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Association of Serotonin Transporter Gene (5-HTTLPR/rs25531) Polymorphism with Comorbidities of Panic Disorder

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Keywords
5-HTTLPR · Agoraphobia · Comorbidity · Depression · Panic disorder

Abstract
Introduction: Panic disorder (PD) has many comorbidities such as depression, bipolar disorder (BPD), and agoraphobia (AG). PD is a moderately heritable anxiety disorder whose pathogenesis is not well understood. Recently, a tri-allelic serotonin transporter (5-HTTLPR/rs25531) polymorphism was reported to be more sensitive to personality traits compared to the bi-allelic 5-HTTLPR polymorphism. We hypothesized that the 5-HTTLPR/rs25531 polymorphism may lead to a pathological anxious state depending on the presence or absence of a comorbidity in PD. ***Methods:*** In this study, we investigated the relationship between comorbidities in PD and tri-allelic 5-HTTLPR polymorphisms. A total of 515 patients with PD (148 males, 367 females) were genotyped, and the Revised NEO Personality Inventory as well as anxiety-related psychological tests were administered. Depression, BPD, and AG were diagnosed as comorbidities. ***Results:*** For the tri-allele 5-HTTLPR genotype, a significant interaction effect was found between openness to experience and comorbid depression. Examination of the interaction between AG and the tri-allelic 5-HTTLPR genotype revealed that L′ allele carriers are associated with higher trait anxiety than the S′S′ genotype group in PD without AG. ***Conclusion:*** Some anxiety and personality traits can be characterized by the tri-allelic gene effect of 5-HTTLPR. These results suggest that tri-allelic 5-HTTLPR genotypes have genetic effects on the presence of comorbidities of PD.

Introduction

Panic disorder (PD) is an anxiety disorder characterized by frequent unexpected panic attacks and anxiety [1]. PD is known to have comorbidity with various mental illnesses, including mood disorders such as depression and bipolar disorder (BPD) and anxiety disorders includ-

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ing agoraphobia (AG) [2–6]. In the relationship between PD and AG, significant changes were observed, as the diagnostic criteria were revised from the Diagnostic and Statistical Manual of Mental Disorders (DSM)-III to DSM-5. In DSM-III, AG was classified as an anxiety disorder and subcategorized into AG with panic attacks and AG without panic attacks [7]. AG was a subcategory of PD in DSM-IV, but became a separate category in DSM-5 [8]. Although the epidemiological and clinical aspects of PD with AG have been described [9], its biological background has not been fully elucidated. Similarly, the biological background of the coexistence of PD with depression and BPD has not been fully elucidated.

Genetic studies have been performed to determine a biological indicator for many psychiatric disorders. Serotonin has been implicated in PD, depression, BPD, and AG based on the epidemiological and therapeutic backgrounds, and research has been conducted targeting serotonin-related genes as biological indicators [10–14]. One of the serotonin-related genes is a serotonin transporter. Serotonin transporters can regulate synaptic cleft serotonin levels by inducing serotonin reuptake [15]. As a result, sequence variants in this gene have been widely investigated for their associations with the risk of mental illness; these variants have included variants in the coding region, some intron regions, and the promoter region [16–18]. The serotonin transporter gene-linked polymorphic region (5-HTTLPR), also known as a 44-base-pair insertion/deletion polymorphism within the promoter region, has two common alleles: a 16-repeat long allele (L) and a 14-repeat short allele (S) [19]. It is reported that 5-HTTLPR has L-type and S-type polymorphisms depending on the length of the allele, and the gene expression level differs between them [20, 21]. The 5-HTTLPR S allele has been reported to have low transcriptional activity, resulting in decreased mRNA levels and reuptake activity [20, 21]. There is an A to G substitution (rs25531) within the L allele, and the L allele with the A variant (La) is associated with increased 5-HTT mRNA expression compared with the S allele and the L allele with the G variant (Lg), thus creating a tri-allelic polymorphism [22]. The conventional classification of the 5-HTTLPR polymorphism, which fails to distinguish between La and Lg alleles, may reduce the likelihood that the appropriate gene effect of the 5-HTTLPR polymorphism will be determined [23]. It would be worthwhile to examine the role of the tri-allelic (S, La, Lg) 5-HTTLPR polymorphism in PD.

