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ARTICLE

Combined analysis of 635 patients confirms an age-related association of the serotonin 2A receptor gene with tardive dyskinesia and specificity for the non-orofacial subtype

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Abstract

Tardive dyskinesia (TD) is an important limiting factor in the use of typical antipsychotic drugs. Genetic variability in the serotonin 2A (5-HT_{2A}) receptor may influence risk for TD but the results of prior studies are not confirmatory. The objective of this study was to determine association of T102C and His452Tyr polymorphisms in the 5-HT_{2A} receptor gene (HTR_{2A}) with TD in a large, multicentre patient sample. The design employed case-control analysis controlling for possible confounders using pooled, original data from published and available unpublished samples and employing logistic regression, analysis of variance and meta-analysis. The study sample consisted of 635 patients with schizophrenia or schizoaffective disorder (256 with TD and 379 without TD) drawn from five research centres, divided into six groups based on population origin. The main outcome measure was association of a categorical diagnosis of TD based on the Research Diagnostic Criteria for TD with HTR_{2A} T102C and His452Tyr genotypes and haplotypes. The findings indicate significant association of TD with HTR_{2A} T102C genotype ($p=0.002$) over and above the effect of population group, also when controlling for age and gender ($p=0.0008$), but not with His452Tyr genotype. The T102C genotype was significantly associated with TD in older (>median age 47 yr, $p=0.002$) but not younger patients and in patients with non-orofacial (limb-truncal) ($p=0.001$) but not orofacial TD. By meta-analysis the Mantel–Haenszel (M-H) pooled odds ratio (OR) across all the available data was 1.64. A T102C-His452Tyr haplotype was significantly associated with TD ($p=0.0008$). These findings confirm that genetic variability in HTR_{2A} contributes a small but significant degree of risk for the expression of TD, particularly in older patients and specifically for the non-orofacial (limb-truncal) type. Together with other genetic variants associated with TD the findings could be used to assess risk in patients who are candidates for treatment with typical antipsychotic medications.

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Introduction

Tardive dyskinesia (TD) is a potentially irreversible, iatrogenic movement disorder that affects 20–50% of patients receiving long-term antipsychotic treatment with dopamine D_2 receptor blocking drugs (Kane and Smith, 1982; Tamminga and Woerner, 2002). Atypical antipsychotic drugs that have a low propensity to induce TD are now widely used (Caroff et al., 2002; Correll et al., 2004). Nevertheless, classical antipsychotic agents are still extensively prescribed, primarily for economic reasons (Emsley et al., 1999). Furthermore, there is growing concern about the adverse effects of atypical antipsychotic drugs on body weight and glucose-lipid metabolism (American Diabetes Association et al., 2004). It would be of great value to clinicians to have the option of prescribing typical antipsychotics by being able to exclude patients at risk for TD.

Age is the strongest known risk factor for the development of TD (Smith and Baldessarini, 1980; Woerner et al., 1998). Other factors include duration and intensity of prior exposure to antipsychotic medication, female gender, organic brain abnormalities, smoking, race and affective disorder (Eastham et al., 1996; Kane and Smith, 1982; Kane et al, 1992; Yassa and Jeste, 1992). A genetic component in the susceptibility to TD is suggested by clinical reports of aggregation of TD cases in families (Waddington and Youssef, 1988; Weinhold et al., 1981; Yassa and Ananth, 1981; Youssef et al., 1989). Strain differences in the susceptibility of rats to antipsychotic-induced repetitive jaw movements (RJM) and vacuous chewing (Rosengarten et al., 1994; Tamminga et al., 1990) and differences among mice strains in up-regulation of striatal dopamine receptors after exposure to dopamine D_2 receptor blockers (Belmaker et al., 1981) provide support for a genetic predisposition.

Although the exact mechanism of TD remains unclear, it is believed that the nigrostriatal dopaminergic tract, which is pivotally involved in the regulation of motor behaviour, may play a key role (Tamminga and Woerner, 2002). The high binding affinity of typical antipsychotic drugs for dopamine D_2 receptors resulting in their up-regulation post-synaptically, is thought to contribute to nigrostriatal dopaminergic overactivity. Several studies have examined the role of dopaminergic receptor and transporter polymorphisms in susceptibility to TD as well enzymes relevant to dopaminergic function (Segman et al., 2003; Segman and Lerer, 2002a). For the most part the results have been negative, with one notable exception – the dopamine D_3 receptor gene (DRD3). A relatively large number of studies have focused on a polymorphic site at position 9 of the first exon of DRD3 that gives rise to a serine (Ser) to glycine (Gly) substitution in the N-terminal extracellular domain of the receptor (Lannfelt et al., 1992). Lerer et al. (2002) published the first pooled and meta-analysis of studies on DRD3 and TD, finding a significant association with DRD3Gly. Reports published since then further implicate DRD3 in susceptibility to TD (Chong et al., 2003; Werge et al., 2003; Woo et al., 2002).

Several lines of evidence implicate brain serotonergic systems as possibly modifying susceptibility to express TD. Dorsal raphe serotonergic projections inhibit dopaminergic neuronal activity through 5-HT_{2A} receptor activation (Kapur and Remington, 1996). 5-HT_{2A} antagonists have been shown to augment endogenous striatal dopamine release in vivo (Dewey et al., 1995). 5-HT_{2A} antagonists reduce neuroleptic-induced catalepsy (Wadenberg, 1996) as well as neuroleptic-induced RJM in rodents (Naidu and Kulkarni, 2001) and atypical antipsychotic agents reduce RJM (Rosengarten et al., 1999). Long-term exposure to typical antipsychotics results in 5-HT_{2A} receptor up-regulation in striatal regions, whereas atypical drugs with low DRD2/high 5-HT_{2A} receptor occupancy do not (Kusumi et al., 2000). Finally, long-term elevations in 5-HT_{2A} receptor binding and mRNA expression in nigrostriatal regions have been documented in response to loss of dopamine neurons following 6-hydroxy dopamine administration (Kostrzewa et al., 1998). Atypical antipsychotic drugs have a lower propensity to induce TD (Caroff et al., 2002; Casey, 1999; Correll et al., 2004) and following exposure to a dopamine D_2 receptor blocker show efficacy in reducing the expression of dyskinesia (Alptekin and Kivircik, 2002; Bassitt and Louza Neto, 1998; Lucetti et al., 2002).

