus, variation in D_2 function and expression may contribute to the risk of TD development.

The dopamine D_2 receptor belongs to the G-protein-coupled receptor family. It activates intracellular signalling by inhibiting the synthesis of cAMP. *DRD2*, the D_2-encoding gene, consists of eight exons and spans ~65 kb (Figure 1). Several in-vivo and in-vitro studies have focused on the effect that DRD2 polymorphisms have on D_2 expression. The −141C Del allele has been reported to decrease DRD2 promoter activity in vitro (Arinami et al., 1997), while one in-vivo study showed that the Del allele is associated with an increase in striatal D_2 binding (Jönsson et al., 1999a); however, another in-vivo study reported no significant difference (Ritchie and Noble, 2003). The B1 allele in intron 1 has been associated with decreased D_2 binding in vivo (Jönsson et al., 1999a; Ritchie and Noble, 2003), as has T957 (Hirvonen et al., 2004). The latter has also been reported to decrease DRD2 mRNA stability in vitro (Duan et al., 2003). The *Taq*IA polymorphism has received much attention recently as it was discovered to reside in an overlapping gene, *ANKK1*, and cause a non-synonymous coding polymorphism (Neville et al., 2004). The A1 allele has been associated with reduced D_2 levels in several studies (Jönsson et al., 1999a; Noble et al., 1991; Pohjalainen

et al., 1998; Ritchie and Noble, 2003; Thompson et al., 1997). Laruelle and co-workers (1998), however, did not find a significant association between *Taq*IA and D_2 expression levels, but this negative finding could be the result of population stratification as subjects with four different ethnic backgrounds were included.

DRD2 has been previously associated with schizophrenia. Several polymorphisms, including the −141C Ins, A2, and B2 variants, as well as their haplotypes, have been reported to be positively associated or over-transmitted in various schizophrenia samples (Arinami et al., 1997; Breen et al., 1999; Dubertret et al., 2001; Golimbet et al., 1998; Inada et al., 1999; Jönsson et al., 1999b). However, other investigators did not replicate the findings, suggesting that further studies are required (Arranz et al., 1998; Hori et al., 2001a; Stober et al., 1998; Suzuki et al., 2000; Tallerico et al., 1999). *DRD2* is one of the first genes to be tested in TD genetic studies. In all, ten studies have been conducted on *DRD2* and TD. Specifically, the A-241G, −141C Ins/Del, *Taq*IB, *Taq*ID, C939T, and *Taq*IA polymorphisms have been analysed for association with TD (Chen et al., 1997; Chong et al., 2003; de Leon et al., 2005; Hori et al., 2001b; Inada et al., 1997, 1999; Kaiser et al., 2002; Lattuada et al., 2004; Segman et al., 2003; Srivastava et al., 2006). Chen et al. (1997) initially detected an association between the *Taq*IA marker and TD particularly in female schizophrenia patients. However, the *DRD2* association with TD could not be replicated in most other studies.

In the present study, we tested for the presence of an association between the *DRD2* gene and TD using both continuous [Abnormal Involuntary Movement Scale (AIMS)] and dichotomous (TD occurrence) measures in relatively large samples of Caucasian and African–American patients with schizophrenia using 12 polymorphisms spanning the *DRD2* gene: A-241G, −141C Ins/Del, *Taq*ID, C939T, C957T, *Taq*IA, rs4648317, rs1125394, rs1079598, rs2242591, rs2242592, and rs2242593 all of which have recently been used in a detailed analysis of *DRD2* in clozapine response (Hwang et al., 2005).

