Short Communication

Association between the Dopamine D2 Receptor A2/A2 Genotype and Smoking Behavior in the Japanese

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Abstract

For the study presented here, we investigated possible links between the dopamine D2 receptor (DRD2) TaqIA genotype (DRD2*A) and smoking behavior in a total of 332 Japanese individuals. For the first time, functional insertion/deletion polymorphism (−141C Ins/Del) in the DRD2 promoter was also examined in relation to smoking behavior. The distribution of the DRD2*A genotype was significantly different among current, former, and never-smokers (P = 0.001; χ² test), and smoking appeared to be associated with the DRD2 A2/A2 genotype, showing marked contrast to previous reports for non-Hispanic whites in the United States. Multivariate logistic regression analysis incorporating age, sex, genotype, and smoking status as variables revealed that DRD2 A2/A2 genotype was significantly associated with an increased risk of predisposition to smoking behavior in the Japanese (odds ratio, 3.680; 95% confidence interval, 1.499–9.052). In contrast, such an increased risk was not observed in terms of association with the −141C Ins/Del polymorphism. These findings suggest an association of the DRD2*A genotype with an increased risk of being predisposed to smoking behavior in the Japanese and suggest the possible existence of ethnic group-specific differences, which warrant additional studies on the underlying molecular mechanism.

Introduction

It is well known that the consumption of nicotine and of other drugs subject to abuse induces pleasurable feelings in users. Dopamine, a major neurotransmitter of the central nervous system, is thought to be involved in the mesolimbic reward pathway (1–3). Nicotinic receptors are present on the dopaminergic cell bodies, and stimulation of these receptors by nicotine has been shown to result in an increased release of dopamine in the nucleus accumbens of the mesolimbic system (4).

Accruing evidence suggests that certain polymorphisms of the dopaminergic system genes may be linked to susceptibility to various neuropsychiatric diseases including alcoholism, polysubstance abuse, cocaine abuse, obesity, and pathological gambling (5–9). Although environmental factors may be important determinants of smoking, the results of association, family, and twin studies suggest that initiation and maintenance of smoking also involve hereditary factors (10–14). To date, three studies on non-Hispanic Caucasian populations in the United States have shown a relationship between either the DRD2*A1 or the *B1 allele and genetic predisposition to smoking behavior (15–17), whereas such an association was not confirmed in a United Kingdom population (18).

In our study, we investigated possible links between the DRD2*A genotype and smoking behavior in a total of 332 Japanese individuals. In addition, a functional polymorphism (−141C Ins/Del), which was identified in the DRD2*A promoter (19), was examined for the first time in relation to smoking behavior.

Materials and Methods

Study Subjects. Blood samples were obtained from a total of 332 Japanese individuals (87% participation rate) who were consecutively recruited when they visited the outpatient clinic of Aichi Cancer Center for either cancer screening (n = 154) or the antibiotic eradication of Helicobacter pylori infection (n = 178). These study populations were chosen simply because of their availability when we initiated the present study. There were 177 females (24 current smokers, 6 former smokers, and 147 never-smokers) and 155 males (53 current smokers, 51 former smokers, and 51 never-smokers). Subjects were categorized as to smoking status as follows: (a) never-smokers who were never-smokers who had never smoked more than 10 cigarettes in their lifetime; (b) former smokers were those who had quit at least 1 year before the interview; (c) current smokers were subjects who either were currently smoking or had stopped smoking within the previous year; and (d) ever-smokers consisted of a combination of current and former smokers. The sex-stratified distribution of smokers and never-smokers in this study cohort was similar to that of the general Japanese population (20). The study was approved by the Ethics Committee of Aichi Cancer Center, and informed consent was obtained from all of the subjects before a structured interview and peripheral blood sampling.

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Short Communication: Association of DRD2*A2 with Smoking in the Japanese

PCR-based Detection of Polymorphisms of the DRD2 Gene. Genomic DNA was extracted from each coded blood sample, and DRD2*A genotypes were determined by means of PCR using sense (5’-CCGTCGACCTCCTCCTGTGTCATCA-3’) and antisense (5’-CCGTCGACGGCGCGCCAAGTGTTGCTCA-3’) oligonucleotide primers (15). The resulting PCR products were digested with TaqI, followed by ethidiumbromide separation on 3% agarose gels. Detection of −141C Ins/Del polymorphism was carried out by ethidiumbromide separation of the BstNI-digested PCR products on 3% agarose gels using sense (5’-ACTGGCGGACAGACGGTAGGACC-3’) and antisense (5’-TGCCGCGGTAGAGTGGGCCTCGG-3’) oligonucleotide primers (19).

