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Pharmacogenetics of antipsychotic-induced Parkinsonism and tardive dyskinesia

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CHAPTER 3

TARDIVE DYSKINESIA AND DRD3, HTR2A, AND HTR2C GENE POLYMORPHISMS IN RUSSIAN PSYCHIATRIC INPATIENTS FROM SIBERIA

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ABSTRACT

Background: Pharmacogenetics of tardive dyskinesia and dopamine D3 (DRD3), serotonin 2A (HTR2A), and 2C (HTR2C) receptors has been examined in various populations, but not in Russians. Purpose: To investigate the association between orofaciolingual (TDof) and limb-truncal dyskinesias (TDlt) and Ser9Gly (DRD3), -1438G>A (HTR2A), and Cys23Ser (HTR2C) polymorphisms in Russian psychiatric inpatients from Tomsk, Siberia.

Methods: In total, 146 subjects were included. Standard protocols were applied for genotyping. TDof and TDlt were assessed with AIMS items 1-4 and 5-7, respectively. Two-part model, logistic and log-normal regression analyses were applied to assess different variables (e.g., allele-carriership status, age, gender, and medication use).

Results and conclusions: TDlt, but not TDof, exhibited an association with Ser9Gly and Cys23Ser (with 9Gly and 23Ser alleles exhibiting opposite effects). However, -1438G>A was not associated with TDof and Dlt. This is the first pharmacogenetic report on tardive dyskinesia in Russians. Subject to further replication, our findings extend and support the available data.

INTRODUCTION

Tardive dyskinesia (TD) is a potentially irreversible antipsychotic-induced movement disorder with a prevalence of about 20-30% in psychiatric patients chronically exposed to antipsychotics. Older age, female gender, race, and family history are several risk factors for the development of TD [Glazer 2000; Kane et al., 1988; Muller et al., 2001; Rosengarten et al., 1994; Wonodi et al., 2004].

Phenotypically, TD can be dissected into two distinct subsyndromes; orofaciolingual (TDof) and limb-truncal dyskinesias (TDlt). TDof involves movements of mouth and face muscles and may impair eating and swallowing, whereas TDlt involves purposeless choreiform movements of trunk and/or limbs and may cause gait disturbances and falls.

Accumulating evidence suggest that TDof and TDlt are two distinct clinical entities with different clinical features, different risk factors, different prognosis, and probably even different genetic liability [Gureje 1988; Gureje 1989; Inada et al., 1990; Paulsen et al., 1996; Waddington et al., 1987; Wilffert et al., 2008].

Dopamine D3, serotonin 2A, and 2C receptors (encoded by DRD3, HTR2A, and HTR2C genes, respectively) are involved, at least partially, in the therapeutic and adverse effects of antipsychotics and genetic variations in these receptors may affect the individual sensitivity to TD [Reynolds 2004]. Several studies suggest, for example, that Ser9Gly polymorphism of DRD3 gene may be associated with TD in humans [Bakker et al., 2006; Lerer et al., 2005; Wilffert et al., 2008] and even in non-human primates [Werge et al., 2003]. Furthermore, accumulating evidence suggests that HTR2A and HTR2C genes may be pharmacogenetically important for TD [Arranz and de Leon J. 2007; Lerer et al., 2005; Segman et al., 1999; Segman et al., 2001; Segman et al., 2003; Segman and Lerer 2002; Wilffert et al., 2008].

Ethnicity is an important, but often underestimated, demographic and pharmacogenetic determinant of the response to antipsychotics [Frackiewicz et al., 1997; Swartz et al., 1997]. African-Americans for instance have been found to be more sensitive to TD [Eastham et al., 1996; Morgenstern and Glazer 1993].

Several studies have examined TD pharmacogenetics in relation to DRD3, HTR2A, and HTR2C gene polymorphisms in various ethnic groups [Liou et al., 2004; Segman et al., 1999; Segman et al., 2000; Segman et al., 2001], but not in Slavonic Caucasians from Siberia. Anthropologically, Siberia forms an important geographic link between the European and Asian continents and between North Asia and the Japanese Archipelago [Karafet et al., 2002]. Southern Siberia particularly is an area where the most ancient contacts occurred between Caucasoid and Mongoloid people, which may have affected the genetic architecture of Eastern Slavonic populations generally and Russian Siberians particularly [Derenko et al., 2006; Karafet et al., 2002; Shorokhova et al., 2005; Verbenko et al., 2005].