Patients and methods

Subjects

Subjects were recruited from four clinical sites in North America: the Center for Addiction and Mental Health in Toronto, Ontario (Dr G. Remington, $n=94$); Case Western Reserve University in Cleveland, Ohio (Dr H. Y. Meltzer, $n=77$); Hillside Hospital in Glen Oaks, New York (Dr J. A. Lieberman, $n=49$), and University of California at Irvine, California (Dr S. G. Potkin, $n=12$). Subjects were selected based on their diagnoses of schizophrenia or schizoaffective disorder according to DSM-III-R or DSM-IV (APA, 1994). All patients had undergone cumulatively at least 1 yr of treatment with typical antipsychotics. For the Meltzer, Lieberman, and Potkin samples, the presence or absence of TD was evaluated before any atypical antipsychotic administration, while patients were on mixed typical and atypical antipsychotics when TD was evaluated in the Remington sample. The presence of TD was assessed using AIMS or the modified Hillside Simpson Dyskinesia Scale (HSDS) for 49 patients recruited from the Hillside Hospital (Basile et al., 1999; Guy, 1976; Schooler and Kane, 1982). The seven body area items and the overall global item of HSDS match those of AIMS, thus the assessment for the presence of TD was the equivalent for all four sites. All four clinicians (G.R., H.Y.M., J.A.L., S.G.P.) are highly experienced in TD severity measurements of which the consistency was further enhanced by exchange visits across sites.

In all, 232 patients were studied. Of these, 202 were European Caucasians, of which 80 were positive for the occurrence of TD. The remaining 30 were African–Americans, of which 11 were TD-positive. AIMS scores were available for 197 patients (171 Caucasians and 26 African–Americans). Because of small sample size, the African–Americans were only used in the test for allele frequency association with TD.

Gene polymorphism analysis

Genomic DNA was purified from whole blood samples using the high-salt method previously described (Lahiri and Nurnburger, 1991). Polymerase chain reactions (10 μl) on 20 ng genomic DNA were performed using TaqMan allele-specific assays with the following conditions: 95 °C for 10 min, followed by 50 cycles of 92 °C for 15 s, and 60 °C for 1 min. Genotyping was done after the subjects completed the follow-up in which all laboratory staff were blind to the AIMS scores. The A-241G, −141C Ins/Del, *Taq*ID, C939T, and *Taq*IA polymorphisms have been used in previous TD studies. The C957T polymorphism was studied because of its functional significance and its high minor allele frequency (Duan et al., 2003; Hirvonen et al., 2004). The remaining polymorphisms, rs4648317, rs1125394, rs1079598, rs2242591, rs2242592, and rs2242593, were selected based on their position within the *DRD2* gene and the

presence of sufficiently high minor allele frequencies (Hwang et al., 2005). The assays with their corresponding polymorphisms and locations are shown in Figure 1. Genotypes were determined using the ABI Prism® 7000 Sequence Detection System with the allelic discrimination program within the ABI software (Applied Biosystems, Foster City, CA, USA). All ambiguous genotypes were retyped, and if they remained ambiguous, they were taken out of the analysis.

Statistics

Statistical analyses were conducted using the Statistical Package for the Social Sciences version 10.0.7, Haploview version 3.2, and UNPHASED version 2.402 (Barrett et al., 2005; Dudbridge, 2003; Hwang et al., 2005; SPSS, 2000). Odds ratio calculations were conducted using program 2BY2 version 2 written by Jurg Ott. Genotype frequency distribution was tested for fitness to Hardy–Weinberg equilibrium using Haploview. The association of genotype frequencies with age and AIMS scores was assessed using ANOVA, and where the variances of AIMS scores among genotypes differed significantly using the Levene Test for Homogeneity of Variance, AIMS scores were examined with the Kruskal–Wallis test on SPSS. Gender differences in genotype frequencies were assessed using the χ^2 test on SPSS. The differences in allele and genotype frequencies between patients with and without TD were analysed by χ^2 test. For contingency tables with at least one expected cell count of <5, two-tailed Fisher's exact tests were performed (http://home.clara.net/sisa/fiveby2.htm). Haplotype analyses and linkage disequilibrium calculations were conducted using UNPHASED and Haploview respectively.