Because of the potential effects of antidepressants and mood stabilizers on transporter expression, serotonin transporters are one of the major targets of antidepressants or mood stabilizers [6, 24] in PD, depression, BPD, and AG patients [6, 12, 13, 25]. Several genetic contributions of the 5-HTTLPR genotype to mental illness comorbidities have been examined, and several positive and negative results have been reported. Positive reports of the involvement of the 5-HTTLPR genotype include depression with alcoholism [26] and depression with posttraumatic stress disorder [27]. Recently, hypermethylation of 5-HTTLPR in PD was reported to be related to comorbid depression with PD [28]. Several papers reported the association of the 5-HTTLPR polymorphism with various personality traits [29, 30]. Differences in the 5-HTTLPR genotype can lead to differences in personality traits and can affect comorbidities of PD. In this context, we assumed that 5-HTTLPR was associated with PD and its comorbidities. In this study, we examined the distribution of 5-HTTLPR and further examined whether differences in the genetic genotype in the presence or absence of comorbidities had a characteristic effect on anxiety traits and personality trends.

Subjects and Methods

Participants

The subjects of this study were 515 PD patients (148 men and 367 women) and 440 healthy controls (146 men and 294 women). The selection criteria for the healthy subjects were as follows: no drug use, no previous diagnosis of mental illness, and no family history of mental illness. We excluded subjects who had lost consciousness due to major physical or neurological disorders or had alcohol abuse, substance abuse, or head trauma. Healthy controls were screened for the presence or absence of disorders listed in DSM-IV [1] using the Japanese version of the Mini International Neuropsychiatric Interview 5.0.0 (MINI) [31].

All of the patients diagnosed with PD based on DSM-IV and DSM-5 were outpatients at Mie University Hospital and Nagoya Mental Clinic in Japan, who were diagnosed by at least two physicians. The MINI was used to evaluate mental illness. Screening was done for psychiatric disorders, including PD. Depression was found in 40 (29.9%) male patients and in 94 (70.1%) female patients. BPD was found in 20 (28.6%) male patients and in 50 (71.4%) female patients. AG was found in 87 (26.5%) male patients and in 241 (73.5%) female patients.

Most patients were on medication and received mood stabilizers, including antidepressants, anxiolytics, or antipsychotics. Most patients were treated with antidepressants such as serotonin reuptake inhibitors, and the mean imipramine conversion was 82.237 ± 2.904 mg (mean ± standard error) [32].

Psychological Tests

Psychological tests were administered to the subjects by questionnaire and by interview. The questionnaire was used to collect clinical information including basic data on family members with

Table 1. Comparison of the sex and mean age of the PD patient and healthy control groups

Group	Sex		*p* value	Mean age, years	*p* value
	male	female			
PD patients	148	367	0.138	38.722±10.258	0.193
Healthy controls	146	294		37.827±10.832	

Continuous variables are listed as means ± standard errors. The χ^2 statistics were used to compare categorical variables and Student's *t* test was used for continuous variables. PD, panic disorder.

