CHAPTER 4

MISSENSE POLYMORPHISMS IN THREE OXIDATIVE-STRESS ENZYMES (GSTP1, SOD2, AND GPX1) AND DYSKINESIAS IN RUSSIAN PSYCHIATRIC INPATIENTS FROM SIBERIA

AF Al Hadithy[1–4]*, SA Ivanova[5], P Pechlivanoglou[6], B Wilffert[1,2], A Semke[5], O Fedorenko[5], E Kornetova[5], L Ryadovaya[5], JR Brouwers[1,2], and AJ Loonen[1,7]

[1] Department of pharmacotherapy and Pharmaceutical Care, University of Groningen, Groningen, the Netherlands
[2] Clinical Pharmacy and Pharmacology, Zorggroep Noorderbreedte and De Tjongerschans, Leeuwarden, the Nether lands
[3] Department of Clinical Pharmacology, University Medical Center Groningen, Groningen, the Netherlands
[4] Department of Psychiatry, University Medical Center Groningen, Groningen, the Netherlands
[5] Mental Health Research Institute, Tomsk, Russian Federation;
[6] Department of pharmacoepidemiology and pharmacoeconomics, University of Groningen, Groningen, the Netherlands
[7] GGZ Westelijk Noord-Brabant, Bergen op Zoom, NL

Human Psychopharmacology : Clinical and Experimental; submitted

ABSTRACT

Background: Neuronal degeneration due to oxidative stress (OS) has been proposed as a mechanism for tardive dyskinesia (TD) pathogenesis. Cellular defense mechanisms against OS may involve detoxification enzymes (e.g., glutathione peroxidase-1, GPX1; superoxide dismutase-2, SOD2; and glutathione S-transferase P1, GSTP1). Several pharmacogenetic studies have examined TD and OS in different ethnic groups, but not in Russians. Here we report the association between orofaciolingual (TDof) and limb-truncal dyskinesias (TDlt) and polymorphisms of GSTP1 (Ile105Val), SOD2 (Ala9Val), and GPX1 (Pro197Leu) genes in Russian psychiatric inpatients from Siberia.

Methods: We applied the AIMS instrument to rate dyskinesias in 146 inpatients. Two-part model analyses, logistic and multivariate parametric regressions were applied to assess the effects of different variables (e.g., genotype, age, gender, and medication use).

Results and conclusions: Our analyses do not suggest that Pro197Leu (GPX1) is associated with TD. However, our analyses suggest that the 105Val-allele of Ile105Val (GSTP1) may be associated with a lower risk and severity of TDof and TDlt and that Ile105Val pharmacogenetics may be different in Slavonic Caucasians from that in American Caucasians. Furthermore, we find evidence for an association between Ala9Val (SOD2) and TDof, but not TDlt. Subject to further replication, our findings extend the available knowledge on the pharmacogenetics of TD and oxidative stress.

INTRODUCTION

Free radicals (FRs) are highly reactive chemical compounds in biological systems. When controlled, their production is important for cells, since it allows for the extraction of usable energy for metabolism and aids in dismantling macromolecules (e.g., proteins and lipids) into their more basic components [Lohr et al., 2003]. However, when uncontrolled, their overproduction (oxidative stress) may cause cell damage and death through the lipid peroxidation cascade; a chain reaction where errant FRs react with polyunsaturated fatty acids and compromise cell membrane structures.

Oxidative stress has been implicated in the pathogenesis of diverse psychiatric disorders, including schizophrenia. A recent literature review [Ng et al., 2008] suggests that the evidence behind oxidative stress mechanisms in schizophrenia can be grouped into three categories: first, those studies that illustrate disturbed oxidative homeostasis (e.g., evidence for glutathione depletion and lipid peroxidation); second, those demonstrating antioxidant mechanisms of antipsychotic drugs (e.g., a differential impact on oxidative stress status may exist between typical and atypical antipsychotic medications and higher levels of lipid peroxidation products have been reported in patients treated with typical than atypical drugs); third, those showing benefits from antioxidant therapies (e.g., supplementation studies with vitamins C and E, and N-acetylcysteine). Furthermore, evidence from genetic studies support the involvement of oxidative-stress disturbances in the schizophrenia pathogenesis [Ng et al., 2008]. In a post-mortem study of prefrontal cortex, about 90% of schizophrenia patients could be differentiated from controls based on a set of genes related to energy metabolism and oxidative stress [Prabakaran et al., 2004].