In the present study, we report for the first time the association between certain polymorphisms in DRD3, HTR2A, and HTR2C (Ser9Gly, -1438G>A, and

Chapter 3

Cys23Ser, respectively) and TD including its two main forms (TDof and TDlt) in Russian psychiatric patients from Siberia.

METHODS

Subjects

Informed consent was obtained from each subject after explanation of the study after approval of the study protocol by the institutional bioethics committee. Subjects were included from two psychiatric departments (for permanent and temporal hospitalization) of the Mental Health Research Institute in Tomsk, Siberia (Russia).

We included subjects with informed consent and clinical diagnosis of schizophrenia or schizotypal disorder (ICD-10: F20 and F21, respectively), and excluded subjects with non-Caucasian physical appearance (e.g., Mongoloid, Buryats, or Khakassians), subjects on clozapine but without TD (clozapine may ameliorate TD), subjects with clinically relevant withdrawal symptoms, and those with organic brain disorders.

Clinical and demographic data were extracted from patients' medical files.

In total, 153 Russian Caucasian patients met the inclusion criteria. Of these, 7 subjects used clozapine but did not exhibit TD (assessed by having an AIMS item ≥ 2 points). Since clozapine may ameliorate TD, these subjects were excluded from the analysis. Therefore, a total of 146 subjects (91 males, 55 females) were included in the analysis.

Assessment instruments

TD was assessed cross-sectionally by the use of the Abnormal Involuntary Movement Scale (AIMS) [Gardos et al., 1977], which scores 7 dyskinesia items (face, lips, jaw, tongue, arms, legs, and trunk) on a 5-point scale (0=none, 1=minimal or extreme normal, 2=mild, 3=moderate, and 4=severe). Four trained raters assessed the presence of TD and, when present, the rating of TD was established by consensus with either one of the two senior doctors. The presence of TDof and TDlt was established by a cutoff score of ≥ 2 (mild but definite) on any of the items 1 through 4 and 5 through 7 of AIMS, respectively. The sum of the first four items and the sum of items 5 through 7 were used as TDof and TDlt severity proxies, respectively. Additionally, we have conducted a full AIMS analyses (i.e., presence of TDof and/or TDlt) by examining items 1-7 of AIMS. In analogy, the sum of these 7 items was used as a proxy of the severity of TD as one entity (TDsum).

Medication

On the day of TD assessment, a complete documentation of the medications utilized was compiled by the raters. The dose of the antipsychotic medication was converted into chlorpromazine equivalents (CPZEq), according to the literature [Davis 1976; Rey et al., 1989; Schulz et al., 1989; Woods 2003].

Genotyping

Blind to the clinical status of the subjects, genomic DNA was extracted from EDTA whole-blood and genotyped with standard TaqMan® Assays-On-Demand ordered from Applied Biosystems (Ser9Gly, C__949770_10; -1438G>A, C__8695278_10; Cys23Ser, C__2270166_10). To reduce the number of classes studied, we chose to underestimate the effects of the polymorphisms by classifying the subjects as carriers or non-carriers of the minor allele, as previously suggested [Al Hadithy et al., 2008].

Statistics

Initially, we applied logistic regression (LR) analysis to investigate the genetic effects on the probability of presence of TDof and TDIt.

Because dichotomizing the data is not reflective of the severity, we also examined the association between these polymorphisms and the severity proxies. However, since clustering of zeros and skewness of data distributions are common problems in psychiatric research [Delucchi and Bostrom 2004], we first examined the distributions of the sums of AIMS items 1-4 (TDof), 5-7 (TDIt), and 1-7 (TDsum) to determine the most appropriate and well-fitting theoretical distribution and to apply an appropriate parametric method thereafter.

To handle the clumping of zeros, we utilized a two-part model (TPM) approach [Delucchi and Bostrom 2004]. In the first part of the TPM analyses we used LR analysis to estimate the probability of having TDof, TDIt, and TDsum sum scores above 0. In the second part of the TPM approach, we used multivariate parametric regression to study the effects of the above mentioned variables on the non-zero part TDof, TDIt, and TDsum variables. Since it is reasonable to consider subjects with zero AIMS values as dyskinesia-free cases, the TPM may explain the difference in the proportion of subjects with and without dyskinesia in relation to allele-carriership status (part 1), and, for those subjects with possible dyskinesia (AIMS-sum>0), whether there is an association between the severity and the carriership of a certain allele (part 2).