Ethical considerations

The scientific work described in the present paper complies with the current laws of Canada and the USA, as well as the ethical standards established in the 1964 Declaration of Helsinki. Informed consent was obtained before subjects' participation, and this study was approved by the Ethics Committee of the Centre for Addiction and Mental Health.

Results

Sample characteristics

The genotype distributions of all the *DRD2* gene polymorphisms in the European Caucasian samples did not differ significantly from Hardy–Weinberg equilibrium ($p>0.10$). RS1125394 was found not to be in Hardy–Weinberg equilibrium in our African–American sample ($p<0.01$). A significant increase in frequency of TD was found in females ($p=0.009$), while no significant association was found between gender and genotype frequencies (Table 1). We also found a significant positive correlation between AIMS scores and age ($r=0.206$, $p=0.007$).

Association study of individual polymorphisms with TD occurrence and AIMS

With our Caucasian sample, we found the C957T allele frequencies to be significantly associated with TD occurrence ($p=0.014$). Specifically, the T957 allele appears to be under-represented in TD-positive patients compared to TD-negative patients (OR_{T957} 0.59, 95% CI 0.39–0.90; Table 2). We also found a significant association for the C939 allele that appears to be present in a lower proportion of TD-positive patients compared to TD-negative patients ($p=0.0085$, OR_{C939} 0.56, 95% CI 0.36–0.86; Table 2). The genotype frequencies of the C957T and C939T polymorphisms were also found to be significantly associated with TD ($p=0.013$, OR_{TT957} 0.30, 95% CI 0.13–0.69; $p=0.022$, OR_{CC939} 0.42, 95% CI 0.23–0.79; Table 1). We further tested for differences in average AIMS scores among genotypes with ANOVA. C957T and C939T showed the same trend as for TD analyses, with the differences being suggestive for C939T ($p=0.150$ and $p=0.071$ respectively; Table 1). Upon close inspection with Student's t tests, we found patients homozygous for C939 to have significantly lower total AIMS scores than patients carrying at least one copy of T939 ($p=0.001$). Patients homozygous for T957 had significantly lower total AIMS scores than patients carrying at least one copy of C957 ($p=0.044$). RS2242592 also showed significant or suggestive association with TD at the allelic and genotypic levels. The RS2242592 findings could be due to generally high linkage disequilibrium observed in the 3′ region of *DRD2* (Figure 2). The other polymorphisms analysed did not show significant associations with TD either for allele, genotype, or AIMS scores analyses.

Association study of haplotypes with TD diagnosis and AIMS

Using the Haploview program with linkage disequilibrium block defined by Gabriel et al. (2002), strong evidence for linkage was found between C939T and C957T, prompting us to test for association between TD and two-marker haplotypes across *DRD2* (Figure 2). RS2242593 and *Taq*IA were also found to be

Table 1. Statistical analyses on demographics (sex, age) as well as total AIMS scores and TD diagnoses with genotypes of the 12 polymorphisms in *DRD2*