Recent insights suggest that tardive dyskinesia (TD), a severe and potentially irreversible movement disorder probably associated with long-term use of typical antipsychotics [Glazer 2000], may form an integral part of schizophrenia and its (genetic) liability [Koning et al., 2008]. Furthermore, animal and clinical studies suggest that long-term blockage of dopamine receptors by antipsychotics may increases the dopaminergic turnover, a process which has been associated with FR overproduction and neuronal toxicity [Lohr et al., 2003]. It is therefore possible that oxidative stress may be involved in the aetiology of TD as well.

Cellular defense mechanisms against FRs may involve enzymes (e.g., glutathione peroxidase-1, GPX1; superoxide dismutase-2, SOD2; and glutathione S-transferase P1, GSTP1) that catalyze the transformation of FRs or FR–producing molecules to non-radical or non-radical producing species.

GPX1 is a selenium-dependent enzyme, expressed in the human brain, and plays an important role in the detoxification of FRs and in reactive nitrogen species-related oxidative stress [Lei et al., 2007]. SOD2 (also known as MnSOD) is a manganese-dependent enzyme found in the mitochondria and encoded by the nuclear DNA. Among all known isoforms of superoxide dismutases, SOD2 scavenges the greatest number of superoxide anions produced in the mitochondria (> 95% of all oxygen consumption in the cells) [Hori et al., 2000]. GSTP1 is a member of glutathione

S-transferases and plays an important role in detoxification by catalyzing the conjugation with reduced glutathione. Furthermore, GSTP1 regulates stress-kinases (e.g., mitogen-activated protein kinases, MAPK) involved in neural plasticity and may suppress dopamine-induced apoptosis in neurons (possibly through MAPK-pathway).

Ethnicity is an important, but often underestimated, demographic and pharmacogenetic determinant of the response to antipsychotics [Frackiewicz et al., 1997; Swartz et al., 1997].

Several studies have examined the association between TD and polymorphisms of oxidative-stress enzymes in various ethnic groups, but not in Slavonic Caucasians from Siberia.

Siberia forms an important geographic link between the European and Asian continents and between North Asia and the Japanese Archipelago. 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 paper we examine –for the first time– the possible effects of three missense polymorphisms (Ile105Val, Ala9Val, and Pro197Leu) in three oxidative-stress enzymes (GSTP1, SOD2, and GPX1, respectively) on orofaciolingual (TDof) and limb-truncal (TDlt) dyskinesias in Russian psychiatric inpatients from Siberia.

METHODS

Subjects

Subjects were included from two psychiatric clinics of the Mental Health Research Institute in Tomsk (Siberia, Russia) after approval of the study protocol by the institutional bioethics committee. 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. TD was assessed cross-sectionally by the use of the Abnormal Involuntary Movement Scale (AIMS) [Gardos et al., 1977]. 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 1-4 and 5-7 of AIMS, respectively. The sum of the first four items was used as a proxy for the severity of

TDof, while the sum of items 5 through 7 was used as a proxy for the severity of TDlt.

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; Rijcken et al., 2003; Schulz et al., 1989; Woods 2003].

Genotyping

DNA extraction and genotyping (standard fluorogenic 5'-exonuclease TaqMan® assays) were conducted according to standard protocols and blind to the clinical status of the subjects. For the determination of Ile105Val we have utilized an Assay on Demand (#C___3237198_20) obtained from Applied Biosystems, whereas for the determination of Ala9Val we have utilized custom-made primers (forward GGCTGTGCTTTCTCGTCTTCA; reverse GGAGGCTGTGCTTCTGCCT) and FAM/VIC -labeled probes with TAMRA as a quencher(CAGATACCCCAAAGCCGGAGCC and CAGATACCCCAAAACCGGAGCCA).

For the determination of Pro197Leu we applied the primers and probes, reported elsewhere [Shinkai et al., 2006].