The 5-HT_{2A} receptor gene (HTR_{2A}) is located on chromosome 13q14-21 (Hsieh et al., 1990). A number of polymorphic sites have been described in the gene (Collier et al., 1997; Erdmann et al., 1996; Ohara et al., 1997; Warren et al., 1993) of which T102C, A-1438G, and His452Tyr are common variations. Following the initial report of Segman et al. (2001), one published study found an association of T102C with TD (Tan et al., 2001) while two did not (Basile et al., 2001; Herken et al., 2003) (Table 1). The two published studies that examined His452Tyr association with TD did not find a relationship (Basile et al., 2001; Segman et al., 2001). Two studies found T102C and A-1438G to be in close linkage disequilibrium (LD), Segman et al. (2001) but not Basile et al. (2001) observing an association with TD. Segman and Lerer (2002b) found that

Table 1. Published studies on HTR$_{2A}$ T102C and other HTR$_{2A}$ polymorphisms and TD

Publication	Subjects	Findings from categorical analysis	Findings from continuous analysis	Other HTR$_{2A}$ polymorphisms examined
Segman et al. (2001)	TD-Y: $n=59$, age $=54.4\pm13.0$ yr (S.D.) TD-N: $n=62$, age $=50.5\pm10.2$ yr (S.D.) Origin: Israeli Ashkenazi and Sephardic Jews	Allelic association – excess 102C allele in TD-Y vs. TD-N and controls ($\chi^2=12.8$, d.f. 2, $p=0.002$) Genotypic association – excess 102C/C genotypes in TD-Y vs. TD-N and controls ($\chi^2=13.3$, d.f. 4, $p=0.01$)	Significant effect of T102C genotype on AIMS trunk ($F=3.9$, d.f. 2, 116; $p=0.02$) and incapacitation ($F=5.0$, d.f. 2, 115, $p=0.006$) scores, by ANCOVA controlling for age at first antipsychotic treatment	His452Tyr: no association with TD A-1438G: in complete linkage disequilibrium with T102C
Basile et al. (2001)	TD-Y: $n=54$ TD-N: $n=82$ Age (combined) $=33.2\pm9.1$ yr (S.D.) Origin: Caucasian, $n=109$, Afro-American, $n=27$	No allelic or genotypic association between HTR$_{2A}$ T102C polymorphism and TD	No effect of T102C genotype on AIMS total scores AIMS subscale scores not reported	His452Tyr: no association with TD A-1438G: in strong but not complete linkage disequilibrium with T102C No association with TD
Tan et al. (2001)	TD-Y: $n=87$, age $=55.9\pm8.4$ yr (S.D.) TD-N: $n=134$, age $=48.7\pm9.4$ yr (S.D.) Origin: Singapore Chinese	No allelic association between HTR$_{2A}$ T102C polymorphism and TD Genotypic association – excess 102T/C and 102C/C genotypes in TD-Y vs. TD-N and controls ($\chi^2=18.81$, d.f. 4, $p=0.001$)	Not reported	Not reported
Herken et al. (2003)	TD-Y: $n=32$ TD-N: $n=111$ Age (combined) $=32.3\pm9.1$ yr (S.D.) Origin: Turkish	No allelic or genotypic association between HTR$_{2A}$ T102C polymorphism and TD	Not reported	A-1438G: no association with TD

AIMS, Abnormal Involuntary Movements Scale; TD-Y, Tardive dyskinesia positive; TD-N, tardive dyskinesia negative.

Table 2. Demographic and clinical details of the sample

Group	TD-Y			TD-N			Origin	TD assessment
	n	Age (yr)	Gender	*n*	Age (yr)	Gender		
Jerusalem	59	54.4±13	F 29 M 30	62	50.5±10.1	F 30 M 32	Israeli Ashkenazi ($n=71$) and non-Ashkenazi ($n=50$) Jews	AIMS
Milan	34	55.8±10.5	F 16 M 18	60	53.4±10.5	F 31 M 29	Northern Italian Caucasian	RSDS
Singapore	88	55.8±8.3	F 18 M 70	133	48.7±9.4[a]	F 32 M 101	Chinese	AIMS
Toronto: African-American	14	36.6±11.9	F 4 M 10	13	29.3±9.8[b]	F 4 M 9	African-American	AIMS
Toronto: Caucasian	40	35.6±8.2	F 13 M 27	69	31.9±8.4	F 17 M 52	North American Caucasian	AIMS
Vienna	21	36.9±13.4	F 9 M 12	42	31.5±9.5	F 17 M 25	Austrian Caucasian	TDRS

Groups: Jerusalem (Hadassah – Hebrew University Medical Center, Jerusalem, Israel); Milan (IRCCS Hospitale San Raffaele, Vita Salute University, Milan, Italy); Singapore (Defence Medical and Environmental Research Institute and Institute of Mental Health, Singapore); Toronto: (Centre for Addiction and Mental Health, University of Toronto, Canada); Vienna (University of Vienna, Austria).

TD-Y, Tardive dyskinesia positive; TD-N, tardive dyskinesia negative; SCZ, schizophrenia; SA, schizoaffective; M, male; F, female; AIMS, Abnormal Involuntary Movements Scale; RSDS, Rockland and Simpson Dyskinesia Scale; TDRS, Tardive Dyskinesia Rating Scale.

[a] $t=5.81$, $p<0.0001$, vs. Singapore TD-Y.

[b] $t=2.26$, $p=0.02$ vs. Toronto Caucasians TD-Y.

the association of T102C with TD was demonstrable in older but not younger patients.

In this paper we present the results of a pooled analysis of original data from three of the four published studies on HTR_{2A} and TD as well as two unpublished samples. Our analysis took into account the possible confounding effects of population origin, differences in the rating of abnormal involuntary movements among centres and the impact of age and gender. In addition a meta-analysis was performed that included all the published and unpublished studies.