PD and genetic factors, and the tests administered and data collected were as follows. The MINI 5.0.0 is a structured interview used to assess mental illness. Psychiatric illnesses including PD were screened. A fact sheet was used to record sex, age, height, weight, blood type, birth place, growth history, body weight at birth, marital status, drinking habits, smoking habits, menstruation, medical health history, family medical history, and disease under treatment. The questionnaire was about the individual's experience with PD, including the symptoms of the first panic attack, the frequency of panic attacks, and avoidance of situations in which a panic attack may occur during the previous month. The Self-Rating Depression Scale (SDS) is a questionnaire used to complement the diagnosis of depression [33]. It includes 20 items that rate the characteristics of depression. Each question is scored on a scale from 1 to 4 (based on replies of "a little of the time," "some of the time," "a good part of the time," and "most of the time." The total is used as a self-evaluation scale. The State-Trait Anxiety Inventory (STAI) is an assessment of state anxiety (STAI-S) and trait anxiety (STAI-T) [34] consisting of 20 items for each type of anxiety. The subjects assessed the degree to which they experienced each state or having each trait as "very much," "likely," "not so much," or "not at all." State anxiety reflects a transitory anxious state or "right now" condition. Trait anxiety represents stable individual differences in anxiety level and refers to a general tendency to respond with "general" anxiety. The Anxiety Sensitivity Index (ASI) is a short questionnaire designed to measure anxiety sensitivity [35]. It contains 16 items, each of which is rated on a 5-point Likert-type scale. The respondents indicate the extent to which each item corresponds to their beliefs about the consequences of their anxiety symptoms. Items are rated from 1 to 5 (1 = "not at all" and 5 = "very much"). Total ASI scores are obtained by totaling the responses to each of the 16 items. The Revised NEO Personality Inventory (NEO-PI-R) is a standard instrument for measuring the personality traits of individuals of widely ranging ages, from the elderly to the young [36]. The inventory is based on a five-factor model of character: neuroticism (N), extraversion (E), openness to experience (O), agreeableness (A), and conscientiousness (C). The participants responded to the 240 items with 5 grades.

5-HTTLPR Genotyping

In each subject, a 7-mL blood sample was collected, centrifuged for 10 min at 2,000 rpm in a collection tube, and separated into a plasma layer, a buffy coat layer (including white blood cells), and a red blood cell layer. A 150-μL sample of buffy coat was subjected to DNA extraction using a fully automatic nucleic acid purification apparatus (BioRobot EZ1; Qiagen, Hilden, Germany). Determination of the 5-HTTLPR genotype (SS type, L/S type, or LL type) was performed according to the method of Wendland et al. [22]. Specifically, amplification was performed with a polymerase chain reaction (PCR) device using a forward primer (5′-TCC TCC GCT TTG GCG CCT CTT CC-3′) and a reverse primer (5′-TGG GGG TTG CAG GGG AGA TCC TG-3′) and detected using a gel electrophoresis device (QIAxcel). In addition, the rs25531 polymorphism (La type, Lg type), which is an L-type single nucleotide polymorphism (SNP), was detected by a real-time PCR method using the Applied Biosystems real-time PCR apparatus StepOne [37].

Statistical Analyses

The statistical analysis software SPSS (Release 17.0)® was used for all statistical analyses. The sex ratio and gene frequency were compared using the χ^2 test. The Hardy-Weinberg equilibrium (HWE) in genotype frequency was calculated using χ^2 tests. Student's *t* test was performed to compare scores among all gene polymorphisms. In addition, an analysis of covariance (ANCOVA) adjusted for age as a covariate was performed using onset age, SDS, STAI, ASI, and NEO-PI-R as dependent variables and comorbidities and genetic genotype as fixed factors. The α level (*p* value) was set to 0.05. In order to compare the results of 10 items (onset age, SDS, STAI-S, STAI-T, ASI, and five factors of NEO-PI-R), Bonferroni correction for multiple testing comparison was carried out. The nominal *p* value (0.05) was divided by the number of analyses (10) for the main hypothesis, resulting in a corrected level of significance of 0.005. False-positive associations were ruled out by Bonferroni correction for multiple testing [38].