Since the separation of zeros from non-zeros in TPM analysis may decrease the sample-size and hence the statistical power, we have also conducted parametric regression analyses on the whole sample to overcome that disadvantage. To make

Chapter 3

the transformation possible, we chose to add 1 to all of the untransformed sums and transform thereafter [Sokal and Rohlf 1994]

In all of these three approaches, we applied a stepwise selection procedure (by means of Akaike's Information Criterion) to select variables (allele-carriership, age, gender, type of psychiatric clinic, use of anticholinergic and antipsychotic medication) that offer the highest explanatory power to the model.

Since there is accumulating evidence suggesting additive effects of these polymorphisms [Segman et al., 2000; Wilffert et al., 2008], we chose to study the effects of 2 genes simultaneously in each model (i.e., Ser9Gly plus -1438G>A, Ser9Gly plus Cys23Ser, or -1438G>A plus Cys23Ser).

For the calculations, the statistical software "R" was used. Where needed, we utilized Fisher's exact test for 2x2 contingency tables. Departure from the Hardy-Weinberg Equilibrium was calculated for all of the polymorphisms, except for Cys23Ser polymorphism (X-chromosomal), using an online tool (<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>).

RESULTS

Demographic and clinical features

Seventy-nine (54%) subjects were included from a psychiatric clinic for permanently hospitalized, severely ill patients. The remaining 46% were less-severely ill inpatients from a clinic for temporal hospitalization. About 95% of the patients had clinically-established schizophrenia (n=138) and only 5% had schizotypal disorder (n=8). All of the subjects, except 2, were using antipsychotics and 35 subjects (24%) were utilizing depot antipsychotics on the day of assessment.

Table 1 presents the main demographic and clinical features of the studied population.

Genotype and hospitalization

Genotype and allele-carriership distribution of the different genotypes are shown in Table 2. Due to technical reasons, we failed to genotype few DNA samples (1-3 samples). The genotype distributions of Ser9Gly and -1438G>A (DRD3 and HTR2A, respectively) were in consistency with the Hardy-Weinberg equilibrium.

Table 2 also presents the distribution of the patients from the two psychiatric clinics (for permanent and temporal hospitalization). The polymorphisms Ser9Gly, -1438G>A, and Cys23Ser are probably not associated with the type of the psychiatric department (Fisher's exact p-values are 1.000, 0.503, and 0.052 respectively).

Table 1. Basic demographic data presented as sample mean \pm standard deviation or as the number (n) and the percentage (%).

	All (146)	Male (91)	Female (55)
Age (years)	46.8 \pm 17.6	45.4 \pm 16.8	49.2 \pm 18.9
Daily dose of antipsychotics (chlorpromazine equivalents)	971.8 \pm 894.3	962.8 \pm 827.0	986.6 \pm 1003.6
Subjects using atypical antipsychotics, % (n)	34.9 (51)	37.4 (34)	30.9 (17)
Subjects using anticholinergics, % (n) ^a	72.6 (106)	78.0 (71)	63.6 (35)
Daily dose of trihexyphenidyl ^a	5.6 \pm 3.9	5.5 \pm 4.0	5.8 \pm 3.6
Temporarily hospitalized (4 months max) inpatients, % (n)	45.9 (67)	44.0 (40)	49.1 (27)
Permanently hospitalized, severely ill inpatients, % (n)	54.1 (79)	56.0 (51)	50.9 (28)
Duration of the psychiatric illness (years)	21.7 \pm 16.1	21.1 \pm 15.9	22.6 \pm 16.4
Orofaciolingual AIMS-score (TDof)	2.3 \pm 2.3	2.5 \pm 2.3	2.1 \pm 2.1
Limb-truncal AIMS-score (TDlt)	1.2 \pm 1.9	1.3 \pm 2.0	1.0 \pm 1.7
Total AIMS-score (TDsum)	3.5 \pm 3.7	3.7 \pm 3.8	3.0 \pm 3.4
Subjects with \geq 2 points on any of AIMS items 1-4, % (n)	23.3 (34)	28.6 (26)	14.5 (8)
Subjects with \geq 2 points on any of AIMS items 5-7, % (n)	11.0 (16)	12.1 (11)	9.1 (5)
Subjects with \geq 2 points on any of AIMS items 1-7, % (n)	29.5 (43)	35.2 (32)	20.0 (11)

^a Trihexyphenidyl was the only anticholinergic medication used by the study population.

