We read with interest a recent study by Pae and colleagues (Pae et al., 2004) regarding a genetic polymorphism Pro187Ser on the NAD(P)H:quinone oxidoreductase (NQO1) gene being associated with tardive dyskinesia (TD). TD is an irregular, non-rhythmic involuntary movement disorder following long-term antipsychotic treatment. Although the pathogenesis of TD is not completely understood, one of the main hypotheses has been focused on neurodegeneration and cell damage in basal ganglia due to the generation of reactive oxygen species (ROS) (Elkashef and Wyatt, 1999; Lohr et al., 2003). NQO1 is a member of the phase II detoxification enzymes and has been suggested to play an important role in the cellular defence against oxidative stress (Ross et al., 2000). Previous studies have also indicated the expression of NQO1 in the substantia nigra, an essential region for movement control in humans (Drukarch and van Muiswinkel, 2000). Because of its expression in human brain and its antioxidant function, it is plausible that the genetic variants on the NQO1 gene that encode proteins with functional differences may be candidates for TD susceptibility. In the following study, we attempted to replicate the study by Pae et al. (2004) in a larger population regarding the association between NQO1 Pro187Ser polymorphism and TD. However, our results were discrepant with regard to the original report.

A total of 282 chronic in-patients with schizophrenia were enrolled in this study. Two certificated psychiatrists made the diagnosis of schizophrenia according to DSM-IV criteria for schizophrenia based on an interview, past history, clinical observation and medical record. All patients had received persistent typical antipsychotic treatments over the past 2 years and were maintained on a stable dosage of antipsychotics for 6 months before the clinical assessment of TD. Patients with the following criteria were excluded: age >65 yr or <18 yr, organic mental disorder, history of mood disorder, neurological illness, diabetes mellitus, history of substance use (alcohol, amphetamine and opioid) and of atypical or second-generation antipsychotic treatment during the past year. Clinical variables that were also measured in this study were gender, age, smoking status (smoker or non-smoker), cumulative duration of antipsychotic exposure, and mean daily antipsychotic dosage over the past 6 months before clinical assessment. All administrated antipsychotic doses were converted to chlorpromazine (CPZ) equivalents. All participants were further stratified according to the presence of TD or not.

TD assessment was conducted with a standardized Abnormal Involuntary Movement Scale (AIMS) examination by experienced psychiatrists who were blinded to the genotypes of each patient. According to the research and diagnostic criteria (RDC) for TD (Schooler and Kane, 1982), TD was defined when moderate involuntary movements were observed over one or more areas, or mild involuntary movements over two or more areas of the body. We rated the first seven items in AIMS to determine the severity of abnormal movement over seven respective regions of the body. For confirmation of diagnosis for TD, all patients were rated twice consecutively over a 3-month interval.

The TD group consisted of 157 schizophrenic patients with ‘persistent TD’, which was defined as TD seen on two or more consecutive visits (Schooler and Kane, 1982). The non-TD group consisted of 125 schizophrenic patients who had no involuntary movements before assessment and with total zero AIMS scores twice after this 3-month interval evaluation. The recorded variables and exclusion criteria were similar for both TD and non-TD groups.

This study was approved by Yuli Veterans Hospital Institutional Review Board. All patients were ethnic Chinese. The study was completely described to the patients and informed consents were obtained. The experiments in this study were performed in the
Molecular Biological Laboratory, Department of Psychiatry, Yuli Veterans Hospital, Hualien, Taiwan, ROC.

Genomic DNA was extracted from the white blood cells in peripheral venous blood with standard methods. \textit{NQO1} Pro187Ser genotypes were determined by the method proposed by Hori et al. (2003) with some modification. After digestion with the restriction enzyme \textit{Hin}fi, the T allele was determined by the presence of 115 bp and 102 bp, while the C allele remained uncut and presented a 217-bp band. Partial digestion was excluded by complete absence of the 217-bp band in each experimental batch. Contaminated amplification was monitored by using a blank sample without genomic DNA in each batch of the experiment.

Analyses with categorical variables were computed with $\chi^2$ test or Fisher’s exact test. Continuous variables were analysed by two-tailed independent \( t \) test or Pearson’s correlation test. Data were presented as mean ± standard deviation (s.d.). Binary logistic regression and analysis of covariance (ANCOVA) were used for controlling for the independent variables with potentially confounding effects. Levene’s test for homogeneity of variance was used to test the assumption of equal variance across each genotypic class. A \( p \) value of <0.05 was regarded as statistically significant. All the analyses were computed using SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

The general characteristics and clinical variables, genotype and allele frequencies of TD and non-TD groups as well as total AIMS scores within the TD group are given in Table 1. The genotype distribution of both groups was within the Hardy–Weinberg equilibrium calculated by $\chi^2$ goodness-of-fit test. There was no statistical difference between TD and non-TD groups in gender, age and smoking status. However, TD patients had significantly longer length of antipsychotic drug exposure but lower dosage of CPZ equivalent dose than the patients in the non-TD group. Hence, in the logistic regression analysis, length of antipsychotic exposure, mean daily antipsychotic dose and the Pro187Ser genotypes were entered as the independent variables. No significant association between \textit{NQO1} Pro187Ser genotypes and TD was detected with this analysis (Wald \( =4.118 \), d.f. = 2, \( p=0.128 \)). The allelic frequencies of the TD and non-TD groups were also not statistically different with a $\chi^2$ test (\( p=0.781 \)).

