Apathy is associated with a single-nucleotide polymorphism in a dopamine-related gene

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\section*{HIGHLIGHTS}

- We focused on 4 functional SNPs in dopamine transmission.  
- The relationship between the 4 SNPs and apathy was analyzed.  
- We selected 963 neurologically normal subjects as participants.  
- A SNP in the COMT gene is associated with a lower risk of apathy.  
- Dopaminergic neurotransmission is important in the pathogenesis of apathy.

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\section*{ABSTRACT}

Dopaminergic neurotransmission is an important factor in the pathogenesis of apathy. In addition, the contribution of genetic factors to the regulation of brain dopaminergic activity is widely acknowledged. Therefore, we hypothesized that genes associated with brain dopaminergic activity may have some effects on apathy. In the current study, we evaluated the association between four functional single-nucleotide polymorphisms (SNPs) in specific genes related to dopaminergic neurotransmission and apathy in a general population. Participants in the health examination at the Shimane Institute of Health Science were recruited for this study (n = 963). Apathy was assessed using the Japanese version of the apathy scale. SNPs were genotyped using the TaqMan method. In our population, 22.1\% had apathy. We confirmed that apathy was associated with decreased cognitive function and depressive state. A significant association was found between an SNP in the catechol-O-methyltransferase (COMT) gene (rs4680) encoding the low-activity Met allele and apathy. This relationship was still significant after adjustment for confounding factors. Our study indicates an association between rs4680, an SNP in the COMT gene, and lower risk of apathy. Considering the function of rs4680, the current study suggests the importance of dopaminergic neurotransmission in the pathogenesis of apathy in a general population.

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1. Introduction

Apathy is a lack of motivation for goal-directed behavior (GDB), and consists of a loss of initiative, interest, and affect for goal-directed events [25]. Apathy may be the most frequent psychobehavioral alteration occurring in patients with brain disease [18,40], although there are no authoritative estimates of its frequency in the general population.

Previously, it was reported that dopamine mediates reward pursuit behavior by attributing incentive salience (“wanting”) to reward stimuli [17,19,38]. Considering Marin's definition of apathy as “the absence or lack of emotion, interest, concern, or motivation” [25], and bearing in mind the relationship between dopamine and reward-seeking, or “wanting” behavior, a hypothesis emerged that dopaminergic neurotransmission is a basis for apathy. In agreement with this hypothesis, functional brain imaging studies [7,30] have suggested a relationship between apathy and abnormal perfusion in the cingulate area, orbital-frontal region, and related fronto-subcortical structures—areas that are crucial components of the brain motivational and reward system [13]. In addition, evidence coming from animal [2] and pharmacological studies [45] have...
suggested that the physiopathology of apathy could be at least partially explained by a dysfunction of the dopaminergic system. Since the contribution of genetic factors to the regulation of brain dopaminergic activity is widely acknowledged, and considering the importance of dopamine neurotransmission on apathy, genes associated with brain dopaminergic activity may have some effects on apathy.

In the present study, we investigated the hypothesis that genes associated with brain dopaminergic activity have some effects on apathy. Candidate genes and single-nucleotide polymorphisms (SNPs) were selected on the basis of the following criteria: (1) genes in pathways implicated in the neurobiology of the dopamine pathway, and (2) within these genes, polymorphisms with well-documented functional effects (in vitro or in vivo) in dopamine transmission. On the basis of these criteria, we focused on 4 SNPs (rs6277, rs6280, rs1800955, and rs4680). rs2677, a SNP in the dopamine D2 receptor gene (DRD2), is thought to affect mRNA stability and, therefore, receptor expression [12]. rs6280, a SNP in the dopamine D3 receptor gene (DRD3), has been associated with altered dopamine binding affinity, thereby predicting a possible functional effect [24]. rs1800955, a SNP in the dopamine D4 receptor gene (DRD4), has been reported to influence transcription efficiency [33]. rs4680 is a common SNP in the COMT gene, and has been associated with decreased enzymatic activity and dopamine catabolism. Since COMT is the major mammalian enzyme involved in the degradation of released dopamine in the brain, and this enzyme is an important factor for regulating brain dopaminergic activity, this SNP is thought to result in increased availability of dopamine in the prefrontal cortex (PFC) [6]. In support of these findings, it has been recently reported that the rs4680-containing haplotype is associated with several negative symptoms, including apathetic social withdrawal, among subjects with schizophrenia [44].