Table 2. Hardy-Weinberg Equilibrium (HWE) and the distribution [presented as % (n)] of genotypes and allele-carriers in the total sample, per gender, and per type of psychiatric clinic, respectively. All of the percentages are column percentages.

Genotype	Total sample	HWE χ^2 (<i>p</i>) values	Allele-carriership status	Total sample	Males	Females	Clinic for permanent hospitalization	Clinic for temporal hospitalization
DRD3 Ser9Gly								
<i>Ser9/Ser9</i>	47.6 (69)		<i>9Gly-allele non-carriers</i>	47.6 (69)	44.4 (40)	52.7 (29)	47.4 (37)	47.8 (32)
<i>Ser9/Gly9</i>	46.2 (67)	1.920 (0.166)	<i>9Gly-allele carriers</i>	52.4 (76)	55.6 (50)	47.3 (26)	52.6 (41)	52.2 (35)
<i>Gly9/Gly9</i>	6.2 (9)							
HTR2C Cys23Ser^a								
<i>Cys23/Cys23</i>	81.1 (116)		<i>23Ser-allele non-carriers</i>	81.1 (116)	86.5 (77)	72.2 (39)	87.3 (69)	73.4 (47)
<i>Cys23/Ser23</i>	9.8 (14)	–	<i>23Ser-allele carriers</i>	18.9 (27)	13.5 (12)	27.8 (15)	12.7 (10)	26.6 (17)
<i>Ser23/Ser23</i>	9.1 (13)							
HTR2A -I438G>A								
<i>-I438G/G</i>	43.1 (62)		<i>-I438A-allele non-carriers</i>	43.1 (62)	45.6 (41)	38.9 (21)	40.3 (31)	46.3 (31)
<i>-I438G/A</i>	47.2 (68)	0.563 (0.453)	<i>-I438A-allele carriers</i>	56.9 (82)	54.4 (49)	61.1 (33)	59.7 (46)	53.7 (36)
<i>-I438A/A</i>	9.7 (14)							

^a For males, the hemizygosities for the X-chromosomal Cys23 and Ser23 alleles are denoted as Cys23/Cys23 and Ser23/Ser23, respectively.

Data distribution

The primary outcome variables (TDof and TDlt) were positively skewed (1.0 and 2.6, respectively) and had Pearson's kurtosis greater than 3 (3.5 and 10.8, respectively).

After formal comparison of a variety of positive skewed distributions (log-normal, Weibull, gamma, generalized gamma, generalized F), we assumed a log-normal distribution for TDof and TDlt.

Two-part model (TPM), log-normal (LNR) and logistic regression (LR)

The results of all of the analyses conducted are presented in Table 3.

Orofaciolingual dyskinesia

The first part of the TPM shows that carriers of -1438A-allele (HTR2A) are 2.4 times more likely to have at least one item of AIMS 1-4 with a non-zero score ($p=0.037$), whereas the propensity of carriers of the 23Ser-alleles (HTR2C) to have a score of ≥ 1 on any of these items is one fifth of that of the corresponding non-carriers ($p=0.034$). However, neither the second part of the TPM nor the LNR analyses provide evidence for an association between the severity (proxied by the sum of AIMS 1-4 items) and the polymorphisms -1438G>A and Cys23Ser. Furthermore, LR analysis did not provide evidence for an association between any of the polymorphisms studied and the presence of at least one out of the first four AIMS items with a score of 2 or more.

Limb-truncal dyskinesia

In all of the analyses conducted, -1438A-allele carriership was not significantly associated with any measure of limb-truncal dyskinesia. However, carriership of the 9Gly-allele (DRD3) was significantly associated with 2.6 - 3.6 times higher risk of having non-zero scores on AIMS items 5-7 ($p=0.007$ and $p=0.001$ for models I and II, respectively), as assessed by TPM part 1. Furthermore, the average sum of AIMS items 5-7 is 1.3 - 1.4 times higher in 9Gly-allele carriers than in non-carriers ($p=0.035$ and 0.019 , respectively), as assessed by TPM part 2. The association observed between 9Gly-allele carriership and limb-truncal dyskinesia is also confirmed by the LNR analysis; the sum of AIMS items 5-7 were on average 1.4 times higher in 9Gly-allele carriers than in non-carriers ($p=0.001$). LR failed however to show an association with 9Gly-allele carriership.