In the tri-allelic analyses, the low-expressing S'S' genotype of the 5-HTTLPR polymorphism consists of SS, S/Lg, and LgLg genotype groups and the high-expressing L' allele carriers are S/La, Lg/La, and LaLa [29]. The tri-allelic 5-HTTLPR genotype could be divided into two groups, a low-expression allele group and a high-expression allele group. Statistical comparisons of the two dichotomous reclassified tri-allelic 5-HTTLPR polymorphisms were carried out in this study. The distributions of the 5-HTTLPR and rs25531 genotypes did not significantly deviate from the HWE in our subjects ($\chi^2 = 2.652$, $p = 0.103$). Solely for the data analyses of the HWE, the participants were sorted into one of three genotype groups: low-expressing (SS, S/Lg), heterozygous (S/La, Lg/La), or high-expressing (LaLa).

Results

Characteristics and Genotyping Frequencies of the 5-HTTLPR Genotypes of the PD Patients and Controls

The average age of the PD group was 38.722 ± 10.258 years and the average age of the healthy control group was 37.827 ± 10.832 years ($t = 1.304$, $p = 0.193$). The sex ratios between the PD group (male 148, female 367) and the healthy control group (male 146, female 294) were not significantly different ($\chi^2 = 2.199$, $p = 0.138$) (Table 1). The 5-HTTLPR/rs25531 genotype distributions of the PD patient and control groups are shown in Table 2. They

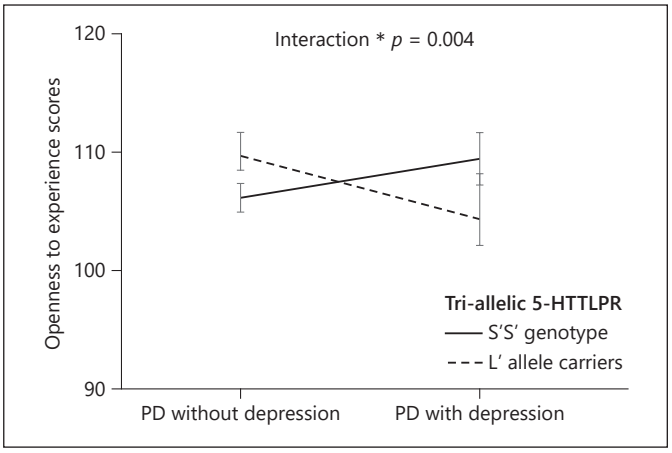

Fig. 1. Effects of tri-allelic 5-HTTLPR genotypes with comorbid depression on NEO-PI-R openness to experience scores in the PD patient group. Data are adjusted for the covariate of age and listed as means ± standard errors. * Bonferroni-corrected $p < 0.005$ was considered statistically significant. 5-HTTLPR, serotonin transporter gene-linked polymorphic region; NEO-PI-R, Revised NEO Personality Inventory; PD, panic disorder.

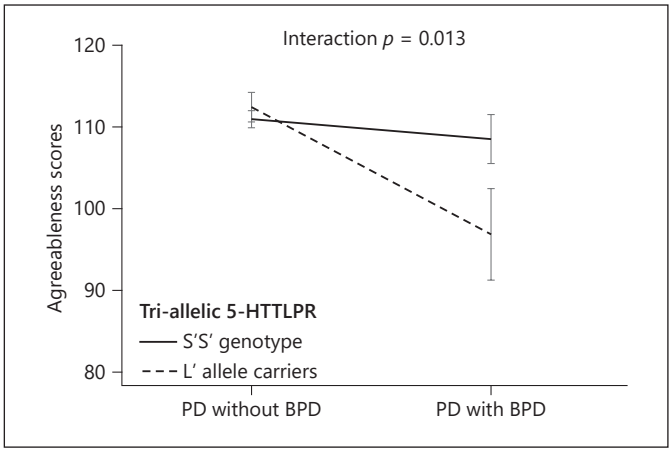

Fig. 2. Effects of tri-allelic 5-HTTLPR genotypes with comorbid BPD on NEO-PI-R agreeableness scores in the PD patient group. Data are adjusted for the covariate of age and listed as means ± standard errors. The interaction was no longer statistically significant after Bonferroni correction. 5-HTTLPR, serotonin transporter gene-linked polymorphic region; BPD, bipolar disorder; NEO-PI-R, Revised NEO Personality Inventory; PD, panic disorder.

