Dopamine D₂ Receptor Gene Polymorphisms Are Associated with Suicide Attempt in the Japanese Population

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Key Words

Dopamine D₂ receptor • –141C Ins/Del • Suicide attempt • *TaqIA* • Polymorphism

$r^2 = 0.016$, $p = 0.10$). **Conclusions:** These findings suggest that DRD2 gene polymorphisms may be involved in the biological susceptibility to suicide.

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Abstract

Background: Some reports have suggested the involvement of the D₂ dopaminergic function in the expression of suicidal behavior. Here, we examined associations between suicide attempts and two kinds of functional polymorphisms in the dopamine D₂ receptor (DRD2) gene, namely, *TaqIA* and –141C Ins/Del. **Methods:** Subjects included 120 suicide attempters and 123 unrelated volunteers. Those who attempted suicide were severely injured and were transferred to the emergency unit in our university hospital. To determine each genotype, we performed polymerase chain reaction and restriction fragment length polymorphism analyses. **Results:** We found significant differences in genotypic and allelic frequencies of –141C Ins/Del and *TaqIA* polymorphisms between suicide attempters and healthy controls (–141C Ins/Del, $p = 0.01$; *TaqIA*, $p = 0.036$). The Ins allele of –141C Ins/Del was significantly more frequent in suicide attempters ($p = 0.011$), as well as the A2 allele of *TaqIA* ($p = 0.017$). Haplotype analysis revealed no significant linkage disequilibrium between –141C Ins/Del and *TaqIA* polymorphisms ($D' = 0.226$,

Introduction

Suicide is an important public health problem thought to be caused by different susceptibility factors. Several data from studies of family history suggest that transmitted factors have partial effects on suicidal behavior [1, 2]. Moreover, investigations on twins including adoption studies strongly support a genetic component in suicidal behavior, and rule out the effects of a shared environment [3–6]. Therefore, previous studies have pursued a possible genetic risk factor for suicide.

On the other hand, some studies have reported low cerebrospinal fluid (CSF) levels of the dopamine metabolite homovanillic acid (HVA) in depressed patients with a history of suicide attempts [7–10]. Bowden et al. [11] provided results supporting reduced dopamine turnover in the basal ganglia in depressed suicide completers by demonstrating decreased levels of dihydroxyphenylacetic acid, which is one of the metabolites of dopamine.

These studies suggest an involvement of the D₂ dopaminergic function in the mechanisms of suicidal behavior.

A number of functional polymorphisms have been identified in the dopamine D₂ receptor (DRD2) gene to date [12]. Considering the possible association of DRD2 with the mechanisms of suicidal behavior, these polymorphisms can be considered as candidate genes for such behavior. Among these, the -141C Ins/Del polymorphism (rs1799732) in the promoter region was suggested to affect promoter activity, as shown by an expression study using human cells [13]. A previous study reported the possible association between -141C Del allele and attempted suicide in alcohol dependents [14]. *TaqIA* (rs1800497), which is located in the 3' flanking region of DRD2 [15], is also associated with reduced DRD2 expression in vitro [16] and in vivo [17, 18], and with reduced D₂ receptor binding as measured by autoradiography [19]. This polymorphism is closely associated with personality traits [20], especially novelty seeking, which has been reported as a risk factor for suicide attempts [21, 22]. Therefore, these two polymorphisms are suspected to have a strong association with suicidal behavior.

In the present study, we screened for two kinds of DRD2 polymorphisms, namely, -141C Ins/Del and *TaqIA*, and assessed genetic associations with the occurrence of suicide attempt. To the best of our knowledge, this is the first association study between functional DRD2 polymorphisms and suicide attempters regardless of psychiatric disorders.

Materials and Methods

The study population consisted of 120 subjects (male = 45, female = 75) who attempted suicide and were admitted to the emergency unit of Yokohama City University Medical Center. The controls consisted of 123 unrelated healthy volunteers (male = 42, female = 81). All controls were recruited after informed consent was obtained, and were diagnosed as healthy using physician-conducted interviews and the Mini-International Neuropsychiatric Interview [23]. Subjects with current and chronic psychiatric disorders and a history of suicidal behavior were excluded.

All subjects were ethnically Japanese. The methods of suicide attempt were extracted from the patient's record, and divided into violent and nonviolent attempts according to the operational criteria of Yamada et al. [24] and subsequently analyzed. The violent suicide group was defined as follows: (1) mechanical ventilation was required for life support; (2) surgery was performed under general anesthesia; (3) the method of attempted suicide carried a high risk of death, specifically, hanging, gunshot, jumping from a high place, inhalation of gas, solvents, or other agricultural chemicals, thermal injury, or drowning.

