SHORT REPORT

Genetic polymorphism of dopamine D2 receptors in Parkinson’s disease and interactions with cigarette smoking and MAO-B intron 13 polymorphism

Paola Costa-Mallen, Lucia G Costa, Terri Smith-Weller, Gary M Franklin, Phillip D Swanson, Harvey Checkoway

Abstract
Genetic polymorphisms of dopamine D2 receptors (DRD2) may be susceptibility factors for Parkinson’s disease due to their influence on dopamine response and association with cigarette smoking, which is inversely related to risk of Parkinson’s disease. Relations of TaqIA and TaqIB DRD2 genotypes with Parkinson’s disease were investigated and tested for interactive effects with smoking and the monoamine oxidase B (MAO-B) intron 13 polymorphism previously found to be related to smoking. Study subjects were 152 cases of idiopathic Parkinson’s disease and 231 controls. The smoking history of all genotyped subjects was known. Subjects of genotype B12 were more frequent among cases than controls (27% and 23.8%, respectively), and were more frequent among “ever smokers” than “never smokers”, among controls (27.8% and 17.2%, respectively), although these associations were not statistically significant. Neither TaqIA or TaqIB genotypes modified the inverse relation of smoking and Parkinson’s disease. When genotypes for DRD2 were considered in combination with genotypes for intron 13 of MAO-B, genotype combinations with high risk of Parkinson’s disease were found; although the MAO-B/DRD2 interaction did not reach statistical significance after Bonferroni correction for multiple comparisons, these results are suggestive of a possible synergism between MAOB and DRD2 genes with respect to Parkinson’s disease.

The A1 allele of the TaqI A polymorphism of the D2 dopamine receptor (DRD2) gene has been shown to be associated with low DRD2 density in human brain both from in vitro and in vivo studies, compared with the A2 allele. Additionally, the A1 allele of the TaqI A polymorphism has been reported to be associated with various addictive behaviours, including tobacco smoking. The TaqIB polymorphism of DRD2 has not been studied as widely as TaqIA, although recently Spitz et al found that the TaqIB polymorphism was a better marker for smoking behaviour than TaqIA. The well known inverse relation between cigarette smoking and Parkinson’s disease provides rationale for studying DRD2 polymorphisms in relation to the disease. Previous studies of polymorphisms of the D2 receptor and Parkinson’s disease have yielded contradictory results.

We have recently found an association of the allele G of the G/A polymorphism in intron 13 of MAO-B with Parkinson’s disease, and an interaction of this polymorphism with smoking with respect to Parkinson’s disease.

In this study we tested for associations of TaqIA and TaqIB DRD2 polymorphisms with Parkinson’s disease, associations of these polymorphisms with smoking, interactions with cigarette smoking, and interaction with MAO-B intron 13 polymorphism and risk of Parkinson’s disease.

Materials and methods
One hundred and fifty two newly diagnosed patients with idiopathic Parkinson’s disease (92 men and 60 women, aged 37 to 88 years), were identified by neurology and general medical practice clinics of Group Health Cooperative (GHC) from the Puget Sound area in western Washington State. Inclusion criteria for the cases were the presence of at least two of the four cardinal signs of Parkinson’s disease: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria were the use of certain medications during the 12 months preceding symptom onset, history of multiple cerebrovascular events, or
new explanation for parkinsonism symptoms (for example, brain injury, brain tumour, encephalitis). Two hundred and thirty one control subjects (149 men and 82 women, aged 45 to 84 years), were identified from GHC enrollees without histories of Parkinson’s disease or other neurodegenerative disorders. Controls were matched as a group to the cases by birth decade, sex, and year of enrollment in GHC. All subjects were of non-Hispanic Caucasian ethnicity. Subjects were considered as “ever smokers” if they had smoked at least 100 cigarettes in their lifetime.

Study subjects were volunteers who were informed of the purpose of the study. Study forms and procedures were approved by the institutional review board committees on human subjects research at the University of Washington and GHC Center for Health Studies.

TaqIA and TaqIB genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described by Grandy et al and Spitz et al respectively.

A total of 383 subjects (152 cases and 231 controls) were genotyped for Taq I B polymorphism, whereas 327 subjects (125 cases and 202 controls) were genotyped for Taq I A. Study subjects genotyped for DRD2 were the same subjects of our previous study on DRD2 polymorphisms, we used the Bonferroni correction and divided the α=0.05 level of significance by 8; every p value>0.00625 was considered as non-significant.