DRD2 markers		*n* (F/M)	Age (yr)	Total AIMS score ± S.D. (*n*)	TD	
					Yes	No
A-241G	1/1 (A/A)	161 (54/107)	37.71 ± 9.46	5.53 ± 7.18 (131)	59	102
	1/2 (A/G)	28 (8/20)	37.25 ± 11.72	7.56 ± 8.75 (27)	12	16
	p	0.605	0.820	0.200	0.531	
−141C Ins/Del	1/1 (Del/Del)	2 (1/1)	38.00 ± 1.41	6.50 ± 6.36 (2)	1	1
	1/2 (Ins/Del)	32 (10/22)	39.06 ± 10.76	5.71 ± 7.84 (28)	14	18
	2/2 (Ins/Ins)	150 (49/101)	37.14 ± 9.76	5.66 ± 7.20 (123)	54	96
	p	0.927*	0.608	0.987	0.554*	
RS4648317	1/1 (C/C)	143 (51/92)	37.83 ± 10.16	5.78 ± 7.21 (124)	56	87
	1/2 (C/T)	38 (8/30)	37.92 ± 8.56	5.32 ± 7.61 (28)	12	26
	2/2 (T/T)	4 (2/2)	26.25 ± 4.35	5.00 ± 7.07 (2)	2	2
	p	0.144*	**0.066**	0.947	0.581*	
RS1125394	1/1 (A/A)	135 (42/93)	37.76 ± 10.36	6.34 ± 8.04 (116)	52	83
	1/2 (A/G)	49 (17/32)	38.31 ± 8.55	4.97 ± 5.89 (38)	18	31
	2/2 (G/G)	6 (2/4)	39.50 ± 10.56	5.60 ± 6.69 (5)	4	2
	p	0.907*	0.878	0.988†	0.372*	
RS1079598	1/1 (C/C)	6 (3/3)	36.33 ± 11.73	6.20 ± 6.65 (5)	4	2
	1/2 (C/T)	48 (16/32)	38.83 ± 8.52	4.74 ± 5.88 (39)	16	32
	2/2 (T/T)	138 (45/94)	37.34 ± 10.33	6.34 ± 7.96 (118)	55	84
	p	0.684*	0.632	0.514†	0.277*	
*Taq*ID	1/1 (C/C)	50 (15/35)	37.42 ± 9.35	6.31 ± 8.00 (45)	20	30
	1/2 (C/T)	83 (30/53)	37.43 ± 9.89	5.22 ± 6.44 (64)	33	50
	2/2 (T/T)	56 (15/41)	37.21 ± 10.30	6.16 ± 8.34 (49)	17	39
	p	0.485	0.991	0.946†	0.467	
C939T	1/1 (C/C)	80 (27/53)	37.51 ± 8.69	3.80 ± 4.91 (69)	21	59
	1/2 (C/T)	85 (29/56)	38.08 ± 9.78	6.69 ± 7.46 (68)	38	47
	2/2 (T/T)	22 (5/17)	34.36 ± 11.21	9.85 ± 11.77 (20)	11	11
	p	0.573	0.263	**0.071**†	***0.022***	
C957T	1/1 (C/C)	44 (10/34)	36.59 ± 10.94	7.29 ± 9.42 (38)	20	24
	1/2 (C/T)	102 (38/64)	38.21 ± 9.32	5.84 ± 6.62 (82)	43	59
	2/2 (T/T)	43 (14/29)	37.95 ± 9.09	3.89 ± 5.86 (38)	8	35
	p	0.229	0.646	0.150†	***0.013***	
RS2242591	1/1 (A/A)	126 (40/86)	37.49 ± 10.32	6.14 ± 8.06 (107)	46	80
	1/2 (A/G)	51 (16/35)	37.45 ± 8.53	5.03 ± 5.99 (40)	18	33
	2/2 (G/G)	8 (4/4)	38.50 ± 11.16	5.14 ± 5.90 (7)	5	3
	p	0.602*	0.960	0.705	0.357*	
RS2242592	1/1 (C/C)	17 (3/14)	35.18 ± 12.60	7.44 ± 10.36 (16)	8	9
	1/2 (C/T)	89 (31/58)	37.69 ± 9.80	6.99 ± 7.94 (72)	40	49
	2/2 (T/T)	79 (29/54)	38.02 ± 9.08	4.37 ± 5.85 (70)	24	59
	p	0.355	0.547	0.135†	**0.070**	
RS2242593	1/1 (A/A)	131 (42/89)	37.63 ± 10.23	6.05 ± 7.97 (111)	47	84
	1/2 (A/G)	50 (15/35)	37.98 ± 8.71	5.27 ± 5.92 (40)	20	30
	2/2 (G/G)	6 (3/3)	36.33 ± 11.73	6.20 ± 6.65 (5)	4	2
	p	0.588*	0.923	0.842	0.291*	
*Taq*IA	1/1 (T/T)	6 (2/4)	38.33 ± 10.35	6.12 ± 8.16 (5)	3	3
	1/2 (C/T)	72 (27/45)	38.47 ± 9.07	4.93 ± 5.88 (60)	25	47
	2/2 (C/C)	111 (32/79)	36.63 ± 10.38	5.20 ± 6.87 (93)	40	71
	p	0.446*	0.458	0.960†	0.781*	

* With at least one expected cell count <5; Fisher's exact test used.

