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Research report

The −67 A/T promoter polymorphism in the dopamine transporter gene affects personality traits of Japanese healthy females

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ABSTRACT

Dopamine transporter (DAT) plays a major role in terminating dopamine neurotransmission, which may be involved in the characterization of personality traits. Recently, polymorphisms of the promoter region (−67 A/T) and intron 8 (40-bp variable number of tandem repeats, VNTRs) in the DAT gene were reported to affect DAT expression. In the present study, we examined the associations of these polymorphisms with personality traits in 654 healthy Japanese. Personality traits were assessed by the Temperament and Character Inventory (TCI), and the DAT polymorphisms were identified by PCR-based methods. Regarding the −67 A/T promoter polymorphism, the females without the A allele predictive of high DAT activity had lower scores of self-directedness ($p = 0.005$) and cooperativeness ($p = 0.038$) than those with the A allele. In males, none of the TCI scores was different between the two genotype groups. The intron 8 VNTR polymorphism did not affect any TCI score either in males or in females. The present study thus suggests that the −67 A/T promoter polymorphism, but not intron 8 VNTR polymorphism, in the DAT gene affects personality traits of Japanese healthy females.

1. Introduction

The Temperament and Character Inventory (TCI) is a comprehensive personality scale developed from biological perspectives by Cloninger et al. [6]. The temperaments describe automatic emotional reactions and habits, and consist of four dimensions, i.e., novelty seeking, harm avoidance, reward dependence, and persistence. Novelty seeking is the activation of behavior in response to novelty and signals of reward or relief of punishment, harm avoidance is the inhibition of behavior in response to signals of punishment or non-reward, reward dependence is the maintenance of behavior that was previously rewarded, and persistence is the perseveration with behavior despite frustration and fatigue. The characters describe the self-concepts about goals and values, and consist of three dimensions, i.e., self-directedness, cooperativeness, and self-transcendence. Self-directedness is the concept of the self as an autonomous individual, cooperativeness is the concept of the self as an integral part of humanity or society, and self-transcendence is the concept of the self as an integral part of the universe and its source. Cloninger et al. [6] hypothesized that novelty seeking, harm avoidance, and reward dependence are associated with activities of dopamine, serotonin, and norepinephrine, respectively.

The dopamine system is implicated in the control of locomotion, cognition, and endocrine function [8]. Dopamine transporter (DAT) plays a major role in terminating dopamine neurotransmission by rapid reuptake of dopamine into presynaptic terminals [8]. The DAT knock-out mice show elevated extracellular levels of dopamine accompanied by enhanced locomotion, rearing and stereotypical behaviors [8]. Therefore, it is possible that DAT function is involved in the characterization of personality traits, especially novelty seeking.

The human DAT gene is located on chromosome 5p15.3, and consists of 15 exons spanning about 50 kb [32]. Firstly, a 40-bp variable number of tandem repeat (VNTR) polymorphism was found in the 3′ untranslated region [32]. However, the effect of this polymorphism on DAT expression in vitro has remained inconclusive [19,20,34]. Little wonder the association studies between this DAT polymorphism and personality traits have produced inconsistent results [12,16,17,30,33].

Recently, Rubie et al. [29] reported an A to T single nucleotide polymorphism located at the −67 position within the core promoter region of the DAT gene (−67 A/T, rs2975226). It has been shown that the DAT expression with the A allele is less than 50% of that with the T allele in a luciferase assay [9]. The −67 A/T promoter polymorphism has been associated with attention-deficit/hyperactivity

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disorder [24] and bipolar disorder [25]. Meanwhile, Guindalini et al. [10] found a VNTR polymorphism in the intron 8 of the DAT gene (intron 8 VNTR), which has 5–6 copies of 30-bp repeat sequences. Functional analyses have shown that the 5-repeat allele induces lower DAT expression than the 6-repeat allele in response to stimuli in a luciferase assay [10] and in postmortem midbrain tissue [4]. The intron 8 VNTR polymorphism has been related to the predisposition to attention-deficit/hyperactive disorder [3] and cocaine abuse [10]. These discussions point to the possibility that the −67 A/T promoter polymorphism and intron 8 VNTR polymorphism affect personality traits, especially novelty seeking, by altering synaptic levels of dopamine. However, no study has reported the associations of these polymorphisms with personality traits. Therefore, in the present study we examined the associations of polymorphisms of the −67 A/T promoter region and intron 8 VNTR in the DAT gene with personality traits in healthy subjects.

2. Methods

2.1. Subjects

The subjects were 654 unrelated Japanese volunteers. The subjects with present psychiatric disorders or past history of psychiatric disorders according to the DSM-IV [1] were excluded after interviews by well-trained psychiatrists. The subjects with serious physical diseases were also excluded. The mean ± SD of age was 27.9 ± 8.8 years. Three hundred and forty-one were males, and 313 were females. The study protocol was approved by the Ethics Committee of Yamagata University School of Medicine, and all subjects provided written informed consent to participate.

2.2. Personality assessment

Personality traits of the subjects were assessed by the Japanese version of the TCI [15], which has been verified to have high reliability and validity.