2. Materials and methods

2.1. Study population

This study was approved by the ethical committee of the Shimane University School of Medicine, Japan. Experiments were undertaken with the understanding and written consent of each subject. From November 2001 to July 2006, a total of 1630 Japanese subjects voluntarily participated in the health checkup system at the Shimane Institute of Health Science. The checkup system included the collection of medical, neurological, and psychiatric history; formal physical and neurological examinations by an experienced neurologist; and neuropsychological testing, magnetic resonance imaging of the head, and blood tests. We selected 963 physically and neurologically normal subjects (513 males and 450 females) aged 41–88 years (mean 59.7 ± 5.2) as participants for this study. On the basis of their detailed medical interview and medication history, we applied exclusion criteria for this study as follows: (1) any history of neurological diseases, including cerebrovascular diseases; (2) any history of psychiatric diseases, such as depression, drug abuse, or other psychiatric diseases; (3) patients taking medications that affect cognitive function or mood state; (4) patients who meet the criteria for dementia as defined by the National Institute of Neurological Disorders and Stroke, probable vascular dementia as defined by the Association Internationale pour la Recherche et l’Enseignement en Neurosciences [35], and/or probable or possible Alzheimer’s disease as defined by the National Institute of Neurological and Communication Disorders-Alzheimer Disease and Related Disorders Association [27]; and (5) missing data. Hypertension was defined as blood pressure levels ≥140/90 mm Hg or the use of antihypertensive drugs. Total serum cholesterol and fasting plasma glucose levels were measured in blood samples taken after overnight fasting. Diabetes was defined as a fasting blood glucose level ≥126 mg/dL, a random blood glucose level ≥200 mg/dL, or the use of oral antidiabetic drugs. Hyperlipidemia was defined as a serum cholesterol level ≥220 mg/dL or the use of lipid-lowering drugs. A smoker was defined as any subject whose smoking index (cigarettes per day × years) exceeded 200. Regular alcohol consumption was defined as ≥58 mL of alcohol consumed per day.

2.2. Cognitive function

Apathy was assessed using the Japanese version of the apathy scale [32,39]. This scale was used in a self-assessment style, with assistance if necessary. We classified the patients into 2 groups according to their apathy scores: an apathy group (≥16 points) and a non-apathy group (<16 points). The cutoff point was determined on the basis of a previous report on Japanese stroke patients, and the scale displayed a high reliability (p = 0.96, p < 0.0001, n = 20) and validity (sensitivity, 81.3%; specificity, 85.3%) with a cutoff point of 16 [32]. Okabe’s Intelligence Scale (Okabe’s Test), which is a shortened and modified Wechsler Adult Intelligence Scale–Revised for the Japanese aged population, was used for assessing general intelligence, including orientation, semantic memory, calculation, forward and backward digit span, and paired association memory [16]. The test is scored out of a total of 60 points, and its reliability and validity have been reported elsewhere [31]. Depression was evaluated using the self-rating depression scale (SDS) [47].

2.3. SNP genotyping

Fasting venous blood samples were obtained from all study participants. Genomic DNA was extracted from peripheral blood leukocytes by using a standard phenol/chloroform method. All SNPs were genotyped using the TaqMan SNP Genotyping Assay in a 384-well microplate format (Applied Biosystems, Foster, CA). Briefly, 20 ng of DNA was amplified in a total volume of 5 μL containing an MGB probe (Applied Biosystems) and 2.5 μL of TaqMan universal polymerase chain reaction master mix (Applied Biosystems). Allelic discrimination analysis was performed on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). To ensure the quality of genotyping, SNP-specific control samples were added to each 384-well plate.

2.4. Statistical analysis

Continuous variables are presented as mean ± standard deviation. Student’s t-test or the χ2 test was applied to compare measurements between the apathy and the non-apathy group. The fitness of the allele and genotype frequencies determined using the Hardy–Weinberg equilibrium were evaluated using the χ2 test. Genotype distributions were analyzed by logistic regression, integrating adjustments for education duration, Okabe’s test score, and SDS. Genotypic associations and odds ratios (OR) with 95% confidence intervals (CI) were estimated by binary logistic regression.

As the SNPs we examined affected biological function of the dopamine receptor and COMT gene, we assumed that the ORs of these SNPs were relatively high. When the OR was 2.0, the statistical power of that study set was 0.97. Statistical analyses were performed using the statistical software package JMP9.0 (SAS Inc., NC, USA). p < 0.05 was considered statistically significant.
Table 1
Clinical characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Apathy (n = 213)</th>
<th>Non-apathy (n = 750)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.4 ± 5.4</td>
<td>59.8 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>41.8</td>
<td>48.1</td>
<td>NS</td>
</tr>
<tr>
<td>Education duration</td>
<td>11.7 ± 2.4</td>
<td>12.3 ± 2.3</td>
<td>&lt;0.0007</td>
</tr>
<tr>
<td>Okabe’s test score</td>
<td>42.6 ± 8.1</td>
<td>45.1 ± 7.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SDS</td>
<td>39.1 ± 6.9</td>
<td>33.3 ± 7.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27.2</td>
<td>22.9</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>47.9</td>
<td>45.3</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10.8</td>
<td>10.1</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>18.8</td>
<td>16.0</td>
<td>NS</td>
</tr>
<tr>
<td>Drinking habits</td>
<td>36.6</td>
<td>37.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation or percentages. SDS, self-rating depression scale.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the study population are shown in Table 1. Of the 963 subjects, 213 (22.1%) had apathy. Subjects in the apathy group were less educated (t(961) = 3.39; p = 0.0007). Moreover, compared to the non-apathy group, those in the apathy group had a lower Okabe’s Test score (t(961) = 4.22; p < 0.0001) and a higher SDS score (t(961) = 10.36; p < 0.0001).