† Variances among comparisons groups differ significantly; Kruskal–Wallis test used.

Bold values indicate $0.05 < p < 0.10$. Bold italic values indicate $p < 0.05$.

Table 2. Results from χ^2 test of allele frequencies of each of the 12 polymorphisms vs. TD diagnoses for both Caucasian and African–American populations

		TD			
		Caucasian		African-American	
DRD2 markers		Yes	No	Yes	No
A-241G	Allele 1 (A)	130	220	15	33
	Allele 2 (G)	12	16	7	5
	p	0.548		0.102*	
−141C Ins/Del	Allele 1 (Del/C)	16	20	9	16
	Allele 2 (Ins/CC)	122	210	13	22
	p	0.365		0.928	
RS4648317	Allele 1 (C)	124	200	19	34
	Allele 2 (T)	16	30	3	4
	p	0.648		0.700*	
RS1125394	Allele 1 (A)	122	197	19	37
	Allele 2 (G)	26	35	3	1
	p	0.521		0.135*	
RS1079598	Allele 1 (C)	24	36	2	0
	Allele 2 (T)	126	200	20	38
	p	0.844		0.131*	
*Taq*ID	Allele 1 (C)	73	110	13	18
	Allele 2 (T)	67	128	9	20
	p	0.266		0.381	
C939T	Allele 1 (C)	80	165	6	14
	Allele 2 (T)	60	69	16	24
	p	***0.0085***		0.449	
C957T	Allele 1 (C)	83	107	20	26
	Allele 2 (T)	59	129	2	12
	p	***0.0135***		***0.0472***	
RS2242591	Allele 1 (A)	110	193	20	34
	Allele 2 (G)	28	39	2	4
	p	0.401		1.000*	
RS2242592	Allele 1 (C)	56	67	14	20
	Allele 2 (T)	88	167	8	18
	p	***0.039***		0.687	
RS2242593	Allele 1 (A)	114	198	21	37
	Allele 2 (G)	28	34	1	1
	p	0.201	1.000*		
*Taq*IA	Allele 1 (C)	31	53	4	8
	Allele 2 (T)	105	189	18	30
	p	0.841		1.000*	

* With at least one expected cell count <5. Fisher's exact test was used.
Bold italic values indicate $p<0.05$.

in linkage disequilibrium in our sample. COCAPHASE analysis within UNPHASED revealed an association for the C939T–C957T haplotype and TD diagnosis (global $p=0.021$; Table 3). Specifically, the C939–T957 haplotype appeared to occur less often while the T939–C957 haplotype appeared more frequently in TD-positive patients ($p=0.007$, $OR_{C939-T957}$ 0.56, 95% CI 0.37–0.86; $p=0.013$, $OR_{T939-C957}$ 1.72, 95% CI 1.11–2.65, respectively). QTPHASE analysis within the UNPHASED program using two-marker haplotypes also showed significant