Materials and methods

Patients and clinical assessment

This project was a collaboration among five research centres (Table 2). Data on the Jerusalem (Segman et al., 2001), Singapore (Tan et al., 2001) and Toronto samples (Basile et al., 2001) had been published previously and data on the patients from Milan (Macciardi et al., 1997) and Vienna (Aschauer et al., 1998) published in abstract form. The existence of additional studies was checked by a MEDLINE search that included the terms tardive dyskinesia, genetics and serotonin 2A (or 5-HT_{2A}) receptors and by contacting known, active researchers on the genetics of TD. All the projects were approved by the local Institutional Review Boards and the patients gave written, informed consent. Clinical and genotypic data were supplied by each of the centres. The minimum criteria for inclusion were information on age and gender; diagnosis of schizophrenia or schizoaffective disorder according to DSM-IV or ICD-9; presence or absence of TD according to the Research Diagnostic Criteria for TD (Schooler and Kane, 1982) and genotypic data for the HTR_{2A} T102C polymorphism. Abnormal Involuntary Movement Scale (AIMS) scores (Guy, 1976) were available the Jerusalem, Singapore and Toronto patients. Patients from the Milan centre were assessed by the Rockland–Simpson Scale (Cavallaro et al., 1993) and patients from Vienna by the Tardive Dyskinesia Rating Scale (Simpson et al., 1979). Since the presence or absence of TD was based on a single evaluation, the TD diagnosis is at a probable level according to the RDC-TD (Schooler and Kane, 1982). The patients from the five centres were divided into six groups (Table 2) since the African-American (A-A) and Caucasian patients from the Toronto centre were considered separately. The Jerusalem sample included Jewish patients of

Ashkenazi and Sephardic origin but there was no significant difference in the frequency of the T102C or His452Tyr polymorphisms between these groups (Segman et al., 2001). Patients from Milan and Vienna were Caucasians of Northern Italian and Austrian origin respectively and the Singapore patients were of Chinese extraction.

Genotyping

The T102C and His452Tyr polymorphisms in HTR$_{2A}$ were genotyped in the laboratories of the five participating centres by standard PCR-based methods. The detailed procedures followed in the Jerusalem (Segman et al., 2001) Singapore (Tan et al., 2001) and Toronto laboratories (Basile et al., 2001) are described in their publications. The same procedures were followed in the Milan and Vienna laboratories and in the Singapore laboratory for His452Tyr (results for which had not been published previously).

Statistical analysis

Maximum-likelihood χ^2 statistics were employed for categorical analyses. For bivariate comparisons of continuous data the Student's t test was used and for multivariate comparisons, analysis of variance (ANOVA) or covariance (ANCOVA). Multinomial logistic regression was applied to analyse the effect of genotypes, considered as multilevel categorical variables. Two sets of analyses were implemented on the pooled sample. For the first set the outcome (dependent) variable was T102C or His452Tyr genotype. The covariates of the model were the clinical phenotype of interest, i.e. TD present or absent and group, given a possible population effect on genotype. In this case, the phenotype of interest is a predictor of outcome and group is a potential confounder. Group was tested by generating a set of dummy variables equal to the number of groups included in the sample in order to evaluate the specific effect of any group. Results from the model are presented as likelihood ratio (LR) tests. In a further set of analyses the outcome variable of the logistic regression was TD phenotype (present or absent) and the covariates of the model were age, gender and HTR$_{2A}$ genotype distribution. The implementation of these procedures for analysis of genetic association in samples from multiple centres and of different population origin has described previously by Lerer et al. (2001, 2002). The STATA 8.0 program (Stata Corporation, College Station, TX, USA) a general package for statistics and genetic epidemiology, was employed for these analyses and also for the meta-analysis. p values <0.05 (two-tailed) were regarded as significant. LD between the polymorphisms and analysis of haplotypes was performed by the program 'hapipf', part of STATA 8.0.

Results

Confounding variables and Hardy–Weinberg equilibrium (HWE)

In order to identify potential confounders in a pooled analysis of the six patient groups, we initially compared age between patients with (TD-Y) and without TD (TD-N) across the groups (by ANOVA with group and TD status as the independent variables). There were significant effects of group ($F=94.38$, d.f. 5, 622, $p<0.000001$) and TD status ($F=24.98$, d.f. 1, 622, $p=0.000001$). Post-hoc Neumann–Keuls tests showed a clearly bimodal distribution with the patients from Jerusalem, Milan and Singapore approximately two decades older than the Toronto A-A, Toronto Caucasian and Viennese patients ($p<0.0001$ in all cases) (Table 2). Comparing TD-Y and TD-N patients within the groups, TD-Y patients were significantly older in the Singapore ($t=5.81$, d.f. 219, $p<0.000001$) and Toronto Caucasian groups ($t=2.26$, d.f. 107, $p=0.03$) but not in the other groups. There was no significant difference in gender distribution between TD-Y and TD-N patients. All the TD-N groups were in HWE for T102C and His452Tyr ($p>0.05$). Among the TD-Y groups, the one from Singapore was not in HWE ($\chi^2=11.92$, $p=0.0006$), due to an excess of heterozygotes. For the His452Tyr polymorphism, only the Toronto A-A TD-Y group was not in HWE ($\chi^2=5.50$, $p=0.02$), due to a small but significant excess of Tyr-Tyr, homozygotes.

Allele and genotype frequencies within groups

Table 3 shows a comparison of the frequency of T102C alleles between patients with and without TD in each of the groups. There was significant excess of C alleles among the TD patients in the Jerusalem ($\chi^2=11.28$, d.f. 1, $p=0.001$) and Singapore ($\chi^2=4.52$, d.f. 1, $p=0.03$) groups and a trend in this direction in the Toronto A-A group ($\chi^2=3.87$, d.f. 1, $p=0.05$). Table 3 also shows HTR$_{2A}$ T102C genotypes in the TD-Y and TD-N patients of each group. Comparison of genotype distribution by the STATA 8.0 subroutine 'genhwcci' showed a significant difference in the Jerusalem group ($\chi^2=12.36$, d.f. 1, $p=0.002$) and in the Singapore group ($\chi^2=17.01$, d.f. 1, $p=0.0002$.). In the Jerusalem group there was an excess of C/C homozygotes in the TD-Y group ($\chi^2=11.45$, d.f. 1, $p=0.003$) and in the Singapore group ($\chi^2=7.23$, d.f. 1, $p=0.03$) an excess of