Peripheral blood was drawn from suicide attempters and healthy controls, and leukocyte DNA was extracted for genotype determination. Polymerase chain reaction and restriction fragment length polymorphism analyses were performed to determine *TaqIA* genotypes according to Grandy et al. [15], and -141C Ins/Del genotypes according to Hori et al. [25]. Differences in the genotypic and allelic frequencies of the two gene polymorphisms between suicide attempters and control subjects were tested for significance using the χ^2 test and Fisher's exact test. The presence of Hardy-Weinberg equilibrium was determined using the χ^2 test. This analysis was performed with SPSS 11.0 for Windows (SPSS Japan, Tokyo). Pairwise linkage disequilibrium and estimated haplotypes were analyzed using Arlequin 2.000 [26]. We investigated the difference in genetic distribution in estimated haplotypes between suicide attempters and controls by the χ^2 test. In addition, logistic regression analysis was performed to evaluate simultaneously the possible associations between suicide attempts and independent variables (DRD2 genotypes, gender, and age). Probability differences of $p < 0.05$ were considered statistically significant.

Results

Table 1 shows the genotypic and allelic frequencies of two polymorphisms investigated in the suicide attempters and healthy controls. No deviation from Hardy-Weinberg equilibrium was observed in either the suicide attempters or healthy controls. We found a significant difference in genotypic distribution in the -141C Ins/Del polymorphism between the suicide attempters and healthy controls ($\chi^2 = 8.429$, d.f. = 2, $p = 0.015$). In addition, the frequencies of the -141C Ins allele were significantly higher in the suicide attempters than in the healthy controls ($p = 0.011$). On the other hand, we found no significant differences in the genotypic and allelic distribution between violent and nonviolent attempters (genotypic distribution, $\chi^2 = 1.827$, d.f. = 2, $p = 0.401$; allelic distribution, $p = 0.295$, detailed data not shown). The odds ratio (OR) for the suicide attempts associated with the Ins allele was 1.85 [95% confidence interval (CI), 1.16–2.94]. In addition, we found a significant difference in the genotypic distribution in *TaqIA* polymorphism between suicide attempters and healthy controls ($\chi^2 = 6.76$, d.f. = 2, $p = 0.034$). The frequency of the A2 allele of *TaqIA* was significantly higher in suicide attempters than in healthy controls ($p = 0.017$), and the OR for the suicide attempts associated with the A2 allele was 1.56 (95% CI, 1.09–2.25). Similarly, as shown in table 2, logistic regression analyses showed a significant association between suicide attempts and the -141C Ins/Del genotypes ($p = 0.014$; OR, 1.95; 95% CI, 1.15–3.32) and *TaqIA* genotypes ($p = 0.014$; OR, 1.58; 95% CI, 1.06–2.35). However, we found no signifi-

Table 1. Genotype distributions and allele frequencies of polymorphisms in the DRD2 gene: -141C Ins/Del and *TaqIA* polymorphisms

	All suicide attempters (n = 120)	Controls (n = 123)	p
-141C Ins/Del			
Genotypes			
Ins/Ins	86 (71.7)	66 (53.7)	0.015*
Ins/Del	33 (27.5)	55 (44.7)	
Del/Del	1 (0.8)	2 (1.6)	
Alleles			
Ins	205 (85.4)	187 (76.0)	0.011*
Del	35 (14.6)	59 (24.0)	
<i>TaqIA</i>			
Genotypes			
A1/A1	13 (10.8)	27 (22.0)	0.034*
A1/A2	63 (52.5)	64 (52.0)	
A2/A2	44 (36.7)	32 (26.0)	
Alleles			
A1	89 (36.5)	118 (48.0)	0.017*
A2	151 (63.5)	128 (52.0)	

Figures in parentheses indicate percentages. p = Significance probability between all suicide attempters and controls; * = significant difference between suicide attempters and controls.

Table 2. Logistic regression analysis of independent variables of suicide attempters

Independent variables	Partial regression coefficients	p	OR (95% CI)
Gender	0.033	0.001	1.033 (1.013–1.053)
Age	-0.141	0.618	0.869 (0.500–1.511)
<i>TaqIA</i> genotype	0.455	0.026	1.576 (1.056–2.350)
-141C Ins/Del	0.668	0.014	1.950 (1.147–3.317)

Table 3. Estimated haplotype distribution of the DRD2 gene polymorphisms between suicide attempters and controls

	Suicide attempters (n = 120)	Controls (n = 123)
A1-Ins	0.272	0.370
A1-Del	0.107	0.098
A2-Ins	0.582	0.395
A2-Del	0.039	0.138
p = 0.07.		

cant genetic and allelic frequency differences between violent and nonviolent attempters (genotypic distribution, $\chi^2 = 0.520$, d.f. = 2, $p = 0.771$; allelic distribution, $p = 0.762$, detailed data not shown).