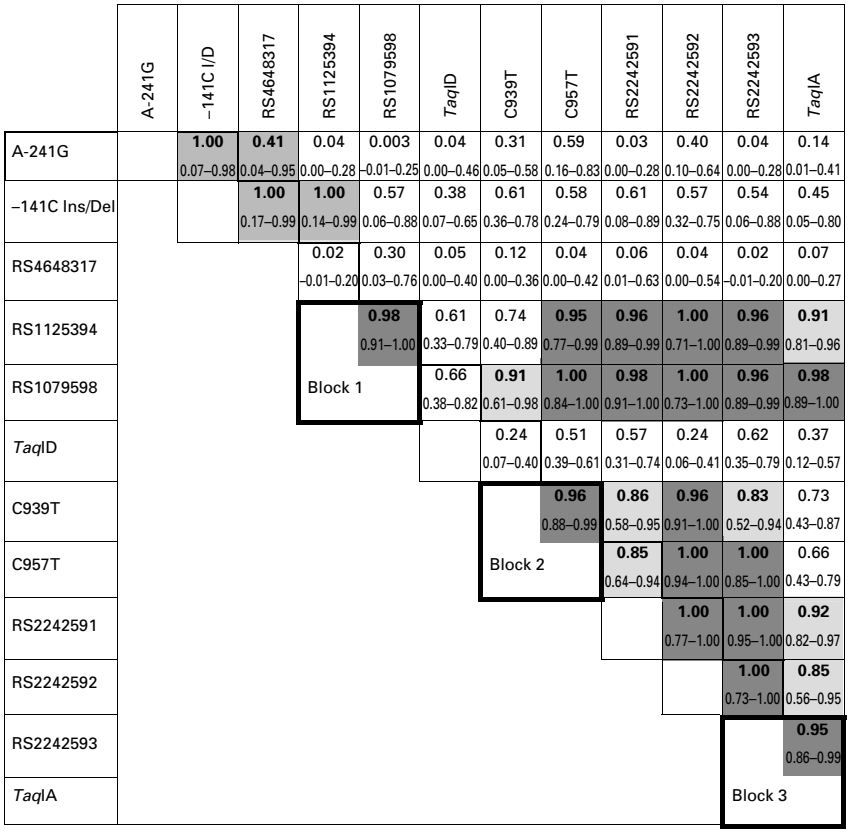

Figure 2. Linkage disequilibrium plot among the 12 DRD2 gene polymorphisms used in the present study. The figures represents D' values and the 95% confidence intervals of D', while the colour darkness within each box corresponds to strength of linkage. The blocks (1, 2, and 3) encompass areas with highest linkage disequilibrium given by $D' > 0.90$ and lower boundary of the 95% confidence intervals of $D' > 0.70$ (Gabriel et al., 2002).

Table 3. Global p values from analyses of two-marker haplotypes in association to TD and AIMS using COCAPHASE and QTPHASE programs respectively. Haplotypes with frequencies of < 0.05 were excluded from the analyses

Haplotype	$p$value (TD±)	p value (AIMS)
A-241G– −141C I/D	0.358	0.326
−141C I/D–RS4648317	0.616	0.924
RS4648317–RS1125394	0.771	0.726
RS1125394–RS1079598	0.869	0.362
RS1079598–*Taq*ID	0.423	0.609
*Taq*ID–C939T	**0.0569**	***0.00655***
C939T–C957T	***0.0206***	***0.00868***
C957T–RS2242591	***0.0339***	**0.0628**
RS2242591–RS2242592	***0.0341***	0.131
RS2242592–RS2242593	***0.0148***	0.122
RS2242593–*Taq*IA	0.536	0.629

Bold values indicate $0.05 < p < 0.10$; bold italic values indicate $p < 0.05$.

association of haplotypes containing C957T and C939T polymorphisms with AIMS scores ($p = 0.0087$, Table 3). Patients carrying the C939–T957 haplotype had significantly lower average AIMS scores than other haplotypes, while those carrying the T939–C957 had significantly higher average AIMS scores ($p = 0.017$ and $p = 0.0013$ respectively).

For the African–American sample, preliminary results indicated a marginally significant association between TD and alleles of the C957T ($p = 0.047$), with the T957 allele appearing to be protective (OR_{T957} 0.22, CI 0.04–1.08). The linkage disequilibrium chart showing D' values calculated using TD status on the 12 polymorphisms is provided as a reference in designing future genetic studies (Figure 2).

