THE A1 ALLELE OF THE D2 Dopamine Receptor Gene IS ASSOCIATED WITH HIGH Dopamine Transporter Density in Detoxified Alcoholics

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Abstract — The A1 allele of TaqI A restriction fragment length polymorphism (RFLP) in the D2 receptor (DRD2) gene locus has been suggested to be associated with low D2 receptor density in man. Striatal dopamine transporter (DAT) densities were studied with [123I]-2-β-carbomethoxy-3β-(4-iodophenyl)tropane and single-photon emission tomography in 29 detoxified alcoholics, who were also genotyped for the two alleles of TaqI A RFLP at the DRD2 receptor gene locus. Alcoholics with the A1/A2 genotypes (n = 10) had statistically significantly higher DAT densities than subjects with the A2/A2 genotypes (n = 19; 8.0 ± 1.2 (mean ± SD) vs 6.9 ± 1.1, P = 0.035). We suggest that the TaqI A RFLP is in linkage disequilibrium with a gene variant modifying DAT density in alcoholics.

INTRODUCTION

The mesocorticolimbic dopaminergic pathway in the human brain is one of the most important systems mediating reward of various substances (Koob, 1992). Genes determining the organization of this system are of current interest. Most of the evidence is available for the role of the A1 allele of the D2 dopamine receptor gene as described by Grandy et al. (1993). Polymerase chain reaction (PCR) was carried out on a total volume of 10 μl containing 1 × reaction buffer supplied with Pfu polymerase (Stratagene, La Jolla, CA, USA), 100 ng of genomic DNA, 40 pmol of each primer, 200 μM of each deoxynucleotide and 0.15 U of Pfu polymerase.

MATERIALS AND METHODS

A total of 29 Caucasian alcoholics (24 males, five females), aged 24–70 years (mean ± SD: 42 ± 10.8) were recruited from an alcoholic inpatient detoxification clinic. For 2 weeks prior to the detoxification process, they had each daily consumed an average (± SD) of 249 g ± 95 g; or 3.4 ± 1.3 g/kg of alcohol.

DNA isolation and TaqI A RFLP

For DNA analyses, blood samples were collected from each subject and frozen at –70°C in glass tubes. DNA was extracted from 10 ml samples of peripheral blood according to standard procedures (Vandenplas et al., 1984). Subjects were genotyped for TaqI A restriction fragment length polymorphism (RFLP) located in the 3’ flanking region of the dopamine D2 receptor gene as described by Grandy et al. (1993). Polymerase chain reaction (PCR) was carried out on a total volume of 10 μl containing 1 × reaction buffer supplied with Pfu polymerase (Stratagene, La Jolla, CA, USA), 100 ng of genomic DNA, 40 pmol of each primer, 200 μM of each deoxynucleotide and 0.15 U of Pfu polymerase.

Montgomery–Åsberg depression rating scale (MADRS; Montgomery and Åsberg, 1979) scores and the first single-photon emission tomography (SPET) scans were performed 1–4 days after cessation. SPET scans were carried out after 4 weeks of sobriety. Weekly meetings and tests of liver function, as well as laboratory markers of alcohol misuse (mean corpuscular volume (MCV), carbohydrate-deficient transferrin (CDT) and gamma-glutamyltransferase (GGT)), were used to monitor sobriety: all of these parameters indicated true abstinence during the follow-up. All subjects fulfilled the DSM III-R criteria (American Psychiatric Association, 1987) for alcoholism, having been clinically examined by a psychiatrist (T.P.J.L.). The use of antidepressant or neuroleptic medications or illegal drugs, monitored by urine tests, and the presence of major CNS diseases or head trauma, evaluated by magnetic resonance imaging, as well as psychotic disorders, were exclusion criteria. (For more detailed information of the study subjects, see Laine et al., 1999a). Written informed consent from all participants was obtained after the procedure had been fully explained. The Ethics Committee of the Oulu University Hospital approved the study protocol.

DAT density was determined by SPET using radioligand β-CIT when 4 weeks of controlled sobriety had elapsed. SPET procedure (Laine et al., 1999a) and some DAT binding data have previously been presented (Laine et al., 1999a,b).

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PCR conditions were: denaturation at 94°C for 3 min followed by 35 cycles at 94°C for 45 s, 64°C for 45 s, 72°C for 45 s, and a final extension of 5 min at 72°C. The digested fragments, separated on a 3% agarose gel containing 0.5 µg/ml ethidium bromide, were then visualized and photographed. The A1 allele remained intact, whereas the A2 allele was cut into one 180 bp and one 130 bp piece.

Statistical analysis

The variables used in the statistical analysis were age, duration of the last drinking bout (days), daily amount of alcohol consumed during the last drinking bout (g/day), number of days of abstinence preceding the first SPET imaging, withdrawal symptom score of the Selected Severity Assessment scale (SSA) (Gross et al., 1973), MADRS during withdrawal and after 4 weeks of abstinence (Montgomery and Åberg, 1979), and the total amount of benzodiazepines (as diazepam equivalents) administered to the patient for detoxification.

Means and SD were used in descriptions of the continuous variables. Student’s t-tests were used in two-tailed independent samples. To reconcile effect of age with DAT density (Volkow et al., 1996a), and to measure differences between the hemispheres, we used a ‘repeated measures analysis of variance’. Statistical analyses were completed using the Statistical Package for the Social Sciences (SPSS), version 6.1, for Microsoft Windows.