Table 2. 5-HTTLPR/rs25531 genotype distribution of the PD patient and healthy control groups

	5-HTTLPR/rs25531 genotype				
	SS	S/Lg	S/La	Lg/La	LaLa
PD patients	338	43	105	11	18
Healthy controls	278	47	97	10	8
Total	616	90	202	21	26

5-HTTLPR, serotonin transporter gene-linked polymorphic region; PD, panic disorder.

show no significant differences in the distribution between the PD and heathy groups ($\chi^2 = 4.369$, $p = 0.358$). We divided the PD group and the healthy control group into two dichotomous groups with the tri-allelic 5-HTTLPR polymorphism (S'S' genotype: 381/325; L' allele carriers: 134/115) (Table 3). No significant difference was observed in the genotypic or allele frequencies of the 5-HTTLPR genotype between the PD group and the healthy control group (tri-allelic 5-HTTLPR groups: $\chi^2 = 1.68 \times 10^{-3}$, $p = 0.967$). The age and sex distributions did not differ significantly between the tri-allelic genotypes. The doses equivalent to the standard imipramine dose did not differ significantly between the tri-allelic 5-HTTLPR groups (S'S' genotype: 81.948 ± 3.282; L' allele carriers: 83.022 ± 6.105; $F = -0.164$, $p = 0.870$).

There were no significant differences in age, sex ratio, onset age, SDS, STAI, ASI, or NEO-PI-R between the tri-allelic 5-HTTLPR genotype groups in the PD patients (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000512699). There were also no significant differences in age, sex ratio, onset age, SDS, STAI, or ASI between the tri-allelic 5-HTTLPR genotype groups in the healthy control group. There was a significant difference in neuroticism in the NEO-PI-R between the tri-allelic 5-HTTLPR genotype groups and the healthy control group (S'S' genotype: 94.670 ± 1.157; L' allele carriers: 102.460 ± 2.152; $t = -3.318$, $p = 0.001$), but there was no significant difference in trait anxiety after Bonferroni correction (S'S' genotype: 40.901 ± 0.535; L' allele carriers: 43.683 ± 1.093; $t = -2.286$, $p = 0.024$) (online suppl. Table 2).

Associations between 5-HTTLPR and the Anxiety Sensitivity and NEO-PI-R Scores

We divided the PD group into three comorbidity groups (depression, BPD, AG) by genotype of 5-HTTLPR. No significant sex differences were observed in the comorbidity ratio within the 5-HTTLPR genotype distribution. The results of the χ^2 test for the tri-allelic 5-HTTLPR genotype were as follows: $F = 0.093$, $p = 0.761$

Table 3. Comparison of the sex, mean age, and tri-allelic 5-HTTLPR genotype distributions between the PD patient and healthy control groups

		Tri-allelic 5-HTTLPR		*p* value
		S'S' genotype	L' allele carriers	
PD patients	*n*	381	134	
	male/female	108/273	40/94	0.741
	mean age, years	38.906±0.536	38.201±0.833	0.495
Healthy controls	*n*	325	115	
	male/female	112/213	34/81	0.388
	mean age, years	37.702±0.582	38.183±1.101	0.683
Total	*n*	706	249	
	male/female	220/486	74/175	0.672
	mean age, years	38.351±0.395	38.193±0.676	0.838

Continuous variables are listed as means ± standard errors. The χ^2 statistics were used to compare categorical variables and Student's *t* test was used for continuous variables. S'S' genotype (low-expression allele homozygotes): SS, S/Lg, LgLg; L' allele carriers (high-expression allele): S/La, Lg/La, LaLa. 5-HTTLPR, serotonin transporter gene-linked polymorphic region; PD, panic disorder.