Table 3. HTR_{2A} T102C allele and genotype frequencies in the individual groups

Group	TD status	No. Patients	Allele freq. T	Allele freq. C	Significance (ML χ^2, d.f. 1)	Genotype freq. T/T	Genotype freq. T/C	Genotype freq. C/C	Significance (LR χ^2, d.f. 2)
Jerusalem	TD-Y	59	37.3	62.7	11.28	16.1	50	33.9	11.45
	TD-N	62	58.9	41.1	$p=0.001$	42.4	40.7	16.9	$p=0.003$
Milan	TD-Y	34	48.5	51.5	0.40	18.3	56.7	25	0.47
	TD-N	60	53.3	46.7	$p=0.53$	23.5	55.9	20.6	$p=0.79$
Singapore	TD-Y	88	64.4	35.6	4.52	3.8	45.1	51.1	7.23
	TD-N	133	73.5	26.5	$p=0.03$	4.5	62.5	33	$p=0.027$
Toronto:	TD-Y	14	17.9	82.1	3.87	30.8	53.9	15.4	4.23
African-American	TD-N	13	42.3	57.7	$p=0.049$	64.3	35.7	–	$p=0.121$
Toronto:	TD-Y	40	52.5	47.5	0.024	21.7	53.6	24.6	0.047
Caucasian	TD-N	69	51.4	48.6	$p=0.88$	20	55	25	$p=0.98$
Vienna	TD-Y	21	54.8	45.2	–	23.8	61.9	14.3	2.10
	TD-N	42	54.8	45.2		33.3	42.9	23.8	$p=0.35$

TD-Y, Tardive dyskinesia positive; TD-N, tardive dyskinesia negative; ML, maximum likelihood; LR, likelihood ratio.

T/C heterozygotes among the TD-Y patients. The frequency of the minor 452Tyr allele varied significantly among the five groups that were assayed (Jerusalem 0.12, Singapore 0.01, Toronto A-A 0.11, Toronto Caucasians 0.13, Vienna 0.05; $\chi^2=53.26$, d.f. 4, $p<0.000001$). There were no significant differences in His452Tyr allele frequency and genotype distribution between the TD-Y and TD-N patients in any of the groups.

Pooled analyses – logistic regressions

Our goal was to evaluate association of the HTR_{2A} T102C polymorphism with TD while controlling for potential confounders: (a) differences among groups in T102C allele frequency; (b) possible group differences in the evaluation of TD; (c) the impact of age and gender. A multinomial logistic regression was performed with the 'dependent' variable being T102C genotype (3 levels) (Table 4). We evaluated the effect of 'group' and 'TD status' as predictors of the dependent variable. The LR test of the two models (group + TD status and group alone) determines the significance of TD status controlled for the potential confounding effect of group (Table 4). There was a significant effect of both the T/C ($Z=0.43$, 95% CI 0.03–0.81, $\chi^2=2.16$, $p=0.003$) and C/C genotypes ($Z=0.14$, 95% CI -0.64 to 0.92, $\chi^2=3.48$, $p=0.001$) vs. the T/T genotype. The LR test for TD status was significant, while controlling for the possible confounding effect of group ($\chi^2=12.46$, d.f. 2, $p=0.002$).

In the second set of logistic regressions the dependent variable was TD status and the predictors evaluated were age, gender and T102C/C genotype (Table 5). There was a significant effect of age ($p<0.001$) and no significant effect of gender ($p=0.35$). The 'net' effect of T102C/C genotype, i.e. the LR test was again significant ($\chi^2=11.23$, d.f. 1, $p=0.0008$). We conducted further, subsidiary analyses in order to identify the most appropriate genotypic model. A co-dominant (additive) model yielded $\chi^2=9.82$, d.f. 1, $p=0.0017$. The dominant model was also significant ($\chi^2=6.0$ d.f. $=1$, $p=0.0093$) while the result for a recessive model was $\chi^2=5.59$, d.f. 1, $p=0.018$.

The same set of logistic regressions was conducted for the His452Tyr polymorphism. There was no evidence for an association between this polymorphism and TD across the five groups (data available on request).

Linkage disequilibrium and haplotypes

To examine the effect of the T102C-His452Tyr haplotype in TD, we first checked whether the two polymorphisms were in LD in our sample, which we found to be true ($\chi^2=8.11$, d.f. 1, $p=0.004$). Therefore, we estimated the effect of the haplotype on TD and found it very significant ($\chi^2=16.7$, d.f. 3, $p=0.0008$; OR 2.8, CI 1.05–12.3) for the TD-Y group with a double risk allele compared to none.

Role of age in HTR_{2A}-related risk for TD

Given the strong relationship between age and susceptibility to TD (Kane et al., 1992; Smith and Baldessarini, 1980; Woerner et al., 1998) we had

Table 4. Multinomial logistic regression for variables predicting T102C genotypes in patients with and without TD from the six groups

Model (Log-likelihood)	(Model) χ^2 (d.f.)	Z (d.f.)	p value	Coefficients Z
Null model	−642.79			
Model A	595.42			
[Genotype T/C] Centre				
Jerusalem		−1.57 (1)	0.11	−0.65 (−1.46 to 0.16)
Milan		−0.68 (1)	0.49	−0.29 (−1.14 to 0.55)
Singapore		−2.77 (1)	0.006	−1.03 (−1.77 to −0.3)
Toronto A-A		0.68 (1)	0.49	0.57 (−1.07 to 2.22)
Toronto Caucasian		−0.94 (1)	0.34	−0.39 (−1.21 to 0.42)
Vienna*		–	–	–
TD status		2.16 (1)	0.003	0.43 (0.03 to 0.81)
[Genotype C/C] Centre				
Jerusalem		−0.98 (1)	0.32	−0.45 (−1.37 to 0.45)
Milan		−1.23 (1)	0.21	−0.62 (−1.6 to 0.36)
Singapore		−5.55 (1)	<0.001	−2.92 (−3.95 to −1.9)
Toronto A-A		1.52 (1)	0.13	1.30 (−0.38 to 2.98)
Toronto Caucasian		−1.32 (1)	0.18	−0.64 (−1.59 to 0.31)
Vienna*		–	–	–
TD status		3.48 (1)	0.001	0.14 (−0.64 to 0.92)
Model B	−601.65			
[Genotype T/C] Centre				
Jerusalem		−1.42 (1)	0.12	−0.58 (−1.39 to 0.22)
Milan		−0.65 (1)	0.51	−0.28 (−1.12 to 0.56)
Singapore		−2.65 (1)	0.008	−0.99 (−1.72 to 0.26)
Toronto A-A		0.76 (1)	0.44	0.63 (−1.0 to 2.28)
Toronto Caucasian		−0.90 (1)	0.36	−0.37 (−1.19 to 0.44)
Vienna		–	–	–
[Genotype C/C] Centre				
Jerusalem		−0.68 (1)	0.49	−0.31 (−1.21 to 0.58)
Milan		−1.17 (1)	0.24	−0.58 (−1.56 to 0.39)
Singapore		−5.40 (1)	<0.001	−2.81 (−3.8 to −1.8)
Toronto A-A		1.69 (1)	0.09	1.44 (−0.23 to 3.11)
Toronto Caucasian		−1.24 (1)	0.21	−0.59 (−1.54 to 0.35)
Vienna*		–	–	–
LR test (−2[(Log L model B) − (Log L model A)]		12.46 (2)	0.002	