Statistics

We first examined the distributions of the sums of AIMS items 1-4 (TDof) and 5-7 (TDlt) to determine the most appropriate and well-fitting theoretical distribution and applied 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 we used logistic regression analysis to estimate the probability of having TDof and TDlt sum scores above 0. In the second part of the TPM, we used multivariate parametric regression to study the effects of the above mentioned variables on the non-zero part TDof, and TDlt variables. Since is it reasonable to consider subjects with zero AIMS values as dyskinesia-free cases, the TPM may explain the genetic difference in the proportion of subjects with and without dyskinesia (part 1), and, for those subjects with possible dyskinesia (AIMS-sum>0), whether there is an association between the severity and the presence of a certain genetic variation (part 2).

Since the separation of zeros from non-zeros in TPM analysis may reduce the sample-size in part 2 analysis, we have also conducted parametric regression analyses on the whole sample. To make the transformation possible, we chose to add 1 to all of the untransformed sums and transform thereafter [Sokal and Rohlf 1994].

Finally, we applied logistic regression (LR) analyses to investigate the genetic effects on the presence of TDof and TDlt.

In all of these three approaches, we applied a stepwise selection procedure (by means of Akaike's Information Criterion) to select variables (genotype, age, gender, type of psychiatric clinic, use of anticholinergic and antipsychotic medication) that offer the highest explanatory power to the model. For the calculations, the statistical software "R" was used. Departure from the Hardy-Weinberg Equilibrium was calculated using an online tool by M. Court (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls).

RESULTS

Demographic and clinical features

A total of 146 subjects (91 males, 55 females) were included in the study. Seventy-nine subjects (54%) 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 schizotypical 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.

Table 1. Sample mean ± standard deviation of age (years), daily dose of antipsychotics (mg chlorpromazine equivalents /day), daily dose of anticholinergics among users (mg trihexyphenidyl /day), Orofaciolingual AIMS-score (TDof), Limb-truncal AIMS-score (TDlt), Total AIMS-score, and the number (n) and the percentage (%) of subjects with atypical antipsychotics, anticholinergic medication, temporal or permanent hospitalization, and those exhibiting at least 2 points on AIMS items 1-4 (orofaciolingual TD), 5-7 (limb-truncal TD), and 1-7 (orofaciolingual and/or limb-truncal TD).

	All (146)	Male (91)	Female (55)
Age	46.8±17.6	45.4±16.8	49.2±18.9
Daily dose of antipsychotics	971.8±894.3	962.8±827.0	986.6±1003.6
Subjects using atypical antipsychotics, % (n)	34.9 (51)	37.4 (34)	30.9 (17)
Subjects using anticholinergics, % (n) *	72.6 (106)	78.0 (71)	63.6 (35)
Daily dose of anticholinergics among users*	5.6+3.9	5.5±4.0	5.8±3.6
Temporally hospitalized (upto 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)
Orofaciolingual AIMS-score (TDof)	2.3±2.3	2.5±2.3	2.1±2.1
Limb-truncal AIMS-score (TDlt)	1.2 ±1.9	1.3±2.0	1.0±1.7
Total AIMS-score	3.5±3.7	3.7±3.8	3.0±3.4
Subjects with ≥ 2 points on any of AIMS items 1-4, % (n)	23.3 (34)	28.6 (26)	14.5 (8)
Subjects with ≥ 2 points on any of AIMS items 5-7, % (n)	11.0 (16)	12.1 (11)	9.1 (5)
Subjects with ≥ 2 points on any of AIMS items 1-7, % (n)	29.5 (43)	35.2 (32)	20.0 (11)

*Trihexyphenidyl is the only anticholinergic medication prescribed and used in the hospital.

Genotype

The genotype distributions of Ile105Val (79 Ile/Ile, 53 Ile/Val, and 11 Val/Val), Ala9Val (37 Ala/Ala, 62 Ala/Val, and 42 Val/Val) and Pro197Leu (72 Pro/Pro, 60 Pro/Leu, and 11 Leu/Leu) were in consistency with the Hardy-Weinberg equilibrium (p-values 0.881, 0.366, and 0.953, respectively).

Data distribution

The 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, we assumed a log-normal distribution for TDof and TDlt.

Association analyses

Results for the Two-part model (TPM), log-normal (LNR) and logistic regression (LR) analyses are presented in Table 2.

