Objective: To establish if there is an association between cigarette smoking and tardive dyskinesia (TD) in patients with schizophrenia and to evaluate the role of the CYP1A2 polymorphism in TD in patients of Chinese descent.

Method: Two-hundred and ninety-one patients diagnosed with schizophrenia according to DSM-IV criteria were included in the study. Dyskinesia was assessed by the Abnormal Involuntary Movement Scale and TD by the criteria of Schooler and Kane. Demographic and clinical data and information on smoking habits were collected, and patients of Chinese descent with a well established smoking history were subsequently genotyped for CYP1A2.

Results: Forty-three (41.3%) of the 104 patients with a history of smoking and 52 (27.8%) of the 187 non-smokers were diagnosed with TD. The prevalence of TD was significantly higher among smokers than non-smokers ($\chi^2 = 5.57, p = 0.018$). Logistic regression using TD as the dependent variable revealed smokers to be at a significantly higher risk for TD ($p < 0.005$). Genotyping of smokers of Chinese descent for CYP1A2 polymorphism revealed no significant differences in the genotypic or allelic distribution between those with and without TD.

Conclusions: Consistent with other studies, the prevalence of TD was significantly higher among smokers than non-smokers; however, we did not find an association between the C→A genetic polymorphism of CYP1A2 and TD.

Smoking and tardive dyskinesia: lack of involvement of the CYP1A2 gene

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Medical subject headings: China; cytochrome P-450 CYP1A2; dyskinesias; genetic predisposition to disease; movement disorders; schizophrenia; smoking.

Introduction

The prevalence of smoking in patients with schizophrenia has been found to be higher than that of the general population.\textsuperscript{1–3} It has been suggested that smoking may lead to a number of consequences, including more severe psychotic symptoms,\textsuperscript{4} higher dose of antipsychotic medication being prescribed\textsuperscript{5–7} and increased risk of tardive dyskinesia (TD).\textsuperscript{8–10}

The pathophysiology of TD — often viewed as a severe and stigmatizing movement disorder — remains to be fully elucidated. Postulated risk factors include age, duration of antipsychotic medication, female sex and organic brain damage.\textsuperscript{11} Genetic factors have also been implicated in TD, as suggested by the interindividual variation in vulnerability.\textsuperscript{12} A particular line of investigation has focused on a genetic contribution to the pharmacodynamic and pharmacokinetic aspects of antipsychotic medications. These include genetic association studies of the dopamine (D\textsubscript{2} and D\textsubscript{3}) receptors\textsuperscript{13,14} and cytochrome P450 2D6 (CYP2D6).\textsuperscript{15–17}

Recently, Basile et al\textsuperscript{18} found an association between a C\textrightarrow{}A genetic polymorphism of the CYP1A2 gene and TD, with the C/C genotype being associated with more severe TD than the A/C or A/A genotypes, and this effect was more pronounced in patients who smoked. The authors suggest that CYP1A2, which is present at higher concentrations in the liver than CYP2D6, would assume greater importance in the metabolism of antipsychotics after the saturation of CYP2D6 with long-term antipsychotic treatment. Pharmacokinetic studies that indicate variability in CYP1A2 activity occurs only in smokers\textsuperscript{19} suggest that this polymorphism would assume functional importance only in smokers. This being the case, it would provide a possible explanation for the association between smoking and TD. Basile et al\textsuperscript{14} proposed another mechanism wherein neurotoxic antipsychotic metabolites produced by alternative metabolic pathways, due to the lowered CYP1A2 activity in patients with the C/C genotypes, may contribute to TD.

The aims of this study were to establish the association of cigarette smoking with TD in patients with schizophrenia and to examine the role of the CYP1A2 polymorphism in TD in patients of Chinese descent.

Method

Patients were recruited from Woodbridge Hospital, which is the only state psychiatric hospital in Singapore and the principal treatment centre for those with severe mental illnesses such as schizophrenia. Most patients with schizophrenia are likely to have received their entire treatment at this hospital, which maintains records from first contact. It is therefore possible to obtain a reasonably detailed lifetime history of patients’ drug treatment, including the daily dose and the cumulative duration of treatment.

Patients in the long-stay wards of Woodbridge Hospital who were willing to give informed consent were recruited into the study. We excluded those with neurological or medical conditions and patients taking medication that could cause dyskinesia (i.e., criterion E of DSM-IV research criteria for neuroleptic-induced TD). Ethnicity of the patients was established by asking patients to state their own ethnicity and their country of birth, as well as that of their parents. We converted dosages of neuroleptic drugs to chlorpromazine equivalents (CPZ eq) using standard guidelines.\textsuperscript{20,21} The only anticholinergic agent used was trihexyphenidyl. All patients were receiving typical neuroleptics, and none had received any atypical agent in the past. The hospital ethics committee approved the study.