A-A, African-American; LR, likelihood ratio.
* Dropped due to collinearity.

proposed that age might influence the relationship between HTR$_{2A}$ and TD (Segman and Lerer, 2002b). Accordingly we performed subsidiary analyses of the current dataset in order to test this hypothesis. First, we re-ran the multinomial logistic regression in which the dependent variable was the T102C genotype (three levels) and evaluated the effect of 'group' and 'TD status' as predictors of the dependent variable with the sample divided into older or younger patient groups based on the median age [younger patients, $\leqslant$47 yr: Y-TD (n=96), N-TD (n=215); older patients, >47 yr: Y-TD (n=159), N-TD (n=164)]. The LR test for TD status predicting T102C genotype was significant for the older (χ^2=13.05, d.f. 2, p=0.002) but not the younger patients (χ^2=1.6, d.f. 2, p=0.45). In a second set of logistic regressions in which the

Table 5. Multinomial logistic regression analysis for variables predicting tardive dyskinesia phenotype in patients from the six groups

Null model	Log L = −427.64			LR χ^2		*p*
Model A	Log L = 0–403.44			LR χ^2 (d.f. 3) = 48.40		$p < 0.0001$
	Variables	OR	S.E.	95% CI	*Z*	$P > \|z\|$
	Age	3.06	0.67	1.99–4.72	5.08	< 0.001
	Sex	1.19	0.21	0.82–1.70	0.94	0.34
	T102C genotypes	0.64	0.08	0.49–0.84	−3.32	0.001
Model B	Log L = −409.05			LR χ^2 (d.f. 2) = 37.17		$p < 0.0001$
	Variables	OR	S.E.	95% CI	*Z*	$P > \|z\|$
	Age	3.11	0.68	2.02–4.78	5.17	< 0.001
	Sex	1.15	0.21	0.83–1.71	–	1.64
	LR test (−2[(Log L Model B) −(Log L Model A)]			LR χ^2 (d.f. 1) = 11.23		$p = 0.0008$

LR, Likelihood ratio; OR, odds ratio; CI, confidence interval.

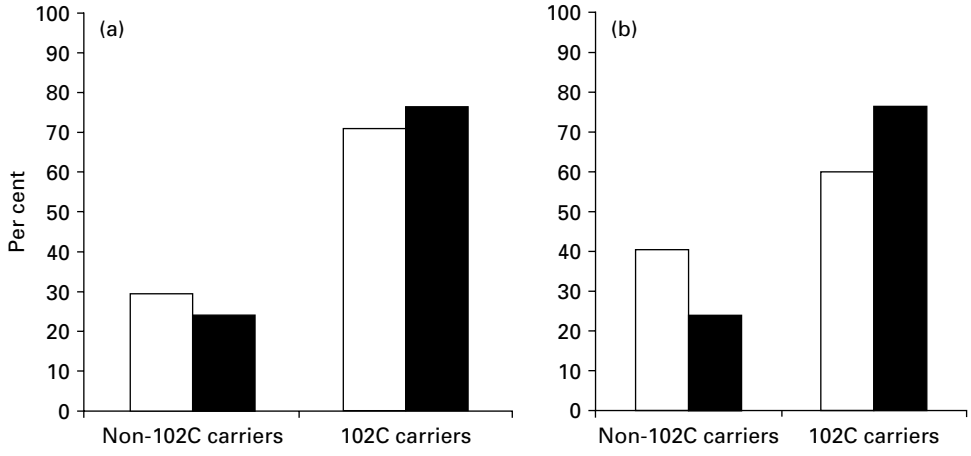

Figure 1. Frequency of 102C allele carriers (T/C heterozygotes and C/C homozygotes) in (a) younger patients (⩽47 yr) with (Y-TD) ($n = 96$) and without TD (N-TD) ($n = 215$) and (b) in older patients Y-TD ($n = 159$) and N-TD ($n = 164$). Likelihood ratio test for TD status predicting T102C/C allele carrier status in younger patients (⩽47 yr): $\chi^2 = 0.18$, d.f. 2, $p = 0.60$; in older patients (>47 years): $\chi^2 = 10.16$, d.f. 1, $p = 0.001$. □, N-TD; ■, Y-TD.

response variable was TD status and the predictors evaluated were age, gender and T102C/C genotype, the LR test for the T102C/C genotype was again significant in the older ($\chi^2 = 12.93$, d.f. 1, $p = 0.0003$) but not the younger patients ($\chi^2 = 1.15$, d.f. 1, $p = 0.28$). The interaction between age and the T102C genotype was not significant suggesting that the underlying model to explain the effect of T102C genotype in the old vs. the young group is a conditional effect of T102C related to age rather than a multiplicative effect. Figure 1 shows the frequency of 102C allele carriers in the younger and older patients with and without TD.