The second parts of the TPM suggest that carriership of the 23Ser-allele may act as a protective factor against limb-truncal dyskinesia; the average sum of AIMS items 5-7 in carriers of this allele is about 0.7 times that of 23Ser-allele non-carriers,

Chapter 3

$p=0.034$ and $p=0.053$ for models II and III, respectively). The LNR also points towards a similar direction; the average sum of AIMS items 5-7 in carriers of this allele is about 0.8 times that of 23Ser-allele non-carriers, $p=0.031$ and $p=0.061$ for models II and III, respectively). Furthermore, LR analysis suggests a non-significant trend towards a lower odds ratio (OR) for having limb-truncal dyskinesia (OR=0.17, $p=0.097$).

Orofaciolingual and/or Limb-truncal dyskinesia (full AIMS-analyses)

TPM part 1 and LNR analyses in models I and II, suggest that 9Gly-allele carriership may be associated with higher AIMS-values. Furthermore, LNR analyses suggest that 23Ser-allele carriership may exhibit a significant (model II) or borderline insignificant (model III) association with lower AIMS-values. Carriership of -1438A-allele may also exhibit an association with higher AIMS-values (TPM part 1 and LR analyses in models I and III).

Table 3. The two-part model (TPM), log-normal regression (LNR), and logistic regression (LR) analyses for orofaciolingual dyskinesia, limb-truncal dyskinesia, and both combined dyskinesias. P-values ≤ 0.05 are printed in **bold**.

Models	Genetic Variation	Two-Part Model Analysis (Tpm)				Log-Normal Regression (Lnr)				Logistic Regression (Lr)				
		Part 1		Part 2						OR		P		
		OR	P	β	P	β	P	β	P	OR	P	OR	P	
Orofaciolingual dyskinesia (AIMS 1-4)														
I	9Gly-allele	2.08	0.084	-	-	1.18	0.143	-	-	-	-	-	-	
	-1438A-allele	2.42	0.037	-	-	1.24	0.065	-	-	-	-	-	-	
II	9Gly-allele	1.27	0.635	1.10	0.499	-	-	-	-	1.85	0.161	-	-	
	23Ser-allele	0.19	0.034	1.36	0.366	-	-	-	-	-	-	-	-	
III	-1438A-allele	1.82	0.156	-	-	1.21	0.103	-	-	-	-	-	-	
	23Ser-allele	-	-	-	-	-	-	-	-	-	-	-	-	
Limb-truncal dyskinesia (AIMS 5-7)														
I	9Gly-allele	2.58	0.007	1.38	0.019	1.41	0.001	-	-	-	-	-	-	
	-1438A-allele	-	-	-	-	-	-	-	-	-	-	-	-	
II	9Gly-allele	3.55	0.001	1.33	0.035	1.40	0.001	-	-	-	-	-	-	
	23Ser-allele	1.76	0.399	0.72	0.053	0.75	0.031	0.166	0.097	-	-	-	-	
III	-1438A-allele	-	-	-	-	-	-	-	-	-	-	-	-	
	23Ser-allele	-	-	0.69	0.0341	0.77	0.061	0.166	0.097	-	-	-	-	
Orofaciolingual and/or Limb-truncal dyskinesia (AIMS 1-7)														
I	9Gly-allele	2.49	0.048	-	-	1.34	0.026	-	-	-	-	-	-	
	-1438A-allele	2.76	0.026	-	-	1.27	0.070	2.18	0.049	-	-	-	-	
II	9Gly-allele	2.71	0.029	-	-	1.36	0.022	-	-	-	-	-	-	
	23Ser-allele	-	-	-	-	0.70	0.035	-	-	-	-	-	-	
III	-1438A-allele	2.38	0.049	1.07	0.634	1.24	0.112	2.36	0.033	-	-	-	-	
	23Ser-allele	-	-	0.17	0.563	0.74	0.075	-	-	-	-	-	-	

Note – Cells without entries indicate that the alleles in question have not been identified by Akaike's criterion as explanatory for the model. All of the estimates are back-transformed estimates (e estimate). All of the models included the variables: age, gender, type of psychiatric clinic, use of anticholinergic and antipsychotics medication. However, the estimates are not presented for the sake of brevity.