Statistical Analyses. Odds ratios and 95% confidence intervals for the genotypes were calculated by using an unconditional logistic model in conjunction with the Statistical Analysis System statistical program package (Ver. 6.12; SAS Institute, Inc., Cary, NC) after completion of the molecular biological evaluation. The χ² test was used to examine the association between DRD2 genotype and smoking status.

Results and Discussion

We first examined a total of 332 Japanese individuals to investigate whether the DRD2*A genotype may be associated with smoking behavior. We consequently found that the DRD2 A2/A2 genotype was seen more frequently in current smokers and former smokers (51% and 61%, respectively) than in never-smokers (31%) and that the difference in the distribution of DRD2*A genotype among current, former, and never-smokers was indeed statistically significant (P = 0.001 as determined by χ² test; Table 1). To our surprise and in marked contrast to previous reports from the United States (15–17), smoking thus appeared to be associated with the DRD2*A2 allele instead of DRD2*A1 allele. Stratification according to sex showed similar relationships and was statistically significant for males (P = 0.044; χ² test). Similar genotype-phenotype relationships were observed regardless of the participant accrual methods (data not shown). A thorough review of the original gels and the coding of genotypes within the database ruled out the possibility of any errors in classification or coding, and genotyping data were confirmed for 19 randomly selected samples by an independent outside researcher (Dr. Kenji Hibi of Nagoya University, Japan) for further validation. It should also be noted that the overall allele frequency observed in this study was very similar to that reported in a study on the association between alcoholism and the DRD2*A2 genotype in the Japanese by an independent Japanese group (21).

Multivariate logistic regression analysis was performed to estimate the age- and sex-adjusted odds ratio for the genotype to examine whether the DRD2*A genotype is independently associated with an increased risk of being predisposed to smoking behavior (Table 2). It was consequently shown that individuals with the A2/A2 genotype had a significantly increased risk of such predisposition when compared with those with the A1/A1 genotype (odds ratio, 3.68; 95% confidence interval, 1.50–9.05). Individuals with the A1/A2 genotype also had a modestly increased, although not statistically significant, risk of predisposition to smoking (odds ratio, 1.65), which suggests that the gene dosage of the DRD2*A2 allele has an effect on the level of risk. Multivariate analysis performed separately for current and former smokers revealed a similarly increased probability for individuals with the DRD2 A2/A2 genotype of being either current smokers (odds ratio, 3.72; 95% confidence interval, 1.23–11.2) or former smokers (odds ratio, 3.58; 95% confidence interval, 1.09–11.8). Stratification by sex revealed the presence of a significant association between the DRD2 A2/A2 genotype and predisposition to smoking behavior in males (odds ratio, 3.19; 95% confidence interval, 1.06–9.60; Table 2). Although it did not reach statistical significance, a similar trend was also seen in females (odds ratio, 7.59; 95% confidence interval, 0.91–63.4).

Our preliminary analysis of TaqIB polymorphism in the intron 1 of the DRD2 gene revealed a highly consistent association between DRD2*A2 and DRD2*B2 alleles in the Japanese population (i.e., only 2 in 154 individuals showed discrepancy), showing a more stringent linkage disequilibrium than that reported for non-Hispanic whites in the United States (17). Both of the DRD2*A and DRD2*B polymorphisms reside outside the coding region, and no direct evidence for their functional significance such as transcriptional regulatory activity has been reported. Therefore, we extended the present study by investigating possible links between smoking behavior and the −141C Ins/Del polymorphism, i.e., a functional insertion/deletion polymorphism recently identified within the promoter region of the DRD2 gene (19). However, in contrast to the DRD2*A genotype, the distributions of −141C Ins/Del genotypes were virtually similar regardless of smoking status (Table 3). We note that although skewing in the genotype distribution was marginally significant for males, multivariate analysis failed to support any independent link of the −141C Ins/Del genotype to smoking behavior (data not shown).

