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Association study between a functional glutathione *S*-transferase (GSTP1) gene polymorphism (Ile105Val) and tardive dyskinesia

Takahiro Shinkai [a,b,*], Vincenzo De Luca [a], Rudi Hwang [a], Chima Matsumoto [b], Hiroko Hori [b], Osamu Ohmori [b,g], Gary Remington [c], Herbert Y. Meltzer [d], Jeffrey A. Lieberman [e], Steven G. Potkin [f], Jun Nakamura [b], James L. Kennedy [a]

[a] *Neurogenetics Section, Centre for Addiction and Mental Health, Clarke Division, Department of Psychiatry, University of Toronto, Toronto, Ont., Canada M5T 1R8*
[b] *Department of Psychiatry, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan*
[c] *Schizophrenia Program, Centre for Addiction and Mental Health, Clarke Division, Department of Psychiatry, University of Toronto, Toronto, Ont., Canada M5T 1R8*
[d] *Department of Psychiatry, Division of Psychopharmacology, Vanderbilt University School of Medicine, Nashville, TN 37212, USA*
[e] *Department of Psychiatry, University of North Carolina, Chapel Hill, NC, USA*
[f] *Department of Psychiatry, University of California, Irvine, CA, USA*
[g] *Wakato Hospital, Wakamatsu-ku, Kitakyushu 808-0132, Japan*

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Abstract

A possible role for oxidative stress in the pathophysiology of tardive dyskinesia (TD) has previously been proposed (reviewed in Andreassen and Jorgensen [O.A. Andreassen, H.A. Jorgensen, Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats Implications for tardive dyskinesia? Prog. Neurobiol. 61 (2000) 525–541]). Long-term administration of antipsychotics alters dopaminergic turnover, which results in increased formation of reactive oxygen species (ROS). This is hypothesized to lead to TD through neuronal toxicity as a consequence of oxidative stress. In the present study, the relationship between TD and a functional polymorphism of the gene coding for human glutathione *S*-transferase P1 (GSTP1), an important antioxidant enzyme involved in the detoxification of ROS, was studied in 225 chronic treatment-refractory patients with schizophrenia. An isoleucine (Ile) to valine (Val) substitution at codon 105 (Ile105Val) in the GSTP1 gene was genotyped. No significant difference in total AIMS scores was found among patients in the three genotype groups ($\chi^2 = 1.47$, d.f. = 2, $p = 0.48$). Moreover, no significant differences in genotype ($\chi^2 = 0.05$, d.f. = 2, $p = 0.98$) or allele frequencies ($\chi^2 = 0.00$, d.f. = 1, $p = 1.00$) were observed between subjects with and without TD. Our results suggest that the GSTP1 gene polymorphism does not confer increased susceptibility to TD, although further studies are warranted before a conclusion can be drawn.

Keywords: Tardive dyskinesia; Schizophrenia; Glutathione *S*-transferase; Oxidative stress; Free radical; Polymorphism; Genetics

Tardive dyskinesia (TD), an involuntary movement disorder, is a serious adverse side-effect which affects roughly 20% of patients chronically treated with typical antipsychotics (AP) [23]. Several mechanisms for the pathophysiology of TD have been hypothesized including dopamine (DA) receptor supersensitivity [37], dysfunction of the serotonergic (5-HT) system [22], and gamma-aminobutyric acid insufficiency [7]. The pathophysiology of TD, however, is still inadequately understood and is likely to be a complex phenotype [15].

Recently, positive associations between TD and several genetic polymorphisms have been reported (reviewed in [25,28]). These included polymorphisms in the dopamine D_3 receptor [3,20,36], cytochrome P450 (CYP) 2D6 [17,29], CYP1A2 [4], 5-HT_{2A} receptor [33], and 5-HT_{2C} receptor

* Corresponding author. Tel.: +81 93 691 7253; fax: +81 93 692 4894.
E-mail address: shinkai@med.uoeh-u.ac.jp (T. Shinkai).

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[34] genes. These data provide some evidence that genetic factors are involved in the development of TD.

Several lines of evidence suggest oxidative stress may play a role in the pathogenetic mechanism of TD (reviewed in [1]). Long-term administration of antipsychotics alters dopaminergic neurotransmissions by increasing DA turnover [32] and dopamine receptor density in the basal ganglia [5]. An important metabolic route for dopamine relies on monoamine oxidase and yields dopamine quinones and hydrogen peroxide (H_2O_2). These, in turn, lead to the formation of reactive oxygen species (ROS). ROS can cause neuronal damage as a consequence of oxidative stress. Several studies have shown that chronic typical antipsychotics (e.g., haloperidol or chlorpromazine) but not atypical antipsychotics (e.g., clozapine) significantly induce lipid peroxidation, a major index of oxidative stress, and decrease levels of antioxidizing enzymes in rat brains [26]. It is also worth noting that quercetin, a potent antioxidant, has been shown to reduce haloperidol-induced vacuous chewing movements in a dose dependent manner [27]. Further support for the oxidative stress hypothesis comes from two studies showing increased lipid peroxidation products in the cerebrospinal fluid of patients with TD [21,39]. In addition, several studies have shown Vitamin E, a free radical scavenger, to have a positive effect on TD symptoms (reviewed in [6]).