DISCUSSION

In this present paper we discuss the possible effects of Ser9Gly, -1438G>A, and Cys23Ser polymorphisms on the emergence of dyskinesias in Russians. For the assessment of TD we utilized the AIMS instrument, which has an acceptable face validity and the inter-rater reliability [Loonen and van Praag 2007], especially when the evaluators are trained as in our study. However, one limitation of AIMS is that its sum does not necessarily reflect severity. For example, a patient with 1 point on each of the 4 first items is certainly less severe than a patient with three 0 scores and a 4 points score on the remaining item, whereas in both cases the severity measured by the sums of the scores would be identical (4 points). Furthermore, as with many other psychiatric instruments [Delucchi and Bostrom 2004], AIMS data are frequently skewed and may contain an abundance of zero values. To mitigate these analytical challenges, we have utilized three different statistical approaches: logistic regression (LR), two-part model (TPM), and log-normal regression (LNR). The purpose of considering multiple statistical methods alternative to the standard classic tests is “not to buy a better result—that will most often not be the case—but rather to buy legitimacy as a safeguard against a type I error” [Delucchi and Bostrom 2004].

In the present study, LR did not provide evidence for an association between clinically present TDof and TDlt and any of the polymorphisms studied suggesting no major effects of these polymorphisms in our subjects. This observation should however be interpreted with caution, since dichotomization discards some of the phenotypic variability and leads to a loss of power to detect association.

Evaluation of TDof’s continuous scores suggests no effects of Ser9Gly polymorphism and only minor, if any, effects of -1438G>A and Cys23Ser polymorphisms. After controlling for possible effects of Ser9Gly we find that the propensity to have non-zero orofaciolingual AIMS scores is significantly higher in carriers of -1438A-allele but significantly lower in carriers of the 23Ser-allele. Notably, these effects disappear when both alleles are studied simultaneously in a model. Furthermore, neither the second part of the TPM nor LNR provide support for effects on the severity. We therefore suggest that the polymorphisms studied are not associated with the orofaciolingual form of TD. In contrast to TDof, analyses with TPM and LNR consistently suggest an association between TDlt-scores and Ser9Gly and Cys23Ser polymorphisms; 9Gly-allele being predisposing for and 23Ser being protecting against higher TDlt-scores (as expressed by AIMS items 5-7). The -1438A-allele is however not associated with TDlt. Therefore, our data as well as those of Wilffert et al. (2008) indicate that the pharmacogenetics of TDof and TDlt may be different and hence suggest a possible difference in the genetic liability.

The present study has several limitations, next to sample size. For example, we did not correct for variation in the psychopathology of the patients. Half of our patients were severely ill patients included from a psychiatric department for permanently hospitalized, while the other half was comprised of less-severely ill inpatients from

a psychiatric department for temporal hospitalization. A bias may exist when a polymorphism that is associated with the severity of psychosis is preferentially overrepresented in either one of the groups. In fact, all of the polymorphisms studied may affect response to antipsychotics and hence possibly also the severity of the psychopathology [Arranz and de Leon J. 2007]. We have examined the distribution of patients with permanent and temporal hospitalization across the three allele-carriership statuses. However, there were no significant differences in the distribution of the carriers and non-carriers between the two psychiatric centers. Furthermore, the impact of any possible differences in the distribution is expected to be marginal as we have allowed for correction of this demographic variable.

Another limitation is that we did not account for possible differences in the cumulative exposure to antipsychotics. However, this important limitation is a common consequence of the retrospective character of the present and most other pharmacogenetic studies. Nevertheless, we assume that the extent of exposure to antipsychotics is similarly distributed between the different carrier and non-carrier groups.

Despite its limitations, the present study has numerous merits. For example, all of the subjects were inpatients from the only psychiatric hospital in Tomsk (Tomsk Mental Health Institute). Full therapeutic-compliance and a good documentation of the medications prescribed were therefore possible.