Pairwise linkage disequilibrium was also analyzed and no significant linkage disequilibrium was detected between -141C Ins/Del and *TaqIA* polymorphisms ($D' = 0.226$, $r^2 = 0.016$, $p = 0.10$). As shown in table 3, no significant difference was found in the estimated haplotype frequencies between suicide attempts and controls ($\chi^2 = 12.12$, d.f. = 3, $p = 0.07$). Using SPSS Sample Power version 2.00, we estimated that the statistical power of our study was 64–74%.

Discussion

The role of the dopaminergic system in the mechanisms of suicidal behavior has not yet been fully clarified. Several studies have reported low CSF HVA levels in depressed patients with a history of suicide attempts compared with healthy controls [7–10]. Low CSF levels of HVA could be a more reliable index of suicidal behavior than low CSF 5-hydroxyindoleacetic acid [10]. Pitchot et al. [27] suggested a smaller growth hormone response to apomorphine, which is a dopaminergic agonist, in depressed patients with a history of suicide attempts compared with nonattempters. This study indicates a specific role for the dopamine system, particularly DRD2, in the pathogenesis of suicide. Therefore, functional polymorphisms of the DRD2 gene, which affect the DRD2 function, should be of great interest in investigating vulnerability to suicide attempts.

In the present study, we performed screening for two functional DRD2 polymorphisms, and assessed the relationship between suicide attempts and these polymorphisms. We found significant differences in genotypic and allelic frequencies of the -141C Ins/Del polymorphism between suicide attempters and healthy controls. The frequencies of the -141C Ins allele were significantly higher in the suicide attempters. Jönsson et al. [17] demonstrated by positron emission tomography that the striatal DRD2 density in healthy subjects with the Del allele was higher than that in those without the Del allele. In view of the functional relationship of DRD2 -141C Ins/Del polymorphisms to DRD2 activity, our results suggest that the -141C Ins variant, which reduces DRD2 density, plays an important role as a risk factor for suicide attempts.

On the other hand, we did not observe any significant differences between high and low lethality of suicide attempts. Some previous studies showed differences in the concentrations of CSF 5-hydroxyindoleacetic acid and HVA between the two groups [28–30]; however, the results of these studies were inconsistent. Pitchot et al. [31, 32] reported that the growth hormone peak responses to apomorphine showed no difference between depressed patients with a history of high-lethal suicide attempt and patients with a history of low-lethal suicide attempt. Our result indicates that violent and nonviolent attempters may have a similar pathogenesis, particularly in the DRD2 function influenced by gene polymorphisms.

We also found a significant difference in the genotypic distribution associated with *TaqIA* polymorphism between suicide attempters and healthy controls. The frequencies of the *TaqIA* A2 allele were significantly higher in the suicide attempters. In previous reports, *TaqIA* polymorphism was suggested to be associated with reduced DRD2 expression in vitro [33] and in vivo [17, 19]. However, Pohjalainen et al. [18] and Laruelle et al. [34] showed no association between *TaqIA* polymorphism and D₂ dopamine binding potential in a positron emission tomography study. The unreplicated result regarding human striatal DRD2 density remains of great interest. However, the results of receptor function studies in vivo have been inconsistent to date, and we could not specifically elucidate the pathogenetic mechanism of suicide in the present study. Since this is the first association study between suicide attempters and *TaqIA* polymorphisms, further studies using larger samples are needed to confirm the present results.

From our findings, the DRD2 –141C Ins/Del and *TaqIA* gene polymorphisms are suggested to affect the pathogenesis of suicide. However, this study has some limitations. First, we examined suicide attempters and not suicide completers. There are some reports suggesting differences between these two subject groups [35]. Previous reports, however, have also pointed out biological similarities between suicide completers and suicide at-

tempters [30]; therefore, we speculated that genetic studies on our subjects who attempted suicide would reflect the possible mechanisms underlying suicidal behavior. Second, we were unable to carry out association studies between psychiatric disorders and suicide attempt because the sample number was too small. Moreover, although it is known that a family history of suicide increases suicide risk [36], we did not investigate the association regarding family history of psychiatric disorders for all 2 degrees of relationship. In the present study, we cannot completely exclude the possibility that the psychiatric disorders and family suicidal histories of the subjects might have affected our results.

Third, our total sample size was relatively small. Replication studies using larger samples are required to specifically clarify the possible effects of –141C Ins/Del and *TaqIA* polymorphisms of the DRD2 gene on the pathogenesis of suicide.

Conclusion

Our findings indicate that –141C Ins/Del and *TaqIA* polymorphisms of the DRD2 gene are involved in the suicidal behavior of attempters. A more conclusive study employing a substantially larger sample may be required to verify the associations between the two polymorphisms and suicide attempts. Moreover, we believe that the gene polymorphisms and physiological processes involved in suicide attempts involve many complex factors. Another approach to fully clarify the above-mentioned associations possibly involves a study of the combination of other genetic factors, such as serotonin-related genes, which has the potential to elucidate genetic risk factors involved in suicide.

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