Clinical assessment

DSM-IV diagnoses were made by a psychiatrist who reviewed medical record data and, where necessary, interviewed the patient. Dyskinesia was assessed by the Abnormal Involuntary Movement Scale (AIMS),\textsuperscript{22} and extrapyramidal side effects were assessed by the Simpson–Angus Rating Scale (SARS).\textsuperscript{23} Such ratings were undertaken by 3 psychiatrists who were blind to the clinical and medical histories of the patients and...
who had jointly assessed a number of patients over 3 sessions before the start of the study. We established inter-rater reliability (intraclass correlation) coefficients of 0.86 for the AIMS and 0.82 for the SARS. All patients received 2 ratings, with an interval of at least 3 months between the ratings. Medications were not changed during the periods of assessment. The criteria used in the diagnosis of TD were those of Schooler and Kane.\textsuperscript{24} Patients were diagnosed to have TD only when 2 assessments fulfilled the criteria of presence of “moderate” abnormal movements in 1 or more body areas or at least “mild” movements in 2 or more body areas. Positive extrapyramidal side effect status was defined by a total SARS score.

The smoking habits of the patients were established by interviews and by obtaining corroborative history from the nursing staff; cases where conflicting histories were obtained were not included in the study.

In the second part of the study, which involved genotyping for the CYP1A2 gene polymorphism, only patients of Chinese descent who were smokers were invited to participate. Patients again gave written consent for this part of the study.

Genotyping

Venous blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) tubes, and genomic DNA was extracted with QIAamp Blood Kit (Qiagen GmbH, Hilden, Germany). The region of interest was first amplified by polymerase chain reaction (PCR), as previously described.\textsuperscript{18} PCR products were incubated with the restriction enzyme \textit{Bsp1201} according to the manufacturer’s instructions (New England Biolabs, Beverly, Mass.). Resulting products were detected by ethidium bromide staining after electrophoresis on a 2% agarose gel.

Statistical analysis

The Mann–Whitney \textit{U} test was used to examine the differences between patients with and without TD (as the variables were not normally distributed). Differences in demographic characteristics, neuroleptic dose, AIMS score and SARS for the various genotypes were determined using the Kruskal–Wallis test. Probability values of 0.05 or less were regarded as statistically significant. Tests for differences of allele frequencies, genotypes and other categorical analysis were performed using the chi-square test. To control for the influence of other variables, logistic regression was applied to the factors associated with TD.

Results

The main demographic and clinical variables of the 291 patients (220 men and 71 women) who were diagnosed with schizophrenia and agreed to take part in the study are listed in Table 1. Patient age ranged from 23 to 83 (mean 52.7, standard deviation [SD] 10.3) years. Of these, 288 (99.0%) were of Chinese origin, and 3 (1.0%) were Malays. There was no sex difference ($\chi^2 = 0.85$, \textit{p} = 0.35) between those with TD and those without TD. Those with TD were significantly older (\textit{p} < 0.005, Mann–Whitney \textit{U} test) and receiving a lower daily dose of antipsychotics than

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients, (n = 291)</th>
<th>Smokers, (n = 104)</th>
<th>Non-smokers, (n = 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With TD, (n = 95)</td>
<td>Without TD, (n = 196)</td>
<td>With TD, (n = 43)</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>75:20</td>
<td>145:51</td>
<td>38:5</td>
</tr>
<tr>
<td>Mean age (and SD), yr\textsuperscript{a}</td>
<td>57.7 (9.5)</td>
<td>50.3 (9.8)</td>
<td>55.3 (10.6)</td>
</tr>
<tr>
<td>Mean cumulative exposure to antipsychotics (and SD), yr</td>
<td>17.9 (10.2)</td>
<td>16.0 (9.6)</td>
<td>17.7 (10.8)</td>
</tr>
<tr>
<td>Mean daily dose of neuroleptic (and SD), CPZ mg eq\textsuperscript{d}</td>
<td>279.1 (299.1)</td>
<td>752.2 (650.1)</td>
<td>322.6 (344.4)</td>
</tr>
<tr>
<td>Mean daily dose of diazepam (and SD), mg</td>
<td>1.73 (3.75)</td>
<td>1.94 (3.87)</td>
<td>2.0 (3.8)</td>
</tr>
<tr>
<td>Mean Simpson–Angus Rating Scale score (and SD)</td>
<td>6.22 (5.99)</td>
<td>5.99 (5.95)</td>
<td>5.9 (6.0)</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; TD = tardive dyskinesia; CPZ mg eq = chlorpromazine milligram equivalent.