More importantly, the present study dissects TD into two phenotypically distinct subsyndromes (orofaciolingual and limb-truncal dyskinesias). With the exception of several studies (Table 4), the majority of pharmacogenetic research so far has examined TD as being one clinical syndrome. Although TD is commonly considered as a unitary entity, accumulating evidence suggests that this assumption is probably false [Brown and White 1992; Glazer and Morgenstern 1988; Kidger et al., 1980; Paulsen et al., 1996]. TDof differs phenotypically from TDlt; the clinical presentation of TDof involves movements of mouth and face muscles, whereas TDlt involves purposeless choreiform movements of trunk and/or limbs. Also the age of onset may be different in TDof and TDlt; whereas TDof maybe more prevalent in older patients, TDlt may occur more often in younger patients [Campbell et al., 1983; Gualtieri et al., 1984; Tarsy et al., 1977]. Rightly, Paulsen et al. [1996] stated “one way to begin to clarify the potentially intricate pathophysiology of TD is to emphasize the clinical heterogeneity in TD to identify subtypes”.

We also discourage the use of the full AIMS instrument in the analyses (pooling TDof with TDlt) and encourage a subsymptom-specific approach for a better understanding of TD pharmacogenetics. Nevertheless, and for the sake of completeness, we have conducted the association analyses after pooling of TDof with TDlt (TDsum). After pooling, the association of lower AIMS values with 23Ser-allele carriership seemed to decrease in statistical significance, which in our opinion may be due to the dilution of the possible effects of TDlt by pooling with TDof. As expected (due to TDlt effects), the pooled analyses indicate that 9Gly allele carriership may be associated with higher AIMS values. The observation that

Chapter 3

9Gly-allele is a risk allele for the development of TD has already been confirmed by a recent meta-analysis in 1610 subjects of several ethnic groups [Bakker, van Os]. Furthermore, the pooled (TDsum) analyses suggest that -1438A-allele carriership is significantly associated with a higher frequency of TD-cases (as judged by TPM part 1 and LR analyses), which may be ascribed to an increase in the number of cases. Alternatively, this finding may be ascribed to a finding by chance. As the severity analyses (TPM part 2 and LNR) do not suggest an association between TDsum and -1438A-allele carriership, the latter explanation seems to be more appropriate. This postulation may be supported by Basile et al. [2001] who have examined -1438G>A and TD in 109 Caucasians and found no evidence for an association with TD as a unitary entity.

However, a more appropriate comparison would be one that compares our results with studies dissecting TD into TDof and TDlt. Therefore, we systematically reviewed any paper investigating Ser9Gly, Cys23Ser, or -1438G>A in relation to TDof and/or TDlt. Additionally, the effects of Ser9Gly, Cys23Ser, and -1438G>A polymorphisms on TDof and TDlt have recently been studied in an African-Caribbean sample by four members of the current research team by comparing the corresponding AIMS values with ANCOVA in carriers versus non-carriers of a combination of two polymorphisms [Wilffert et al., 2008]. To make comparison with our results possible, we have re-analyzed the data obtained from that study sample by applying the same statistical procedures discussed in the present study.

Table 4 presents an overview of the findings obtained from studies that have dissected TD into TDof/TDlt and findings obtained from re-analysis of the African-Caribbean sample.

Table 4. Ethnicity and the association of orofaciolingual and limb-truncal dyskinesias with the polymorphisms studied.

Ethnicity	Reference	TD-assessment	Ser9Gly (DRD3)	-1438G>A (HTR2A)	Cys23Ser (HTR2C)
Orofaciolingual dyskinesia (AIMS items 1-4)					
Slavonic Caucasians	This report	Categorical (any item ≥ 2 points)	NS	NS	NS
		Severity (continuous)	NS	NS	NS
African-Caribbeans	Recalculation of Wilffert et al. (2008)	Categorical (any item ≥ 2 points)	NS	NS	NS
		Severity (continuous)	NS	NS	possible association (23Ser-allele protective)
Jewish	Segman et al. (1999, 2000, and 2001)	Severity (continuous)	possible association (9Gly-allele predisposing)	NS	possible association (23Ser-allele predisposing)
Chinese	Liou et al. (2004)	Severity (continuous)	NS	-	-

Table 4. Continued.