Results

The distribution of genotypes followed the expected Hardy-Weinberg equilibrium. The frequency for the A1 allele of the Taq I A polymorphism was 0.180 in cases and 0.186 in controls; for Taq I B polymorphism, the frequency of allele B1 was 0.140 in cases and 0.136 in controls. As shown in the table, no significant differences in Parkinson’s disease risk were present for the genotypes A11, A12, or A22 of the Taq I A polymorphism, nor for the B11, B12, or B22 genotypes of the TaqIB polymorphism. The associations with Parkinson’s disease were also tested in a logistic regression model and were non-significant. For the association of TaqIA with smoking, the odds ratios for ever compared with never smoked were: 1.00, 0.94 (95%CI 0.43–2.03), and 1.1 (95%CI 0.15–8.17) for genotypes A22, A12, and A11, respectively in cases. In controls the odds ratios for genotypes A22, A12, and A11 were: 1.00, 1.1 (0.58–2.08), and 0.61 (0.15–2.53). For TaqIB polymorphism the odds ratios for ever versus never smoked were, in cases: 1.00 and 0.93 (95%CI 0.45–1.89) for B22 and B12 genotypes, respectively (OR could not be calculated for B11 as only one subject was present in cases with this genotype). For controls the OR was: 1.00, 1.79 (0.92–3.48), and 0.23 (0.02–2.19), for B22, B12, and B11, respectively. The association with smoking was not significant from a likelihood ratio test in a logistic regression model.

We confirmed the inverse association between smoking and Parkinson’s disease; 53% of the cases never smoked compared with 38% of the controls, with a relative risk of 0.55 for Parkinson’s disease for subjects who ever smoked compared with subjects who never smoked. The presence of a possible interaction between smoking and Taq I A or Taq I B genotype on risk for Parkinson’s disease was tested.

<table>
<thead>
<tr>
<th>Genotype combination</th>
<th>MAO-B/DRD2 TAQIA:</th>
<th>MAO-B/DRD2 TAQIB:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+AA/A11+A12</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>G+GG+GA/A11+A12</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>A+AA/A22</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>G+GG+GA/A22</td>
<td>48</td>
<td>48</td>
</tr>
</tbody>
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<tr>
<td>G+GG+GA/B11+B12</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>A+AA/B22</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>G+GG+GA/B22</td>
<td>57</td>
<td>57</td>
</tr>
</tbody>
</table>

Table 1 DRD2 genotype distribution in Parkinson’s disease cases and controls and distribution of MAO-B intron 13 and DRD2 genotype combinations in Parkinson’s disease cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A22</td>
<td>84</td>
<td>67.2</td>
<td>135</td>
<td>66.8</td>
</tr>
<tr>
<td>A12</td>
<td>37</td>
<td>29.6</td>
<td>59</td>
<td>29.2</td>
</tr>
<tr>
<td>A11</td>
<td>4</td>
<td>3.2</td>
<td>8</td>
<td>4.0</td>
</tr>
</tbody>
</table>

For both TaqIA and TaqIB genotypes the odds ratios for ever versus never smoked were: 1.00, 1.42 (0.72–2.79) for A12, and 1.1 (95%CI 0.15–8.17) for A11, respectively in cases. In controls the odds ratios for genotypes A22, A12, and A11 were: 1.00, 1.1 (0.58–2.08), and 0.61 (0.15–2.53). For TaqIB polymorphism the odds ratios for ever versus never smoked were, in cases: 1.00 and 0.93 (95%CI 0.45–1.89) for B22 and B12 genotypes, respectively (OR could not be calculated for B11 as only one subject was present in cases with this genotype). For controls the OR was: 1.00, 1.79 (0.92–3.48), and 0.23 (0.02–2.19), for B22, B12, and B11, respectively. The association with smoking was not significant from a likelihood ratio test in a logistic regression model.
in a logistic regression model by the likelihood ratio test, and was non-significant (p=0.55).

As shown in the table, particular combinations of genotypes for MAO-B and DRD2 resulted in increased risk for Parkinson’s disease, in particular where the allele G for MAO-B cooccurred with the allele A1 or B1 of DRD2. Interactions between MAO-B and TaqIA or TaqIB genotypes of DRD2 were tested in logistic regression models, resulting in $\chi^2=3.63$, p=0.058 for TaqIA and $\chi^2=4.38$, p=0.038 for TaqIB. These values were not significant after Bonferroni corrections.

**Discussion**

The allelic frequencies we found in this study for Taq I A and Taq I B polymorphisms of DRD2 for both cases and controls were consistent with those previously given in the literature, as the frequency of allele A1 and B1 alleles had been previously reported as 0.20 and 0.16, respectively, among Caucasians.\(^6\) The TaqIA and TaqIB polymorphisms did not seem to be associated with Parkinson’s disease in this study. Contrary to some previous reports,\(^5 \)\(^7\) but in agreement with others,\(^8\) we did not find associations of TaqIA or TaqIB polymorphisms of DRD2 with smoking in either cases of Parkinson’s disease or controls. Also, we did not find evidence that either the TaqIA or the TaqIB polymorphism modified the inverse relation between smoking and Parkinson’s disease. The results from MAO-B and DRD2 genotype combinations, although not significant after correcting for multiple testing, are suggestive of a possible synergism on Parkinson’s disease risk between MAOB and DRD2 genes, which are both involved in the dopamine pathway. The effect of the MAO-B polymorphism in functional terms is not known. Studies of larger populations and functional studies on the effects of these genetic variants will help clarify the interpretation of these results.

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