Specificity of HTR_{2A} polymorphism as a risk factor for non-orofacial TD

We examined whether the HTR_{2A} T102C polymorphism is differentially related to orofacial dyskinesia as opposed to dyskinesia affecting the limbs or trunk (non-orofacial dyskinesia) because of a preliminary

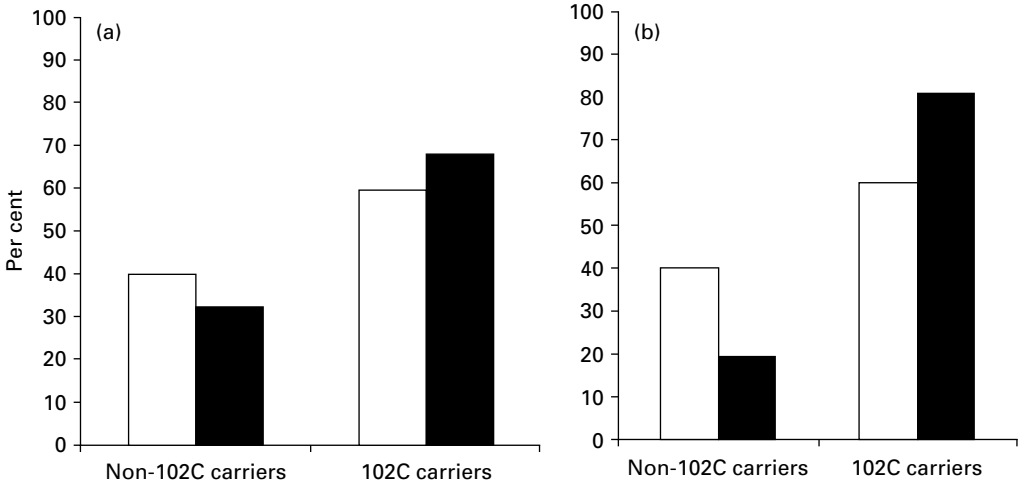

Figure 2. Frequency of 102C allele carriers (T/C heterozygotes and C/C homozygotes) in (a) patients with (Y-TD) ($n=100$) and without orofacial TD ($n=255$), excluding patients with concomitant non-orofacial TD ($n=34$) from the group without TD and (b) in patients with ($n=31$) and without non-orofacial TD ($n=255$), excluding patients with concomitant orofacial TD ($n=34$) from the non-orofacial TD group. Likelihood ratio test for TD status predicting T102C/C allele carrier status in patients with orofacial TD: $\chi^2=2.1$, d.f. 1, $p=0.15$; in patients with non-orofacial TD: $\chi^2=5.10$, d.f. 1, $p=0.02$. □, N-TD; ■, Y-TD.

observation by the Milan group (Macciardi et al., 1997) that the T102C polymorphism was not related to TD in an overall way but was related to limb dyskinesia. In the data from three of the five centres (Jerusalem, Milan and Singapore) separate TD ratings were available for orofacial, limb and trunk dyskinesia, according to the Schooler and Kane criteria (Schooler and Kane, 1982). Because of the rarity of trunk dyskinesia (only 13 cases in all three groups and only three cases in patients without concomitant orofacial TD), patients with limb or trunk dyskinesia were combined into a single non-orofacial TD group. We examined the contribution of the T102C genotype to susceptibility to non-orofacial TD by examining patients with non-orofacial TD ($n=31$) and patients without TD ($n=255$) separately, excluding patients with concomitant orofacial TD ($n=34$) from the non-orofacial TD group. A significant effect of the T102C genotype was observed in the non-orofacial TD group only (Global test: $\chi^2=15.0$, d.f. 2, $p=0.001$). For C/C vs. T/T ($\chi^2=14.5$, d.f. 1, $p=0.0001$); for T/C vs. T/T ($\chi^2=1.98$, d.f. 1, $p=0.16$). Non-orofacial TD (in the absence of orofacial TD) was almost exclusively observed in the patients older than 47 yr (29 out of 31 patients with non-orofacial TD) and the effect was significant in this group alone when compared to the older patients without TD. Performing the opposite analysis we compared patients with orofacial TD ($n=100$) to patients without TD ($n=255$), excluding patients with concomitant non-orofacial TD ($n=34$) from the group without TD. In this analysis there was no effect of the T102C genotype in patients with orofacial TD compared to the patients without TD ($\chi^2=4.65$, d.f. 2, $p=0.1$). Figure 2 shows the proportion of 102C allele carriers and non-carriers with non-orofacial TD and without TD.

Relationship of HTR$_{2A}$ polymorphism to severity of dyskinetic movements

In addition to classifying TD status in a dichotomous fashion we performed analyses of the relationship of HTR$_{2A}$ T102C to abnormal involuntary movement as a continuous variable in the four groups that had AIMS ratings (Jerusalem, Singapore, Toronto A-A and Toronto Caucasians). In an ANCOVA, AIMS total score was the dependent variable; group, gender and T102C/C allele carrier status were the independent variables and age was a covariate. There were significant main effects of group ($F=35.7$, d.f. 3, $p<0.0001$), T102C allele carrier status ($F=5.2$, d.f. 1, $p=0.023$) and of the covariate age ($F=31.8$, d.f. 1, $p<0.0001$) but not of gender. The group × allele interaction was not significant ($F=2.02$, d.f. 3, $p=0.11$). The same elements were included in a second ANCOVA except that the T102C genotype was substituted for T102C/C allele carrier status. In this model, the effects of group ($F=30.6$, d.f. 3, $p<0.0001$), T102C genotype ($F=3.61$, d.f. 2, $p=0.028$) and of the covariate age ($F=31.1$, d.f. 1, $p<0.0001$) but not gender were again significant. There was no group × genotype interaction ($F=1.19$, d.f. 3, $p=0.31$). Post-hoc comparison with a Scheffé test

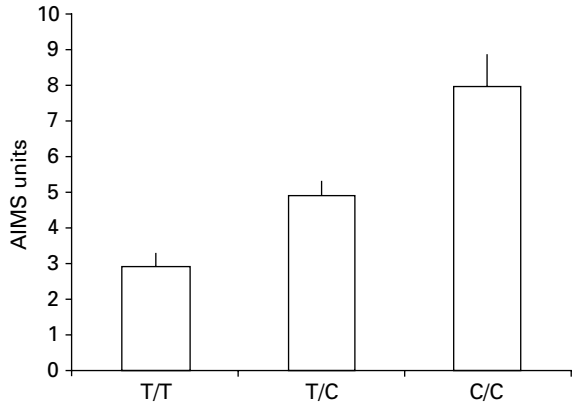

Figure 3. Adjusted mean AIMS total scores by T102C genotype (derived from ANCOVA with group, gender and T102C genotype as independent variables and age as covariate). Bars show standard error of the mean. ANCOVA results given in the text. By post-hoc Scheffé test: C/C vs. T/C genotype, $p=0.001$; C/C vs. T/T genotype, $p<0.0001$.

of adjusted mean AIMS total scores (Figure 3) showed significantly higher scores for the C/C vs. T/C ($p=0.001$) and C/C vs. T/T ($p<0.0001$) genotypes.