\textsuperscript{a}p < 0.005, Mann–Whitney \textit{U} test (for all patients with TD v. without; smokers with TD v. without; non-smokers with TD v. without)
those without TD ($p < 0.005$, Mann–Whitney $U$ test). The prevalence of TD was significantly higher in smokers than non-smokers ($41.3\%$ v. $27.8\%$, $\chi^2 = 5.5$, $p = 0.018$).

Among the 104 (35.7%) patients who smoked, there were also significant differences in age and daily antipsychotic dose between those with TD and those without (Table 1).

The distribution of the alleles and genotypes for the patients who were smokers is presented in Table 2. Testing for Hardy–Weinberg equilibrium showed that the genotype frequencies did not deviate significantly from the frequencies expected under random mating conditions for those with TD ($\chi^2 = 0.19$, $p = 0.67$) and those without TD ($\chi^2 = 0.33$, $p = 0.56$). Genotype frequencies did not differ significantly ($\chi^2 = 0.90$, $p = 0.63$), and allele frequencies did not differ significantly ($\chi^2 = 0.38$, $p = 0.56$). The median AIMS score was not significantly different between the 3 genotypes ($\chi^2 = 1.8$, $p = 0.40$), and the genotypes did not differ significantly in other demographic or clinical characteristics.

On performing a logistic regression, with TD as the dependent variable and age, neuroleptic dose, cumulative exposure to neuroleptics, smoking status and genotype as the independent variables, age ($p < 0.05$) and smoking status ($p < 0.005$) remained independently associated with a higher risk of TD, whereas lower daily neuroleptic dose was associated with a significantly lower risk of TD ($p < 0.005$). The odds ratio of developing TD for smokers relative to non-smokers was 2.7 (95% confidence interval, 1.5–5.0).

**Discussion**

Consistent with other studies, we found a significantly higher prevalence of TD in smokers than non-smokers.

**Table 2: Genotypic and allelic distribution with relation to tardive dyskinesia (TD) status and AIMS score in 103 patients of Chinese descent who smoked**

<table>
<thead>
<tr>
<th>Test result</th>
<th>Genotypic distribution, no. of patients</th>
<th>Allelic distribution, no. of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/A</td>
<td>A/C</td>
</tr>
<tr>
<td>TD-positive, $n=43$</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>TD-negative, $n=60$</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>Median AIMS score</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mean AIMS score (and SD)</td>
<td>(2.7) (2.3)</td>
<td>(2.7) (2.7)</td>
</tr>
</tbody>
</table>

Note: AIMS = Abnormal Involuntary Movement Scale; SD = standard deviation.
*Chi-square test.
†Kruskal–Wallis test.
‡Analysis of variance.

Although there are also negative reports in the literature, the smokers in many of those studies were taking significantly higher doses of antipsychotic medication, and this could have masked the presence of dyskinetic movements.  

Suggested mechanisms for the association of smoking and TD include increased dopaminergic activity from nicotine, leading to nigrostriatal hypersensitivity to dopamine, and neurotoxicity from the free radicals in cigarette smoke, causing damage to catecholaminergic neurons in the basal ganglia. Smoking also increases the risk of cerebrovascular pathology, which may lead to increased risk of developing TD.

Consistent with the findings of Schulze et al and Shimoda et al, we did not find any difference in the genotypic or allelic distribution between patients with and without TD, and there was no significant difference observed in the mean AIMS scores of the different genotypes. However, there were differences in antipsychotic dose and duration of exposure in patients with and without TD, which could have confounded our results. Smoking-induced CYP1A2 activity may result in lower antipsychotic plasma levels, which may in turn lead to inadequate control of psychotic symptoms and higher dosages of antipsychotics being prescribed; thus, these patients may eventually be exposed to higher plasma antipsychotic levels. In our study, the lower daily antipsychotic dose of those with TD may have been due to a deliberate dose reduction after the diagnosis of TD was made. Conversely, the significantly higher mean antipsychotic dose taken by patients who did not have TD could have masked dyskinetic movements, although this is unlikely given that there were no significant differences in the mean SARS scores between the 2 groups.

A limitation of this study is the lack of a structured interview to establish our diagnosis of schizophrenia. Another limitation is that we did not quantify the amount of smoking, which may have confounded our results, because a positive correlation between CYP1A2 activity and amount smoked has been reported. Our patients also received a variety of typical antipsychotics, with some being prescribed more than 1 type. The diversity of antipsychotics is an important consideration given that the role of CYP1A2 in the disposition of antipsychotics is limited to a few (i.e., clozapine, haloperidol and olanzapine).

Although we did not find an association between the C→A genetic polymorphism of CYP1A2 and TD, we...
did not investigate the role of the G→A polymorphism, which has been associated with decreased activity of CYP1A2 in Japanese subjects. Such studies should, however, be limited to patients who are taking antipsychotics that are substrates for CYP1A2.

Competing interests: None declared.

References