Next, we examined the difference in AIMS total scores of each genotype using the ANCOVA test in TD patients. Because patient age (Pearson correlation, \( p=0.001 \)) and smoking status (Mann–Whitney \( U \) test, \( p=0.004 \)) were significantly correlated with the AIMS total scores in our TD sample, both variables were entered in the subsequent ANCOVA analysis. The mean AIMS scores of each genotype were 9.9 ± 5.2 for C/C type, 10.7 ± 4.8 for C/T type and 12.1 ± 6.4 for T/T type. After controlling for the confounding effects of smoking status and age, the AIMS total scores were not significantly different across the three genotypes of \textit{NQO1} Pro187Ser polymorphism (\( F=0.99 \), d.f. = 2).

<p>| Table 1. Demographic characteristics, genotype distribution and allelic frequencies in TD and non-TD groups |
|-------------------------------------------------|---------------------------------|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Gender (male/female)</th>
<th>TD (( n=157 ))</th>
<th>non-TD (( n=125 ))</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (s.d.)</td>
<td>47.0 (9.2)</td>
<td>46.4 (9.4)</td>
<td>0.866a</td>
</tr>
<tr>
<td>Years of APD exposure (s.d.)</td>
<td>22.3 (7.9)</td>
<td>20.0 (8.0)</td>
<td>0.016b</td>
</tr>
<tr>
<td>CPZ equivalent dose, mg (s.d.)</td>
<td>574.4 (471.8)</td>
<td>826.9 (549.2)</td>
<td>( &lt;0.001 ) b</td>
</tr>
<tr>
<td>Smoking status (smoker/non-smoker)</td>
<td>72 (23.5)</td>
<td>54 (71)</td>
<td>0.655a</td>
</tr>
<tr>
<td>Pro187Ser genotypes (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>50 (31.8)</td>
<td>30 (24.0)</td>
<td>0.128c</td>
</tr>
<tr>
<td>C/T</td>
<td>70 (44.6)</td>
<td>72 (57.6)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>37 (23.5)</td>
<td>23 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Pro187Ser alleles (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>170 (54.1)</td>
<td>132 (52.8)</td>
<td>0.781a</td>
</tr>
<tr>
<td>T</td>
<td>144 (45.9)</td>
<td>118 (47.2)</td>
<td></td>
</tr>
</tbody>
</table>

APD, Antipsychotic drug; CPZ, chloropromazine.

\( a \) \( \chi^2 \) test: gender (\( \chi^2=0.03 \), d.f. = 1); smoking status (\( \chi^2=0.08 \), d.f. = 1); alleles (\( \chi^2=0.10 \), d.f. = 1).

\( b \) Two-tailed independent test.

\( c \) Binary logistic regression analysis, adjustment for years of APDs exposure and CPZ equivalent dose.
and C/C types (comparing the T/T type with the combination of C/T). However, we excluded the patients with a history of atypical antipsychotic treatment during the past year. Compared with typical antipsychotics, atypical antipsychotic drugs have been suggested to have a lower risk for TD development (Correll et al., 2004) and have been shown to be beneficial in the treatment of TD (Bai et al., 2003). Finally, although both studies recruited from the Oriental population, the allelic frequencies of NQO1 Pro187Ser polymorphisms were different because of different descendants residing in Eastern Asia. In our study, the frequency of the Ser187 allele was similar to another report in Taiwan (47.2% vs. 47.4%) (Lin et al., 2003) but was higher than that of the report by Pae et al. (2004). It may be that NQO1 Pro187Ser genetic polymorphism is in linkage disequilibrium with other TD susceptibility loci among Korean but not Taiwanese subjects. Therefore, haplotype analyses that include the Pro187Ser and other polymorphisms over the NQO1 genetic region would be more informative. However, several limitations deserve further consideration regarding our findings. One is the possibility of a false-negative in the present study. Based on the reported odds ratio of 1.84 (Pae et al., 2004), our sample size had the power (0.57) to exclude T/T type as a risk genotype. Accordingly, the likelihood that we had a type II error with our sample size could not be excluded. Another limitation is the lack of a second independent group for further confirmation of our results. In addition, the average antipsychotic dose in the non-TD group was significantly higher than that in the TD group. Although we recruited patients maintained on a stable antipsychotic dose for 6 months prior to the clinical assessment of TD, this could mask dyskinetic movements and be a possible bias. Therefore, we suggest further studies with similar study design in a larger population will be necessary to draw any conclusions about this issue.

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Statement of Interest

None.

References


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