The study presented here shows for the first time that the DRD2 A2/A2 genotype is significantly associated with an increased risk of predisposition to smoking behavior in the Japanese

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**Table 1** Distribution of DRD2*A genotypes in relation to smoking status

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>DRD2*A genotype</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1</td>
<td>A1/A2</td>
</tr>
<tr>
<td>All of the subjects (n = 332)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>5 (6)*</td>
<td>33 (43)</td>
</tr>
<tr>
<td>Former smokers</td>
<td>5 (9)</td>
<td>17 (30)</td>
</tr>
<tr>
<td>Never-smokers</td>
<td>31 (16)</td>
<td>105 (53)</td>
</tr>
<tr>
<td>Males (n = 155)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>4 (8)</td>
<td>22 (42)</td>
</tr>
<tr>
<td>Former smokers</td>
<td>5 (10)</td>
<td>14 (27)</td>
</tr>
<tr>
<td>Never-smokers</td>
<td>8 (16)</td>
<td>26 (51)</td>
</tr>
<tr>
<td>Females (n = 177)</td>
<td>current smokers</td>
<td>1 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 (16)</td>
</tr>
</tbody>
</table>

*χ² test.
* Numbers in parentheses represent percentage of each genotype among individuals with the indicated smoking status.

**Table 2** Multivariate analysis of the association between smoking status and DRD2*A genotype

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>DRD2*A genotype</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1</td>
<td>A1/A2</td>
</tr>
<tr>
<td>Ever-smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.65 (0.67–4.04)*</td>
<td>3.68 (1.50–9.05)*</td>
</tr>
<tr>
<td>Current smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.90 (0.63–5.70)*</td>
<td>3.72 (1.23–11.2)*</td>
</tr>
<tr>
<td>Former smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.16 (0.35–3.91)*</td>
<td>3.58 (1.09–11.8)*</td>
</tr>
<tr>
<td>Ever-smoking males</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.27 (0.43–3.77)*</td>
<td>3.19 (1.06–9.60)*</td>
</tr>
<tr>
<td>Ever-smoking females</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.58 (0.43–29.8)*</td>
<td>7.59 (0.91–63.4)*</td>
</tr>
</tbody>
</table>

* Age- and sex-adjusted odds ratio; 95% confidence interval is shown in parentheses.
* Age-adjusted odds ratio; 95% confidence interval is shown in parentheses.
population. It is interesting that the observed association is in inverse relation to that reported for non-Hispanic Caucasians in the United States (15–17), indicating the possible existence of ethnic differences in the association of the DRD2 A2/A2 genotype with an increased risk of being predisposed to smoking behavior. Thus, additional studies are needed to clarify the molecular mechanism, which can account for the observed ethnic group-specific difference. One possibility is that DRD2*A alleles may be involved in a different linkage disequilibrium, depending on the ethnic population, with a multiallelic polymorphism of DRD2, which has a stronger influence on the function of DRD2 than \( -141C \) In/Del. An alternative explanation would be that DRD2*A alleles may be differentially linked to a multiallelic functional polymorphism of a neighboring gene, as yet to be identified, in an ethnic group-specific manner. It will be interesting to investigate whether a similar association exists in other Asian populations such as Koreans and Chinese, because ethnic variation has been reported in the strength of both intragenic and intergenic linkage disequilibriums (22, 23). The lack of apparent association of the DRD2*A genotype with either cigarettes/day or pack-years smoked was noted in contrast to its significant link to the acquisition of smoking behavior (data not shown). These findings suggest that there may not be a simple relationship between DRD2*A genotype and the degree of nicotine dependence in the Japanese population. Nevertheless, although replication of the present findings by other groups using independent samples is necessary, the present study suggests the potential usefulness of DRD2*A genotyping for the assessment of individual risk profiles for smoking among Japanese. Thus, future studies are warranted, especially those which focus on the gene–gene interaction between DRD2 and other mesolimbic dopamine systems such as the dopamine D4 receptor gene (24) as well as on social, behavioral, and personal factors that may influence the acquisition of smoking behavior.

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References

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