Discussion

Previous studies yielded conflicting results with regard to the *DRD2* gene and TD. Besides the positive findings by Chen et al. (1997), only a few others

have found a significant association using *DRD2* polymorphisms. There are several reasons for these mixed results. First, different polymorphisms were used in many of the studies, and in most cases only a few polymorphisms were tested without haplotype analyses. Only Kaiser et al. (2002) analysed nine polymorphisms spanning the entire 65-kb-long *DRD2* gene. Second, populations with different ethnic backgrounds were used in the studies, making findings difficult to compare due to potentially undetected stratification effects. Further, in some studies, the sample sizes were small, limiting the power to detect an association. Finally, many studies did not take into account statistical advantages of the continuous AIMS scores and only used the dichotomous TD occurrence for the analyses. The aim of our study has been to investigate 12 DRD2 gene polymorphisms for genetic association with TD in a relatively large sample involving haplotype analyses.

Results from the present study are consistent with most previous studies in that the A-241G, −141C Ins/Del, *Taq*ID, and *Taq*IA polymorphisms were found to be not significantly associated with TD. The previous positive finding with *Taq*IA could be due to higher linkage disequilibrium in the 3′ portion of *DRD2* as shown in our sample and others (Figure 2; Kaiser et al., 2002; Ritchie and Noble, 2003), and that the causative variant may reside within *DRD2*. Indeed, we found a significant association between TD and the C957T polymorphism as well as its neighbouring C939T polymorphism in our Caucasian sample. To our knowledge, the C957T polymorphism has not been investigated previously for its effect on TD. Although the polymorphism does not affect the amino-acid sequence, the T variant has been associated with decreased striatal DRD2 levels in vivo and decreased DRD2 mRNA stability in vitro (Duan et al., 2003; Hirvonen et al., 2004). Using MFOLD, Duan and co-workers showed that the predicted mRNA folding structures were different between the two alleles (Duan et al., 2003). They hypothesized that T957 may decrease DRD2 expression through its effect on D_2 mRNA secondary structure. The change in secondary structure may affect binding of mRNA-stabilizing proteins at the 5′-cap and 3′-poly(A) as well as translation initiation factors, thus decreasing both translation efficiency and mRNA half-life (Duan et al., 2003; Perkins et al., 2005). An under-representation of the T allele in patients with TD and a decrease in TD severity in T-allele carriers suggest that decreased DRD2 levels may decrease TD susceptibility and severity. Our present study also reported a positive association with the nearby C939T, a polymorphism not found to be associated with TD in a previous study on a Japanese sample (Inada et al., 1997). It is possible, though, that TD susceptibility and risk factors may be different among different ethnic groups. Ethnic differences were reflected by findings in our African–American sample, in which a significant association was only detected between C957T alleles and TD.

TD occurrence and severity were found to increase with age in the current sample, supporting previous studies from our laboratory and others (Basile et al., 1999; Jeste, 2000; Kaiser et al., 2002; van Os et al., 1997). Age and gender were unlikely to be responsible for the positive findings in this study, because mean age and gender proportions did not differ significantly among the genotypes of C939T and C957T.

Since both C939T and C957T are located in exon 7, it is possible that they are linked to a region that affects splicing around exon 6, resulting in a different ratio of the long (D_2L) and short (D_2S) isoforms. The two isoforms have distinct functions in vivo, and only the post-synaptic D_2L isoform appears to be a major target of haloperidol (Centonze et al., 2004; Usiello et al., 2000). Thus, splicing changes could have contributed to changes in DRD2 function, expression levels and patterns, affecting antipsychotic response and adverse effects. Understanding the mechanisms that regulate splicing of the *DRD2* gene will help answer the question of the locations of polymorphisms in the *DRD2* gene that affect the splicing efficiency. From the epigenetics standpoint, the C957T polymorphism may have arisen from deamination of methylated C957 to T957. Although C957T has been reported to regulate D_2 expression through its effect on mRNA stability, it is possible that C957 could be methylated in a subset of individuals. Methylated C957 may be functionally different from unmethylated C957 at the DNA level, thus opening another dimension of regulation of D_2 expression and function. This may also be the case for other polymorphisms in *DRD2*, especially in the promoter region where methylation has been reported (Popendikyte et al., 1999). Because association studies of *DRD2* have not considered CpG methylation and genetic polymorphisms do not capture the variability of regional CpG methylation (Flanagan et al., 2006), it may have given rise to the mixed results in previous findings. Understanding the role of epigenetics in the regulation of gene expression will help resolve at least some of the variability of results from genetics studies.