3.2. Association of the four SNPs with apathy

The relationship between the four SNPs and apathy was analyzed in the study population. The genotype frequencies of the four SNPs were in agreement with the Hardy–Weinberg equilibrium. As shown in Tables 2 and 3, for the SNP in the COMT gene (rs4680), the frequency of the minor allele and minor allele-containing genotypes were lower in the apathy group than in the non-apathy group. We noted that the apathetic subjects had a lower incidence of heterozygous and homozygous COMT genotypes with the risk allele than expected by chance, indicating a dominant mode of inheritance. Moreover, logistic regression analysis that included education duration, Okabe’s test score, and SDS score as confounding factors, demonstrated significantly lower frequency of the minor allele-containing genotypes for the SNP in the COMT gene (rs4680) in the apathy group than in the non-apathy group (GA: OR = 0.59, p = 0.003, CI = 0.42–0.84 and AA: OR = 0.34, p = 0.002, CI = 0.15–0.67) (Table 3). We found no correlation between the other three SNPs and apathy.

Table 2
Allele frequency of DRD2, DRD3, DRD4, and COMT in our subjects.

<table>
<thead>
<tr>
<th>Gene (rs number)</th>
<th>Allele</th>
<th>Apathy, n (%)</th>
<th>Non-apathy, n (%)</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRD2 (rs2277)</td>
<td>C</td>
<td>406 (95.3)</td>
<td>1410 (94.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>20 (4.7)</td>
<td>90 (6.0)</td>
<td>NS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DRD3 (rs2680)</td>
<td>A</td>
<td>317 (74.4)</td>
<td>1074 (71.6)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>109 (25.6)</td>
<td>426 (28.4)</td>
<td>NS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DRD4 (rs1800955)</td>
<td>C</td>
<td>255 (59.9)</td>
<td>904 (60.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>171 (40.1)</td>
<td>596 (39.7)</td>
<td>NS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>COMT (rs4680)</td>
<td>G</td>
<td>325 (76.3)</td>
<td>1033 (68.5)</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>101 (23.7)</td>
<td>467 (31.1)</td>
<td>0.003</td>
<td>0.68</td>
<td>0.53–0.87</td>
</tr>
</tbody>
</table>

NS, not significant; OR, odds ratio; CI, confidence interval.

Table 3
Effect of four SNPs on apathy.

<table>
<thead>
<tr>
<th>Gene (rs number)</th>
<th>Genotype</th>
<th>Apathy, n (%)</th>
<th>Non-apathy, n (%)</th>
<th>Unadjusted p value</th>
<th>Multivariate analysis, adjusted for education duration, Okabe’s test score, and SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td>OR</td>
</tr>
<tr>
<td>DRD2 (rs2277)</td>
<td>CC</td>
<td>194 (91.0)</td>
<td>664 (88.5)</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>18 (8.5)</td>
<td>82 (11.0)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>1 (0.5)</td>
<td>4 (0.5)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>DRD3 (rs2680)</td>
<td>AA</td>
<td>119 (55.9)</td>
<td>375 (50.0)</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>79 (37.1)</td>
<td>324 (43.2)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>15 (7.0)</td>
<td>51 (6.8)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>DRD4 (rs1800955)</td>
<td>CC</td>
<td>76 (35.7)</td>
<td>277 (36.9)</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>103 (48.4)</td>
<td>350 (46.7)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>34 (15.9)</td>
<td>123 (16.4)</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>COMT (rs4680)</td>
<td>GG</td>
<td>123 (57.7)</td>
<td>349 (46.5)</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>79 (37.1)</td>
<td>335 (44.7)</td>
<td>0.01</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>11 (5.2)</td>
<td>66 (8.8)</td>
<td>0.02</td>
<td>0.47</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism; NS, not significant; OR, odds ratio; CI, confidence interval; SDS, self-rating depression scale.