Meta-analysis

Figure 4 shows the results of the meta-analysis and presents the observed distribution of odds ratios for TD among carriers of the T102C/C allele (T/C heterozygotes and C/C homozygotes) in the six groups included in the pooled analysis (Toronto A-A excluded) and in an additional published report (Herken et al., 2003). The Mantel–Haenszel (M-H) pooled odds ratio (OR) across all the available data was 1.64 (95% CI 1.17–2.32, $\chi^2=8.30$, d.f. 1, $p=0.004$), pointing to a small but significant effect of the HTR_{2A} T102C/C allele as a risk factor for TD. There was no evidence for heterogeneity ($\chi^2=7.17$, d.f. 5, $p=0.21$). The meta-analysis was also significant with the Toronto A-A patients included (OR 1.67, 95% CI 1.19–2.36; $\chi^2=9.23$, d.f. 1, $p=0.002$) but the 95% CI of the A-A group individually was exceedingly large (0.27–144.70, OR 6.30), well outside the 95% CI limits of the groups combined.

Comment

HTR_{2A} and modification of the propensity to express TD

The pooled analysis of 635 patients reported here demonstrated a small but significant increase of risk for TD in patients with schizophrenia who carry the C allele of the HTR_{2A} T102C polymorphism, over and above the potential confounding effects of population group, age and gender. A meta-analysis, which included the results of a published paper that was not part of the pooled analysis supported this finding with an odds ratio of 1.64. We did not find an effect of the His452Tyr polymorphism considered alone. However, the joint contribution of T102C and His452Tyr was significant. While this effect could be carried by the strong effect of T102C, the p value of the two-way haplotype seems to suggest that the combination of the two SNPs is in fact greater than T102C alone. An explanation for the non-detectable effect of His452Tyr alone might be the low minor allele frequency in several of the groups, which would require much larger samples for the identification of an association.

Limitations of the study must be borne in mind. Evaluations of TD were conducted by different clinicians in different centres. While inter-rater reliability was achieved within centres, this was not the case between centres. Moreover, different rating scales were used to rate abnormal involuntary movements as a basis for applying the RDC-TD. These limitations are inevitable in a post-hoc analysis of data collected in different centres. Nevertheless, there was standardization in applying the RDC-TD, and also, the inclusion of group as a confounding variable in the logistic regression controls for this problem to a certain extent. Data were not available from all the centres on the type of antipsychotic drugs that the patients were administered nor on dose and duration of use. These are potentially important variables and our findings must be considered in this context. A further limitation is that in all the centres the diagnosis of TD was based on a single assessment. Abnormal involuntary movements may fluctuate over time and the diagnosis of TD in the sample must, therefore, be considered to be at a 'possible' level according to the RDC-TD (Schooler and Kane, 1982). Genotyping was done in different laboratories; however, methods are standard and the margin of error small. We did not correct statistical analyses for multiple testing since it is not clear that the two SNPs examined should be regarded as independent in that they are located in the same gene and are in LD. The same consideration of non-independence may be applicable to the two subsidiary phenotypes that we examined (age effect and body region specificity). However, the significance levels are such that in almost all cases the p value would remain significant even after correction.

The HTR_{2A} T102C polymorphism is an intronic variant that does not code for a change in the structure of the receptor protein. It is in close LD with an

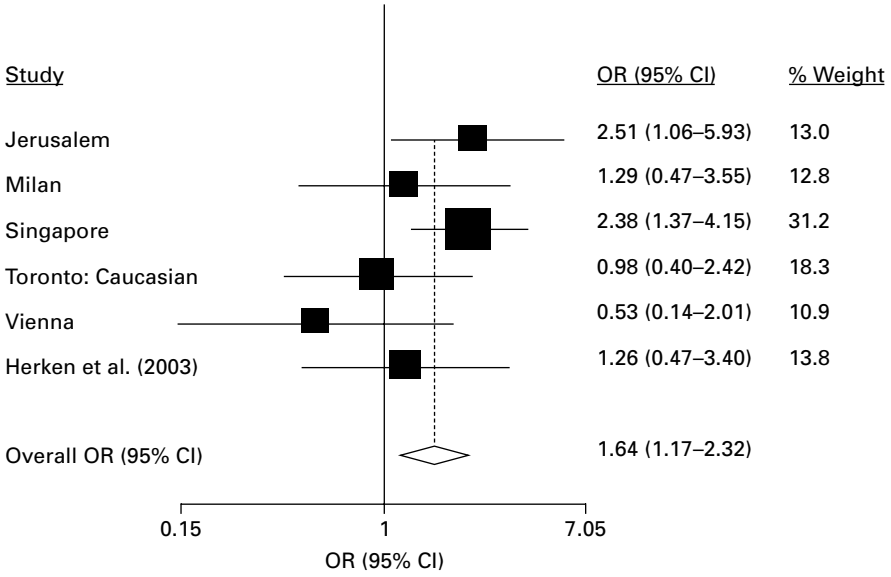

Figure 4. Results of meta-analysis showing the odds ratios (OR) and 95% confidence intervals (CI) for TD among carriers of the T102C/C allele (T/C heterozygotes and C/C homozygotes) in six of the groups included in the pooled analysis (Toronto A-A excluded) and in an additional published report (Herken et al., 2003). Mantel–Haenszel pooled OR = 1.64 (95% CI 1.17–2.32; $\chi^2 = 8.30$, d.f. 1, $p = 0.004$).