Table 2. Estimates of the two-part model (TPM), log-normal regression (LNR), and logistic regression (LR) analyses for orofaciolingual and limb-truncal dyskinesia. All of the estimates (β) are back-transformed coefficients (e^β). Cells without entries indicate that the variable in question has not been identified by Akaike's criterion as explanatory for the model. Polymorphisms with significant p-values are **bold** printed.

DETERMINANT	TPM PART 1	TPM PART 2	LNR	LR
Orofaciolingual dyskinesia (AIMS 1-4)				
Intercept	3.03	1.15	1.74**	0.04***
GSTP1 (*105Ile / 105Val*)	**0.41***	-	**0.78***	-
GSTP1 (*105Val / 105Val*)	**0.21***	-	**0.65▪**	-
SOD2 (*9Ala / 9Val*)	-	-	**1.30▪**	**4.38****
SOD2 (*9Val / 9Val*)	-	-	0.97	0.82
GPX1 (*197Pro / 197Leu*)	-	-	-	-
GPX1 (*197Leu / 197Leu*)	-	-	-	-
Age	0.97	-	-	1.04▪
Gender	-	-	-	0.28**
Psychiatric clinic	12.29***	0.44***	1.28*	0.16**
Use of anticholinergics	2.04	1.70**	1.49**	4.04*
Daily exposure to antipsychotics	-	-	-	-
Limb-truncal dyskinesia (AIMS 5-7)				
Intercept	1.10	1.91*	2.31****	0.17
GSTP1 (*105Ile / 105Val*)	-	0.88	0.93	0.51
GSTP1 (*105Val / 105Val*)	-	**0.46****	**0.63***	-
SOD2 (*9Ala / 9Val*)	-	-	-	6.32
SOD2 (*9Val / 9Val*)	-	-	-	4.06
GPX1 (*197Pro / 197Leu*)	-	-	-	-
GPX1 (*197Leu / 197Leu*)	-	-	-	-
Age	-	1.01	-	0.95**
Gender	0.55▪	-	0.81▪	-
Psychiatric clinic	-	0.33****	0.74***	-
Use of anticholinergics	-	-	-	3.57▪
Daily exposure to antipsychotics	-	$1000.27x10^{-3}$**	-	-

Note: significance codes have been assigned in the following manner; p-value ≤ 0.0001 '****', p-value ≤ 0.001 '***', p-value ≤ 0.01 '**', p-value ≤ 0.05 '*', p-value ≤ 0.09 '▪'.

Orofaciolingual dyskinesia (TDof)

Although LR analysis do not support the association, TPM part 1 and LNR analyses suggest that GSTP1 polymorphism (Ile105Val) is significantly associated with a lower risk and severity of TDof, since heterozygous and homozygous subjects (105Ile/105Val and 105Val/105Val genotypes, respectively) had estimates <1.

Regarding SOD2 polymorphism (Ala9Val), heterozygosity (9Ala/9Val), but not homozygosity (9Val/9Val), for the 9Val-allele was associated with significantly higher

TDof risk and severity as shown by LR (OR=4.38, p=0.014) and LNR (β=1.30, p=0.064) analyses, respectively, compared to the 9Ala/9Ala genotype.

In contrast to Ile105Val and Ala9Val polymorphisms, GPX1 Pro197Leu polymorphism was not selected as an explanatory variable in any of the analyses conducted, even after correction for the other relevant variables.

Limb-truncal dyskinesia (TDlt)

As show in Table 2, TPM part 2 and LNR analyses suggest that homozygosity, but not heterozygosity, for the 105Val allele of the GSTP1 polymorphism is associated with a lower severity of TDlt. However, Ala9Val (SOD2) and Pro197Leu (GPX1) polymorphisms did not exhibit a significant association with any measure of TDlt.

DISCUSSION

In the present paper we examine in Slavonic Caucasians from Siberia the association between orofaciolingual (TDof) and limbtruncal (TDlt) dyskinesias and three missense polymorphisms in three oxidative-stress enzymes (isoleucine→valine, alanine→valine, and proline→leucine substitutions in GSTP1, SOD2, and GPX1 genes, respectively).