4. Discussion

The present study demonstrated that the minor allele and minor allele-containing genotype for SNP in the COMT gene (rs4680) were associated with lower risk of apathy. We confirmed that apathy was associated with decreased cognitive function and depressive state. Previous studies have suggested that apathy and depression might be distinct but partially overlapping symptoms [21,22]. Notably, apathy was still significantly associated with that SNP after adjustment for confounding factors, such as education duration, Okabe’s score, and SDS score. To the best of our knowledge, this is the first report revealing the association between the SNP in the COMT gene (rs4680) and apathy.

The SNP in the COMT gene (rs4680) refers to a G-to-A change at codon 158, resulting in a valine (Val) to methionine (Met) substitution. The Met allele leads to a three- to four-fold reduction in COMT activity, which results in increasing levels of dopamine in the PFC [6]. Because other regulators of synaptic dopamine are scarce in the PFC, COMT plays a central role in determining prefrontal dopamine levels [29]. Taken together, the current study suggests...
that dopaminergic neurotransmission in the PFC combined with other related brain areas is an essential part of the pathogenesis of apathy.

Apathy is often present after direct lesions of the PFC [15,41]. In previous studies, an average of 61% of those with focal lesions involving the PFC manifested apathy [23,37]. Similarly, focal damage to the associative and limbic territories of the basal ganglia is frequently associated with an apathetic symptom [3,14,28]. This is also observed in patients with neurodegenerative diseases associated with direct lesions of the basal ganglia, such as Parkinson’s disease [1,34], Huntington’s disease [8], and progressive supranuclear palsy [1]. According to the proposed definition, apathy is a pathology of voluntary action or GDB, and the underlying mechanism(s) responsible for apathy may be seen as dysfunctions occurring at the level of elaboration, execution, and control of GDB [5]. Since the PFC and its connections with the basal ganglia are essential to GDB [11], apathy is considered a clinical consequence of the disruption of the PFC-basal ganglia system. Thus, the PFC plays central roles in neural circuits associated with apathy, and our results reinforce the notion that the PFC is responsible for the emergence of apathy.

The causal significance of dopamine in apathy was also confirmed by the current data. A dopaminergic deficit has previously been used to explain the pathogenesis of apathy [20]. In support of this hypothesis, levodopa has been reported to improve apathy in Parkinson’s disease [9]. Similarly, dopaminergic drugs, such as bromocriptine and amantadine, have also been reported to be successful in the treatment of apathy in patients with other neurological conditions, including traumatic brain injury and post-stroke patients [26,43]. In neuroimaging studies, an association between the dysfunctions of dopaminergic transmission and apathy has been demonstrated. One study showed that apathy severity was related to striatal dopamine uptake, as assessed by a single photon emission computed tomography ligand [10]. Another study found an inverse correlation between apathy and dopamine transporter levels in the caudate nucleus of patients with dementia with Lewy bodies [36]. Although these data do not directly show the association between apathy and dopamine neurotransmission in the PFC, reduced connectivity between the PFC and its basal ganglia inputs may disrupt dopaminergic signals associated with apathy within the PFC. This notion is compatible with recent functional imaging evidence that dopamine agonists might alter decision-making and risk-taking in susceptible individuals with Parkinson’s disease via the actions on the PFC [42]. Taken together, the SNP in the COMT gene (rs4680), which results in an increased level of dopamine in the PFC, may act through a modulation of the PFC-basal ganglia circuit. Since not only dopamine, but also other neurotransmitters have a significant impact on the PFC, it is also important to consider the possibility that the effects of dopamine on apathy might arise from secondary effects. In fact, there is evidence of interactions between dopamine and several other neurotransmitters in the PFC [4].

Several limitations of the present study must be noted. First, because this is an institute-based case-control study, a bias in selecting subjects cannot be fully excluded. It would therefore be important to confirm these findings in a population-based study before definitive conclusions can be made about the role of COMT genes in apathy. Second, SDS is a self-report symptom scale that measures depressive symptoms. Diagnosis of depression can be set only with a structured interview. However, SDS is commonly used to screen for depression in larger patient groups and to measure the severity of depression with good internal consistency and validity that encompasses most DSM-IV criteria for major depression [46,48]. Finally, although several polymorphisms have been identified in each gene, we analyzed only one SNP for each gene. Nevertheless, we believe that the choice of the four SNPs investigated here is justified because of their putative functional significance, and does not invalidate our findings.

In conclusion, our study highlights the contribution of COMT (rs4680) to lowering the risk of apathy. Considering the function of the SNP (rs4680), the current study suggests the importance of dopaminergic neurotransmission in the pathogenesis of apathy. Further functional analyses are warranted to elucidate the biological plausibility of the relationship between the COMT gene and apathy identified in this study.

References