RESULTS

Statistical analyses revealed higher DAT densities after 4 weeks of sobriety in subjects heterozygous with regard to the A1/A2 allele than in homozygotes with A2/A2 alleles (Fig. 1, Table 1). When DAT densities of left and right hemispheres with age as a covariant were used, subjects with the A1 allele still had higher DAT densities ($P = 0.026$). Age alone also had a significant effect on DAT density ($P = 0.002$), but left and right hemispheres did not differ from each other ($P = 0.834$).

Subjects with the A1/A2 genotype had significantly more depressive symptoms under withdrawal than A2/A2 patients. After 4 weeks of sobriety, the MADRS scores were similar in both groups. Two patients with the A1/A2 and two with the A2/A2 alleles were still clinically depressed (MADRS score $>17$; Mittmann et al., 1997). Later MADRS scores were not available for two patients.

DISCUSSION

Our main finding was that alcoholics, independent of age, with the dopamine DRD2 A1/A2 genotype, have statistically significantly higher striatal DAT densities after 4 weeks of sobriety, than alcoholics with the A2/A2 genotype. Taq I A RFLP does not represent functional gene variation per se. However, endophenotypic studies link this gene variation to low $D_2$ receptor density in vivo (Pohjalainen et al., 1998; Jönsson et al., 1999). In our earlier article involving the same database (Laine et al., 1999a), we reported that DAT availability in alcoholics increased after alcohol withdrawal. The degree of recovery during the first 4 days reached levels similar to those found in healthy controls. We therefore believe that 4 weeks into the sobriety period striatal DAT availability is free of any lingering effects of the earlier alcohol consumption (Laine et al., 1999a).

Table 1. Differences between alcoholics with A1/A2 and A2/A2 genotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A1/A2</th>
<th>A2/A2</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>10</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Antisocial personality disorder ($n$)</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Females ($n$)</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>DAT in withdrawal</td>
<td>7.3 ± 1.7</td>
<td>6.4 ± 1.0</td>
<td>0.088</td>
</tr>
<tr>
<td>DAT density after 4 weeks of sobriety</td>
<td>8.0 ± 1.2</td>
<td>6.9 ± 1.1</td>
<td>0.036</td>
</tr>
<tr>
<td>Alcohol dose before cessation (g/day)</td>
<td>245 ± 104</td>
<td>253 ± 94</td>
<td>0.085</td>
</tr>
<tr>
<td>Benzodiazepine equivalents needed for detoxification</td>
<td>110 ± 83</td>
<td>94 ± 80</td>
<td>0.63</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.5 ± 11.2</td>
<td>44.4 ± 10.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Length of drinking bout (mean; days)</td>
<td>40 (median 21)</td>
<td>640 (median 120)</td>
<td>0.15</td>
</tr>
<tr>
<td>MADRS scores under withdrawal</td>
<td>28.5 ± 11.2</td>
<td>17.8 ± 12.3</td>
<td>0.012</td>
</tr>
<tr>
<td>MADRS scores after 4 weeks of sobriety</td>
<td>8.9 ± 13.4</td>
<td>7.9 ± 12.3</td>
<td>0.85</td>
</tr>
<tr>
<td>Withdrawal symptom scores (SSA)</td>
<td>12.9 ± 5.8</td>
<td>9.6 ± 5.4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise stated.
DAT, dopamine transporter; MADRS, Montgomery–Åberg depression rating scale; SSA, Selected Severity Assessment scale.
The mechanism linking the A1 allele to increased DAT levels is unclear. In principle, low post-synaptic D₂-receptor density among patients with the A1 allele may result in low net dopamine neurotransmission. DAT density is relatively stable, possibly reflecting the condition of the dopaminergic tract (Kuikka et al., 1995; Moody et al., 1996; Scheffel et al., 1996). In diseases like Parkinson’s, a decrease of DAT density is indicative of cell losses (Menza et al., 1995). In our earlier studies, we found increases in striatal DAT densities following remissions of alcohol withdrawal (Laine et al., 1999a). Cocaine has previously been found to up-regulate DAT density by blocking dopamine transporters (Little et al., 1993; Malison et al., 1998). In a study involving rats, decreased dopamine flow did not decrease DAT density (Moody et al., 1996; Scheffel et al., 1996), and dopaminergic medication, used in Parkinson’s disease, was not found to affect β-CIT SPECT imaging (Ahlskog et al., 1999; Innis et al., 1999).

According to our observations, aromatization with the A1/A2 allele are more depressed during acute withdrawal. Two of the patients were clinically depressed after 4 weeks of sobriety, suffering from either primary depression or long-lasting secondary depression. Alcoholics with higher recoveries in DAT densities during alcohol withdrawal had more severe depressive symptoms possibly because of fragility of their mood system (Laine et al., 1999b). This is also in line with the reported increase of DAT levels in depressed patients (Laasonen-Balk et al., 1999).

We conclude that the DRD2 A1/A2 genotype can be associated with relatively higher β-CIT binding to DAT in dopaminergic nerve terminals of alcohol-dependent patients than the A2/A2 genotype. One limitation of this study is the small sample size, which is unfortunate, but typical in studies of functional neuroimaging. The carriers of the A1 allele also displayed increased depressive symptoms during alcohol withdrawal, suggesting that this DRD2 gene variant (or a functional variation in linkage disequilibrium with the allele) can be associated with the symptom phenotype in alcohol withdrawal.

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REFERENCES