For the assessment of TD we utilized the AIMS instrument, which data is frequently skewed and may contain an abundance of zeros. To mitigate these and other 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 (e.g., t test and ANOVA) 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].

Our study suggests that 105Val-allele of GSTP1 Ile105Val polymorphism is significantly associated with a lower risk and a lower severity for dyskinesia (TDof and TDlt). This finding is plausible, since it has been suggested that Ile105Val polymorphism is functional with the 105Val-allele leading to an increased enzymatic activity and an enhanced detoxification capacity in vitro [Sundberg et al., 1998]. However, the association with Ile105Val observed in our study has not been reported by Shinkai et al. [2005] who have studied 197 Caucasian subjects from the United States and Canada. Interestingly, the frequency of the 105Val-allele was 1.5-fold higher in our sample than in those in Shinkai et al. sample (0.52 versus 0.35, respectively). If the results obtained from both studies are genuine and if the differences in the association are not caused by analytical differences or by differences in the linkage disequilibria, then this discrepancy may suggest that the pharmacogenetics of GSTP1 may be different in Slavonic Caucasians from Siberia from that in American Caucasians.

The Ala9Val (also known as Ala16Val) polymorphism is thought to lead to changes in the structural conformation of the mitochondrial targeting sequence and consequently to changes in SOD2 activity in the mitochondria (9Ala-allele is the high activity allele in vitro) [Shimoda-Matsubayashi et al., 1996; Sutton et al., 2005]. Our study suggests that heterozygosity for the 9Val-allele –as opposed to 9Ala/9Ala homozygosity– is associated with a significant increase in the risk (OR=4.38, p=0.014) and severity of TDof. Our study therefore suggests that 9Val-allele confers susceptibility to TDof.

This finding supports, at least partially, previously published studies [Akyol et al., 2005; Galecki et al., 2006; Hitzeroth et al., 2007; Hori et al., 2000; Zhang et al., 2002; Zhang et al., 2003]. Galecki et al. [2006] have reported a 10-times higher risk for tardive dyskinesia in Polish subjects with the 9Val/9Val genotype. Furthermore, Hori et al. [2000] and Hitzeroth et al. [2007] report in Japanese as well as in South African (Xhosa) subjects, respectively, a protective role of 9Ala-allele, suggesting that the 9Val variant is a risk-allele for dyskinesia.

However, Zhang et al. [2002] did not report an association between Ala9Val and dyskinesia in their Chinese subjects. Moreover, a study in Korean subjects similarly did not find evidence for this association [Pae et al., 2007]. In fact, the findings from both studies are of considerable importance, not because they suggest no association, but rather because none of these 2 studies contained subjects with the 9Ala/9Ala genotype despite sufficient sample sizes (151 Chinese and 525 Korean subjects, respectively). The lack of association reported [Pae et al., 2007; Zhang et al., 2002] may therefore be attributed to the lack of an appropriate reference group without the 9Val-allele (9Ala/9Ala subjects). In our Russian sample, 24% of the subjects were carriers of the 9Ala/9Ala genotype; a finding that highlights the importance of ethnicity when studying pharmacogenetics.

Interestingly, Zhang et al. [2002] measured SOD2 activity in plasma of Chinese subjects and found that SOD2 activity is about 40% higher in subjects with the 9Val/9Val genotype than in those with the 9Ala/9Val genotype, suggesting that 9Val-allele is the high activity allele. Furthermore, there was a positive correlation between SOD2 plasma activity and AIMS values (i.e., the higher the activity, the higher the severity of dyskinesias) [Zhang et al., 2002]. Since SOD2 converts superoxide anions ($O2\cdot-$) to hydrogen peroxide ($H2O2$), Zhang et al.'s [2002] observations may suggest that the 9Val-allele confers protection against superoxide-anions related oxidative stress. On one hand, this is true if $H2O2$ is rapidly reduced to water by GPX1. On the other hand, $H2O2$ can be converted to the highly toxic hydroxyl radical ($\cdot OH$) in the presence of reduced transition metals. An increased SOD2 activity (especially when combined with a reduced GPX1 activity) may therefore induce oxidative stress. In our study we have allowed the correction for the possible effects of a putatively functional GPX1 polymorphism (Pro197Leu).