A-1438G polymorphism in the HTR$_{2A}$ promoter (Basile et al., 2001; Segman et al., 2001) that was found by Segman et al. (2001) to be associated with TD. The silent T102C site could reflect the impact of the promoter site through LD. However, there is evidence that the T102C polymorphism may directly affect expression of 5-HT$_{2A}$ mRNA and protein (Polesskaya and Sokolov, 2002) and cortical ketanserin binding (Turecki et al., 1999) although the results of other studies are variable (Kouzmenko et al., 1999).

While the focus of the current study was on HTR$_{2A}$ as a risk factor for TD, an alternative explanation is possible. A small but significant contribution of T102C and more recently A-1438G to risk for schizophrenia has been reported although not consistently (Hawi et al., 1997; Lohmueller et al., 2003; Shinkai et al., 1998; Spurlock et al., 1998; Williams et al., 1997) and also association of the T102C polymorphism with response to clozapine (Arranz et al., 1998) not replicated in other studies (Malhotra et al., 1996; Masellis et al., 1998). The HTR$_{2A}$ T102C polymorphism may be a genetic marker for a subtype of schizophrenia that is characterized by increased susceptibility to TD or reduced responsiveness to antipsychotic drugs (Chakos et al., 1996). The latter situation would result in patients receiving higher doses of antipsychotics for longer periods, thus being exposed to increased risk for TD on this basis. This explanation would account for the reported association of the polymorphism with schizophrenia, clozapine response and TD in different studies. Studies that focus on a refined phenotype that includes drug responsiveness are needed.

Age-related association of HTR$_{2A}$ with TD

The influence of age on the association of the HTR$_{2A}$ polymorphism with TD is an intriguing aspect of the present study. Segman and Lerer (2002b) re-analysed their data for three polymorphisms that were associated with TD in their sample. For two serotonin receptor polymorphisms, HTR$_{2C}$-Cys23Ser and HTR$_{2A}$-T102C, but not for DRD3, there was a significant effect on AIMS scores only in subjects $\geqslant$47 yr. Segman and Lerer (2002b) suggested that genetically based variations in receptors might become functionally relevant at an older age when receptor reserve is reduced below a critical threshold for reasons related to the ageing process (Versijpt et al., 2003). Another possible explanation is that older patients will generally have been exposed to antipsychotic drugs for a longer period. However, Segman and Lerer (2002b) controlled for years of exposure and found that the age effect on the genetic associations that they observed remained significant. Of the four published studies on the relationship of HTR$_{2A}$ T102C to TD, in the two that found an association the mean age of the subjects was $>$50 yr (Segman et al., 2001; Tan et al., 2001) and in the two which did not find an association the mean age

of the subjects was <35 yr (Basile et al., 2001; Herken et al., 2003) (Table 1). In the present study, the hypothesis of Segman and Lerer (2002b) was confirmed in a large sample of patients. HTR_{2A} T102C genotype was significantly associated with TD in the older patients only (>47 yr). These findings have important implications for the design and interpretation of pharmacogenetic studies in psychiatry. If the increase in risk conferred by a genetic variant is manifested primarily in older patients, studies confined to younger samples could lead to its unwarranted exclusion. In developing a pharmacogenetic test the age relatedness of genetic variants included would need to be taken into account. The same would apply to gender-related genetic variants; while these have not been identified for TD, they could exist for other pharmacogenetic phenotypes.

Genetic refinement of the TD phenotype

Whereas TD is usually regarded as a unitary entity, its clinical manifestations and course show considerable heterogeneity. Previous studies have described two major subtypes with predominantly orofacial or limb-truncal presentations (Gureje, 1988; Muscettola et al., 1999; Paulsen et al., 1996). Distinct clinical and demographic risk factors have been reported to associate with these subtypes (Gureje, 1988; Muscettola et al., 1999; Paulsen et al., 1996). Our results point to the possibility that genetic variability in the 5-HT_{2A} receptor may differentially modulate the risk for these TD subtypes with a specific emphasis on the non-orofacial subtype.

Implications for the pharmacogenetics of TD

Case-control designs require large samples if they are to identify and in particular replicate small gene effects and identify interactions among genes. Therefore, it is frequently necessary to pool samples from different centres. While increasing power, this can lead to spurious results if allele frequencies for the genes being studied vary among the populations. On the other hand, there is a distinct advantage to studying pharmacogenetic issues across populations so that it can be determined to what extent findings are generally applicable. In the current analysis, we applied a statistical approach, logistic regression, in order to address the potentially confounding effects of population origin and also those of age and gender. Previously, Lerer et al. (2001) applied this approach to an analysis of the 5-HT_{2C} receptor gene in large samples of patients with recurrent major depression (MDD) bipolar disorder (BPD) and in their analysis of the DRD3 Ser9Gly polymorphism in relation to TD (Lerer et al., 2002).

The results of the present study support an association of the T102C polymorphism and T102C-His452Tyr haplotypes in the 5-HT_{2A} receptor gene with TD that is more strongly manifested in older patients and in patients with non-orofacial (limb-truncal) TD. In itself, this identified risk factor has low predictive value and does not have clinical utility. It should be considered in the context of a panel of genetic polymorphisms that have been associated with TD such as the Ser9Gly polymorphism in DRD3 (Lerer et al., 2002) and the 5-HT_{2C} receptor gene (HTR_{2C}) (Segman et al., 2000; Zhang et al., 2002). While the individual contribution of each polymorphism to the risk for TD is small, association of this complex phenotype with several genes is consistent with a polygenic basis. The potential for developing a pharmacogenetic test based on a combination of risk alleles is feasible once a sufficient number of such variants have been identified. Such a test would need to take into account complex interactions among different variants (Segman et al., 2002; Zhang et al., 2003) as well as the contribution of variables such as age, gender and ethnic origin. Significant association with TD of two genes across populations – DRD3 in our previous study (Lerer et al., 2002) and HTR_{2A} in this paper – is an important step in the direction of establishing a pharmacogenetic test for susceptibility to TD.

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Statement of Interest

Bernard Lerer is the Editor-in-Chief of the *International Journal of Neuropsychopharmacology*. Therefore, the

entire review process of this paper was conducted outside the Editorial Office of the Journal by a Field Editor.

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