Based on the oxidative stress hypothesis of TD, we have previously reported a positive association between a functional single nucleotide polymorphism (SNP) (Ala-9Val) in the gene of manganese superoxide dismutase (MnSOD), an important antioxidant enzyme, and occurrence of TD in a Japanese sample of schizophrenia [11]. In that study, we found fewer allele counts of the −9Ala allele in patients with TD compared with patients without TD ($p = 0.02$; OR, 0.29; 95% CI, 0.10–0.83). This finding suggests that the −9Ala allele may play a protective role against developing TD. Although Zhang et al. [42] failed to replicate our findings, a significant positive correlation between total AIMS score and MnSOD activity was found. The finding of Zhang et al. [42], thus, implies a general role for oxidative stress-related genes in the pathogenesis of TD.

In order to further investigate the oxidative stress hypothesis, this study focuses on the gene coding for human glutathione *S*-transferase P1 (GSTP1; MIM #134660). GSTP1 binds to reduced glutathione and is involved in the detoxification of ROS, which consequently maintains cellular redox balance [30]. Alterations in GSTP1 may be involved in oxidative stress and apoptosis that affect neurodegenerative processes. In addition, GSTP1 has a pivotal role in the regulation of stress kinases, such as MAP, p38, ERK, and JNK/stress-activated protein kinase (SAPK), kinases which are involved in the intracellular signal transduction pathways that control brain function and neural plasticity against stress [8,18,41]. Furthermore, GSTP1 has been found to suppress dopamine-induced apoptosis in neurons through the modulation of MAP activity in vitro [14]. Given that TD has been postulated to arise from dopaminergic supersensitivity due to long-term antipsychotic treatment and/or the degeneration of nigrostriatal dopaminergic neurons [14,35], GSTP1 may play an important role in the development of TD.

A SNP in exon 5 of the GSTP1 gene at nucleotide 313, an adenosine to guanosine substitution, which causes an isoleucine (Ile) to valine (Val) substitution at codon 105 (Ile105Val), has been identified (rs947894) [10,12,24]. The valine allele has been shown to have a possible functional effect on GSTP1 enzyme activity by decreasing enzymatic activity and detoxification capacity [12].

These findings provide us with a solid rationale to investigate the association between the GSTP1 Ile105Val polymorphism and TD. In this study we therefore genotyped this polymorphism in our sample of schizophrenic patients chronically treated with typical APs and examined a possible association with TD.

Our sample included 225 unrelated patients (156 men and 69 women) with DSM-III-R diagnoses of schizophrenia recruited from four independent research clinics: Case Western Reserve University (H.Y. Meltzer, $n = 78$); Hillside Hospital in Long Island (J.A. Lieberman, $n = 25$); University of California in Irvine (S.G. Potkin, $n = 14$); and the Centre for Addiction and Mental Health in Toronto (G. Remington, $n = 108$). As for patients from the former three clinics, information about clinical assessment is described elsewhere [4]. Written informed consent was obtained from each patient included in this study. The average patient age was 39.2 years (S.D. = 9.7) with ages ranging from 16 to 66. The sample was 87.6% Caucasians and 12.4% African-Americans. Ethnicity was determined from the place of birth of the patient, their parents and grandparents, as well as mother tongue and religion. This information came from a standardized form filled out by the clinician for each patient. None of these patients had previously been treated with atypical antipsychotics. Patients were assessed for TD severity using the Abnormal Involuntary Movement Scale (AIMS) [9]. A diagnosis of TD according to the Schooler–Kane criteria [31] was also performed using the AIMS scores. All four clinicians (HYM, JAL, SGP, and GR) have extensive experience in assessing TD severity and exchange visits across clinical sites were arranged to ensure inter-rater consistency.

Three 10 ml EDTA tubes of blood were drawn from patients and their parents. Genomic DNA was extracted using the high salt method of Lahiri and Nurnberger [19]. Genotypes were assessed using the TaqMan allele-specific assay method (Applied Biosystems, Foster City, CA) according to manufacturer's protocols. The Ile105Val polymorphism site was amplified by polymerase chain reaction (PCR) using the following primers: 5′-CCTGGTGGACATGGTGAATGAC-3′ (forward) and 5′-CAGATGCTCACATAGTTGGTGTAGA-3′ (reverse). Two dual labeled probes centered on the SNP and differing in sequence by the 1-bp polymorphism of the SNP site itself were designed by Applied Biosystems Inc. The probes were labeled with 5′ reporter fluors VIC or 6-FAM and 3′ quencher. The probe sequences were: VIC-CTGCAAATACATCTCC