Another interesting aspect of the Ala9Val association observed in our analyses, is that heterozygosity, but not homozygosity, for the 9Val-allele exhibited the greatest difference with the 9Ala/9Ala genotype. This observation triggered us to compare the presence of TDof and TDlt in 9Ala/9Ala subjects versus 9Val/9Val subjects (n=37 and 42, respectively). Comparison showed that TDof and TDlt cases are equally represented in both homozygosity groups (Fisher's exact p=1.000 for both dyskinesias).

Since our sample consists of permanently hospitalized severely ill patients and less severely ill temporally hospitalized inpatients, a bias may potentially exist when a particular group of subjects (e.g., severely ill patients) is preferentially overrepresented in either one of the homozygosity groups. However, we have also examined the distribution of permanently and temporally hospitalized inpatients across the two homozygosity groups and we could not find significant differences in the distribution (χ^2=1.142, d.f.=1, p=0.285).

Our study indicates furthermore that SOD2 Ala9Val polymorphism exhibit differences in its associations with TDof and TDlt. If the associations are genuine (i.e., not a result of type I and II errors) then our study may imply differences in the genetic predisposition to TDof and TDlt; a plausible postulation since it has already been shown that these subsyndromes may exhibit different susceptibilities to risk factors and genetic polymorphisms [Paulsen et al., 1996; Wilffert et al., 2008]. However, we can not exclude Type II error particularly, since the prevalence of TDlt is 2-timers lower than that of TDof.

For GPX1 Pro197Leu polymorphism we found in the current study -in contrast to Ile105Val and Ala9Val polymorphisms- no evidence for an association with TDof or TDlt. Since GPX1 enzyme plays an important role in the detoxification of FRs and since Pro197Leu has been shown to be a functional polymorphism in vitro (197Leu-allele may decrease GPX1 activity) [Hu and Diamond 2003], this finding is interesting. Our findings are, however, in line with those of the only published pharmacogenetics study on this topic [Shinkai et al., 2006]. In that study, Shinkai et al. [2006] examined the association in 68 subjects of predominantly Caucasian origin and found –as in our study– no evidence for an association. The frequency of the 197Leu minor allele observed in our study is similar to that reported by Shinkai et al. [2006]. Since the sample size of our study is twice the sample size of Shinkai et al. [2006] study, we assume that GPX1 Pro197Leu is a clinically unimportant polymorphism.

A possible limitation of our study, next to the sample size, is that we did not correct for variation in the psychopathology of the patients. However, to the best of our knowledge, to date no association with psychopathology has been reported for any of the polymorphisms studied. Furthermore, we have allowed for correction of the type of the hospitalization, which can be considered as a surrogate for psychopathology. Therefore, differences in psychopathology are unlikely to affect our results.

Despite the limitations, our 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. Furthermore, this study is probably the first published study on the pharmacogenetics of TD and oxidative stress in Russian psychiatric patients from Siberia. Sine ethnicity is an important (genetic) determinant of the response to antipsychotics [Frackiewicz et al., 1997; Swartz et al., 1997], our study valuably extends the available findings.

In conclusion, this is the first published study on the pharmacogenetics of TD and oxidative stress enzymes in Russian psychiatric subjects from Siberia. The present study suggests that (a) the pharmacogenetics of Ile105Val polymorphism (GSTP1)

may be different in Slavonic Caucasians from that in American Caucasians, (b) 105Val-allele of Ile105Val polymorphism may be associated with a lower risk and a severity of TDof and TDlt, (c) 9Val-allele of Ala9Val (SOD2) may confer susceptibility to TDof, but not TDlt; an observation that warrants investigation in larger samples, and (d) that Pro197Leu polymorphism (GPX1) is a clinically irrelevant polymorphism for the study of TD.

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

ACKNOWLEDGMENTS

This work would not have been possible without the kind assistance of the Clinical Laboratory of the University Medical Center Groningen (UMCG) and the Laboratorium voor Volksgezondheid (LVF), Groningen and Leeuwarden, respectively, the Netherlands.