2.3. Genotyping assay

DNA was extracted from peripheral leukocyte using a QIAamp DNA Blood Kit (Qiagen, Tokyo, Japan). For genotyping the −67 A/T promoter polymorphism, the 332-bp in the promoter region of the DAT gene was amplified using a set of primers (forward: 5′-GGA AGG CGC CCG TCT AGA TC-3′ and reverse: 5′-CTG GGC TTT GCA CGC GAG TC-3′) in the 25 μl volume containing 100 ηg of genomic DNA, 0.5 μM of each primer, 200 μM of each dNTP, 1.5 μM of $MgCl_2$, 5 μl of Q solution (Qiagen, Tokyo, Japan), and 1.25 U of HotStar Taq DNA polymerase (Qiagen, Tokyo, Japan). After an initial denaturation step at 95 °C for 15 min, 40 cycles were performed at 94 °C for 0.5 min, 62 °C for 0.5 min, and 72 °C for 0.5 min. Finally, an elongation step was performed at 72 °C for 10 min. Five microliters of the PCR product was digested overnight with 10 U of Tth 111 I (Takara Bio Inc., Otsu, Japan). The fragments were electrophoresed on a 5% agarose gel with ethidium bromide staining and visualized using ultraviolet light. After digestion by Tth 111 I, the A allele gives 220- and 112-bp fragments, and the T allele gives 189-, 112-, and 31-bp fragments.

For genotyping the intron 8 VNTR polymorphism, PCR was performed by using a set of primers (forward: 5′-GCA CAA ATG AGT GTT CGT GC-3′ and reverse: 5′-GAC ATC TGC TAA TGT CCT TC-3′) in the 20 μl volume containing 100 ηg of genomic DNA, 1 μM of each primer, 200 μM of each dNTP, 1.5 μM of $MgCl_2$, 4 μl of Q solution (Qiagen, Tokyo, Japan), and 1 U of HotStar Taq DNA polymerase (Qiagen, Tokyo, Japan). After an initial denaturation step at 95 °C for 15 min, 35 cycles were performed at 95 °C for 0.5 min, 57 °C for 0.5 min, and 72 °C for 0.5 min. Finally, an elongation step was performed at 72 °C for 10 min. The fragments were electrophoresed on a 5% agarose gel with ethidium bromide staining and visualized using ultraviolet light. The 5-repeat and 6-repeat alleles give 219- and 249-bp fragments, respectively.

2.4. Data analysis

Since our previous studies have shown significant effects of age and sex on some TCI scores [13,26], the effects of the −67 A/T promoter polymorphism and intron 8 VNTR polymorphism on TCI scores were analyzed by the two-factor analysis of covariance with age as a covariate in males and females separately. All statistical analyses were performed using SPSS 14.0J for Windows, and a p value of less than 0.05 was regarded as significant.

3. Results

Regarding the −67 A/T promoter polymorphism, 10 subjects were homozygous for the A allele, 150 were heterozygous for the A and T alleles, and 494 were homozygous for the T allele. According to the study by Ohadi et al. [25], the subjects were divided into two genotype groups for statistical analysis, i.e., the group with the A allele (A/A and A/T) and that without the A allele (T/T). Regarding the intron 8 VNTR polymorphism, 31 subjects were homozygous for the 5-repeat allele, 212 were heterozygous for the 5- and 6-repeat alleles, and 411 were homozygous for the 6-repeat allele. According to the study by Guindalini et al. [10], the subjects were divided into two genotype groups for statistical analysis, i.e., the group with the 5-repeat allele (5/5 and 5/6) and that without the 5-repeat allele (6/6). The genotype distributions of the −67 A/T promoter and intron 8 VNTR polymorphisms were in the Hardy–Weinberg equilibrium.

In males, neither the −67 A/T promoter polymorphism nor intron 8 VNTR polymorphism affected any TCI score (Table 1). In females, regarding the −67 A/T promoter polymorphism, the group without the A allele had lower scores of self-directedness and cooperativeness than that with the A allele (Table 1). In females, the intron 8 VNTR polymorphism did not affect any TCI score. There was no significant interaction effect between the two DAT polymorphisms on any TCI score either in males or in females (Table 1).

4. Discussion

Cloninger et al. [6] have hypothesized that novelty seeking is associated with dopamine activity. More specifically, high novelty seeking may be related to low synaptic levels of dopamine and compensatory increase in sensitivity of postsynaptic dopamine receptors [6]. In relation to DAT polymorphism, it is revealed that the polymorphisms of −67 A/T promoter region [9] and intron 8 VNTR [4,10] affect DAT expression level. It is also of note that these polymorphisms have been associated with attention-deficit/hyperactive disorder [3,24], in which dopamine activity is implicated [27]. Therefore, we expected that the −67 A/T promoter polymorphism and intron 8 VNTR polymorphism would affect scores of novelty seeking, but the results obtained show no associations between these polymorphisms and novelty seeking. In this regard, we should take into consideration the putative brain region involved in novelty seeking and the distribution of DAT. Several studies have tried to specify the brain region involved in novelty seeking using neuroimaging techniques, e.g., single photon emission computed tomography (SPECT) [31] and magnetic resonance imaging [28]. Although the results are not necessarily consistent, some reports [28,31] have suggested that the cingulate is involved in the characterization of novelty seeking. On the other hand, it is demonstrated that DAT is distributed exclusively to the striatum and substantia nigra, but not to the cingulate [11,14]. If the locus of novelty seeking does not overlap with the distribution of DAT in this way, our negative finding may not be surprising.

In the present study, the females without the A allele of the −67 A/T promoter polymorphism predictive of higher DAT activity [9] show lower scores of self-directedness and cooperativeness. It is described that low scorers of self-directedness are immature, fragile, blaming, unreliable, purposeless, inert, ineffective, and self-striving, and low scorers of cooperativeness are socially intolerant, critical, unhelpful, revengeful, destructive, and opportunistic [6]. The present results that suggest that DAT activity affects self-directedness and cooperativeness are rather unexpected, since to date these personality traits have not been related to any neurotransmitter [6]. A recent study by Cloninger et al. [7] shows that individuals with depressive symptoms are low in self-directedness and cooperativeness. On the other hand, some SPECT studies demonstrate that striatal DAT density is higher in depressed patients than in controls [5,18]. Furthermore, Newberg et al. [23] found a positive correlation between striatal DAT density and depressive symptom scores even in healthy subjects. The