The present study encourages further examinations into C957T, C939T, as well as adjacent polymorphisms and TD, but it has several limitations. First, not

all clinical data were available for our study. These include medication history such as antipsychotic dose and duration, schizophrenia disease history including age of onset, clinical subtypes, psychopathology, and comorbidities. These factors or variables have been previously associated with TD (reviewed in Müller et al., 2004). Medications taken by patients for other adverse effects such as parkinsonism could have masked the TD phenotype (Egan et al., 1997; Glazer, 2000; Shale and Tanner, 1996). Moreover, we did not have information on tobacco, alcohol, or substance use for our entire sample. Further, our sample was drawn from four different clinical sites. Even though the study Caucasian population was in Hardy–Weinberg equilibrium for all 12 polymorphisms and the gender ratios among the four geographical groups do not differ significantly ($p=0.165$), the mean ages differ significantly among them ($p<0.001$). Therefore, the possibility of ascertainment bias cannot be ignored. About half of the present sample had been analysed previously for other genes with TD, with DRD3 findings being replicated (Bakker et al., 2006; Basile et al., 1999; Lerer et al., 2002). The other half of the sample has not been published previously for TD. Nonetheless, when the two halves were analysed separately, the trend remained for C939T and C957T (data not shown). Also, heterogeneity of the TD phenotype could have confounding effects; only total AIMS scores, but not the subscores, were available for most of our sample for genetic analyses, preventing further dissection of the phenotype. Finally, the marginally significant association could be due to the possibility that the polymorphisms have only a small contributing effect to the risk for TD as expected in complex phenotypes. The sample size in the current study was not large enough to provide sufficient power to detect a significant difference in AIMS scores between the genotypes. False-positive results from multiple testing are possible; indeed, if we corrected for multiple testing taking linkage disequilibrium into account using the online SNPSpD program, the significance threshold (α) in order to keep the Type I error rate at 5% would have become 0.005 (Nyholt, 2004). As a result, only our findings from haplotype analyses would have remained statistically significant. Larger sample sizes are required to detect small effects of genotypes on TD risk and severity, especially for our African–American sample where we did not detect significant associations between TD and any DRD2 polymorphisms after correction for multiple testing. With α set at 0.005, the Caucasian portion of the sample used for the present study has only 18% power to detect the differences in AIMS scores observed among the C939T genotypes (Glantz, 1992). To detect such differences, a sample size of at least 120 per genotype group will be needed for future studies.

The *DRD2* gene is probably not the only genetically determined factor for TD, as other genes have been associated with TD as well. Genetic studies have identified *DRD3* to be reproducibly associated with TD (Basile et al., 1999; Garcia-Barcelo et al., 2001; Lerer et al., 2002; Liao et al., 2001; Lovlie et al., 2000; Segman et al., 1999; Steen et al., 1997). Studies in other genes such as *HTR2A*, *HTR2C*, *CYP1A2*, and manganese superoxide dismutase require further investigation (Basile et al., 2001; Hori et al., 2000; Schulze et al., 2001; Segman et al., 2000, 2001; Tan et al., 2001). As nearly all antipsychotics target more than one receptor, it is likely that TD is not related to one receptor gene, but rather it is a polygenic condition with each gene contributing a small proportion of the risk to the disorder. Gene–gene interaction studies may help in identifying and clarifying pathways that contribute to TD. TD risk is also likely to be influenced by several environmental factors (Müller et al., 2004), and acquiring this information will help immensely in increasing the statistical power and limiting the effects of potential confounders in genetic studies of TD.

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Statement of Interest

None.

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