References

Akyol O, Yanik M, Elyas H, Namli M, Canatan H, Akin H, Yuce H, Yilmaz HR, Tutkun H, Sogut S, Herken H, Ozyurt H, Savas HA, and Zoroglu SS (2005). Association between Ala-9Val polymorphism of Mn-SOD gene and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 29, 123-131.

Davis JM (1976). Comparative doses and costs of antipsychotic medication. Arch Gen Psychiatry 33, 858-861.

Delucchi KL and Bostrom A (2004). Methods for analysis of skewed data distributions in psychiatric clinical studies: working with many zero values. Am J Psychiatry 161, 1159-1168.

Derenko MV, Malyarchuk BA, Wozniak M, Dambuveva IK, Dorzhu CM, Luzina FA, Lee HK, Miscicka-Sliwka D, and Zakharov IA (2006). The diversity of Y-chromosome lineages in indigenous population of South Siberia. Dokl Biol Sci 411:466-70., 466-470.

Frackiewicz EJ, Sramek JJ, Herrera JM, Kurtz NM, and Cutler NR (1997). Ethnicity and antipsychotic response. Ann Pharmacother 31, 1360-1369.

Galecki P, Pietras T, and Szemraj J (2006). [Manganese superoxide dismutase gene (MnSOD) polimorphism in schizophrenics with tardive dyskinesia from central Poland]. Psychiatr Pol 40, 937-948.

Gardos G, Cole JO, and La BR (1977). The assessment of tardive dyskinesia. Arch Gen Psychiatry 34, 1206-1212.

Glazer WM (2000). Review of incidence studies of tardive dyskinesia associated with typical antipsychotics. J Clin Psychiatry 61 Suppl 4, 15-20.

Hitzeroth A, Niehaus DJ, Koen L, Botes WC, Deleuze JF, and Warnich L (2007). Association between the MnSOD Ala-9Val polymorphism and development of schizophrenia and abnormal involuntary movements in the Xhosa population. Prog Neuropsychopharmacol Biol Psychiatry 31, 664-672.

Hori H, Ohmori O, Shinkai T, Kojima H, Okano C, Suzuki T, and Nakamura J (2000). Manganese superoxide dismutase gene polymorphism and schizophrenia: relation to tardive dyskinesia. Neuropsychopharmacology 23, 170-177.

Hu X, Xia H, Srivastava SK, Pal A, Awasthi YC, Zimniak P, and Singh SV (1998). Catalytic efficiencies of allelic variants of human glutathione S-transferase P1-1 toward carcinogenic anti-diol epoxides of benzo[c]phenanthrene and benzo[g]chrysene. Cancer Res 58, 5340-5343.

Hu YJ and Diamond AM (2003). Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. Cancer Res 63, 3347-3351.

Karafet TM, Osipova LP, Gubina MA, Posukh OL, Zegura SL, and Hammer MF (2002). High levels of Y-chromosome differentiation among native Siberian populations and the genetic signature of a boreal hunter-gatherer way of life. Hum Biol 74, 761-789.

Koning JP, Tenback DE, van OJ, Aleman A, Kahn RS, and van Harten PN (2008). Dyskinesia and Parkinsonism in Antipsychotic-Naive Patients With Schizophrenia, First-Degree Relatives and Healthy Controls: A Meta-analysis. Schizophr Bull (doi:10.1093/schbul/sbn146).

Lei XG, Cheng WH, and McClung JP (2007). Metabolic regulation and function of glutathione peroxidase-1. Annu Rev Nutr 27:41-61.

Lohr JB, Kuczenski R, and Niculescu AB (2003). Oxidative mechanisms and tardive dyskinesia. CNS Drugs 17, 47-62.

Ng F, Berk M, Dean O, and Bush AI (2008). Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol 11, 851-876.

Pae CU, Kim TS, Patkar AA, Kim JJ, Lee CU, Lee SJ, Jun TY, Lee C, and Paik IH (2007). Manganese superoxide dismutase (MnSOD: Ala-9Val) gene polymorphism

may not be associated with schizophrenia and tardive dyskinesia. Psychiatry Res 153, 77-81.

Paulsen JS, Caligiuri MP, Palmer B, McAdams LA, and Jeste DV (1996). Risk factors for orofacial and limbtruncal tardive dyskinesia in older patients: a prospective longitudinal study. Psychopharmacology (Berl) 123, 307-314.

Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, Wayland M, Freeman T, Dudbridge F, Lilley KS, Karp NA, Hester S, Tkachev D, Mimmack ML, Yolken RH, Webster MJ, Torrey EF, and Bahn S (2004). Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 9, 684-97, 643.

Rey MJ, Schulz P, Costa C, Dick P, and Tissot R (1989). Guidelines for the dosage of neuroleptics. I: Chlorpromazine equivalents of orally administered neuroleptics. Int Clin Psychopharmacol 4, 95-104.

Rijcken CA, Monster TB, Brouwers JR, and de Jong-van den Berg LT (2003). Chlorpromazine equivalents versus defined daily doses: how to compare antipsychotic drug doses? J Clin Psychopharmacol 23, 657-659.

Schulz P, Rey MJ, Dick P, and Tissot R (1989). Guidelines for the dosage of neuroleptics. II: Changing from daily oral to long acting injectable neuroleptics. Int Clin Psychopharmacol 4, 105-114.

Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, and Mizuno Y (1996). Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun 226, 561-565.

Shinkai T, Luca VD, Hwang R, Matsumoto C, Hori H, Ohmori O, Remington G, Meltzer HY, Lieberman JA, Potkin SG, Nakamura J, and Kennedy JL (2005). Association study between a functional glutathione S-transferase (GSTP1) gene polymorphism (Ile105Val) and tardive dyskinesia. Neurosci Lett 388, 116-120.

Shinkai T, Muller DJ, De L, V, Shaikh S, Matsumoto C, Hwang R, King N, Trakalo J, Potapova N, Zai G, Hori H, Ohmori O, Meltzer HY, Nakamura J, and Kennedy JL (2006). Genetic association analysis of the glutathione peroxidase (GPX1) gene polymorphism (Pro197Leu) with tardive dyskinesia. Psychiatry Res 141, 123-128.

Shorokhova DA, Stepanov BA, Udovenko I, Novoselov VP, and Pusyrev VP (2005). Genetic variability and discriminating power of four microsatellite loci in Russian population. Mol Biol (Mosk) 39, 965-970.

Sokal R and Rohlf F (1994). Biometry: The Principles and Practices of Statistics in Biological Research (3rd edition). W.H. Freeman and Co.: New York, USA.

Sundberg K, Johansson A-S, Stenberg G, Widersten M, Seidel A, Mannervik B, and Jernström B (1998). Differences in the catalytic efficiencies of allelic variants of glutathione transferase Pl-l towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19, 433-436.

Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, and Degoul F (2005). The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics 15, 311-319.

Swartz JR, Burgoyne K, Smith M, Gadasally R, Ananth J, and Ananth K (1997). Tardive dyskinesia and ethnicity: review of the literature. Ann Clin Psychiatry 9, 53-59.

Verbenko DA, Knjazev AN, Mikulich AI, Khusnutdinova EK, Bebyakova NA, and Limborska SA (2005). Variability of the 3'APOB minisatellite locus in Eastern Slavonic populations. Hum Hered 60, 10-18.

Wilffert B, Al Hadithy A, Jangbahadoer Sing V, Matroos G, Hoek H, van Os J, Bruggeman R, Brouwers J, and van Harten P (2008). The role of dopamine D_3, $5HT2_A$ and $5HT2_C$ receptor variants as pharmacogenetic determinants for tardive dyskinesia in African-Caribbean patients with chronic antipsychotic treatment. J Psychopharmacol 23(6):652-659.

Woods SW (2003). Chlorpromazine equivalent doses for the newer atypical antipsychotics. J Clin Psychiatry 64, 663-667.

Zhang Z, Zhang X, Hou G, Sha W, and Reynolds GP (2002). The increased activity of plasma manganese superoxide dismutase in tardive dyskinesia is unrelated to the Ala-9Val polymorphism. J Psychiatr Res 36, 317-324.

Zhang ZJ, Zhang XB, Hou G, Yao H, and Reynolds GP (2003). Interaction between polymorphisms of the dopamine D3 receptor and manganese superoxide dismutase genes in susceptibility to tardive dyskinesia. Psychiatr Genet 13, 187-192.

PART II

Pharmacogenetics of Parkinsonism