Ethnicity	Reference	TD-assessment	Ser9Gly (DRD3)	-1438G>A (HTR2A)	Cys23Ser (HTR2C)
<i>Limb-truncal dyskinesia (AIMS items 5-7)</i>					
Slavonic Caucasians	This report	Categorical (any item ≥ 2 points) Severity (continuous)	NS possible association (9Gly-allele predisposing)	NS NS	NS possible association (23Ser-allele protective)
Chinese	Liou et al. (2004)	Severity (continuous)	NS	-	-
African-Caribbeans	Recalculation of Wilfert et al. (2008)	Categorical (any item ≥ 2 points) Severity (continuous)	NS possible association (9Gly-allele protective)	NS possible association (-1438A-allele predisposing)	NS NS
Jewish^a	Segman et al. (1999, 2000, and 2001)	Severity (continuous)	-	possible association (-1438G-allele predisposing)	NS

Note – Cells are left without entries if the study did not investigate the allele in question. If a study fails to report a significant association then NS (non-significant) is filled in.

^a Limb-truncal dyskinesia was dissected into distal and trunk dyskinesias. Only trunk dyskinesia exhibited a significant association with -1438G>A polymorphism.

Examining Table 4 reveals a number of interesting findings. Firstly, Ser9Gly – a polymorphism that has been extensively studied in relation to TD – may exhibit an association with TDof in Jewish (Ashkenazi and non-Ashkenazi), but not in Slavonic Caucasians, African-Caribbeans, or Chinese subjects.

Secondly, there is no evidence for an association between -1438G>A polymorphism and TDof in any of the ethnicities examined. Since this lack of association has also been observed in our study, our findings are in line with the literature.

Thirdly, the polymorphism Cys23Ser may be associated with TDof in African-Caribbeans and Jewish subjects, but not in Slavonic Caucasians. Strikingly, the possible effects of this polymorphism may be different in African-Caribbeans and Jewish subjects (23Ser-allele may protect against higher TDof values in African-Caribbeans, but in Jewish subjects the same allele may predispose to higher TDof values). Although type I and type II errors can not be excluded, these conflicting findings may indicate differences in epistasis and linkage disequilibrium patterns, due possibly to differences in ethnicity (the extent of linkage disequilibrium is ethnicity-sensitive). Indeed, this postulation is plausible, since for example a recent gene expression study suggests that the -1438G>A polymorphism becomes functional only with co-presence of a certain polymorphism in the promoter region [Myers et al., 2007]. Fourthly, Slavonic Caucasians and African-Caribbeans may exhibit an association between TDlt and the Ser9Gly polymorphism, the association is however opposite in direction (9Gly-allele is predisposing or protective, respectively), probably due to differences in the linkage disequilibrium.

Fifthly, African-Caribbeans and Jewish subjects (but not Slavonic Caucasians) may exhibit an association between TDlt and the -1438G>A polymorphism. The association is however opposite in direction, as with Cys23Ser and Ser9Gly.

Sixthly, since the effects of Ser9Gly, -1438G>A, and Cys23Ser polymorphisms vary in different ethnic groups, it is highly plausible that these polymorphisms are non-functional and that polymorphisms other than these polymorphisms may confer susceptibility for TD.

Lastly, analysis of categorical data (presence of at least one AIMS-item with a score of 2 or higher) suggests that none of the polymorphisms studied can predict clinically present TDof or TDlt in Slavonic Caucasians or in African-Caribbeans. It is therefore plausible that Ser9Gly, -1438G>A, and Cys23Ser polymorphisms may affect the phenotypic variability of TD only after its occurrence. However, the effects of these polymorphisms are expected to be generally of a limited magnitude.

In conclusion, this is the first published pharmacogenetic study of TD in Russian psychiatric patients from Siberia. Although type I or II errors can not be excluded, the present study suggests that the pharmacogenetic effects of Ser9Gly, -1438G>A, and Cys23Ser polymorphism on TD may be different in Russians from those in other ethnic groups. Our data suggest that none of the polymorphisms studied predict clinically present TDof or TDlt. However, Ser9Gly and Cys23Ser polymorphisms may affect the phenotypic variability of TDlt after its occurrence, but not that of

Chapter 3

TDof. Future studies in larger samples of comparable ethnicity are warranted to support our findings.

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Chapter 3

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Chapter 3

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