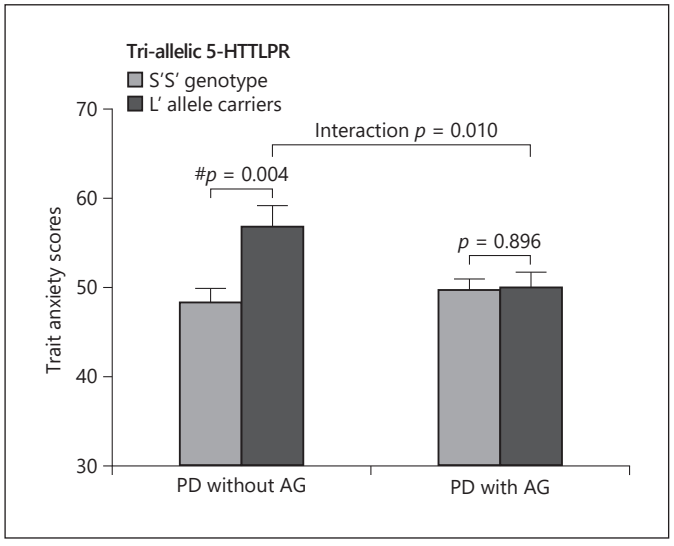

Fig. 3. Effects of tri-allelic 5-HTTLPR genotypes with comorbid AG on trait anxiety scores in the PD patient group. Data are adjusted for the covariate of age and listed as means ± standard errors. # $p < 0.01$ was considered statistically significant in Student's *t* test. A Bonferroni-corrected $p < 0.005$ was considered statistically significant. 5-HTTLPR, serotonin transporter gene-linked polymorphic region; AG, agoraphobia; PD, panic disorder.

for depression; $F = 0.001$, $p = 0.974$ for BPD; and $F = 2.161$, $p = 0.142$ for AG (online suppl. Tables 3–5).

A significant interaction effect was found between openness to experience and comorbid depression in the tri-allelic 5-HTTLPR genotype ($F = 8.256$, $p = 0.004$) (Fig. 1; online suppl. Table 3). After Bonferroni correction, this interaction effect remained statistically significant ($p < 0.005$). ANCOVA showed that there were nominally significant effects of the interaction between comorbid BPD and the tri-allelic 5-HTTLPR genotype on the agreeableness scores ($F = 6.255$, $p = 0.013$) (Fig. 2; online suppl. Table 4). This interaction was no longer statistically significant after Bonferroni correction.

ANCOVA, adjusted for the covariate of age, revealed a nominally significant effect of the interaction between comorbid AG and the 5-HTTLPR genotype on trait anxiety ($F = 6.677$, $p = 0.010$) (Fig. 3; online suppl. Table 5), but this result did not survive Bonferroni correction for multiple testing. Student's *t* test was used for post hoc comparison, and significant differences were found in trait anxiety between the two tri-allelic 5-HTTLPR genotype groups (S'S' genotype: 49.458 ± 1.482; L' allele carriers: 58.500 ± 2.671; $t = -2.908$, $p = 0.004$) in the PD patients without AG (Fig. 3). In PD patients with AG, no significant difference in trait anxiety was observed (S'S' genotype: 50.369 ± 1.152; L' allele carriers: 50.660 ± 1.943; $t = -0.131$, $p = 0.896$) (Fig. 3).

Discussion

In PD, comorbid diseases play an important role, and the diagnostic criteria of DSM-IV divided PD into two types based on a specific comorbidity, i.e., PD with or without AG. Therefore, we hypothesized that the gene genotype related to anxiety may have different gene effects depending on the presence or absence of comorbidities in PD. In the present

study, we were able to shed light on the underlying genetic pathogenesis with regard to comorbidities in PD. However, there was no significant difference in the distribution of the 5-HTTLPR genotype in association with the presence of depression, BPD, and AG as comorbidities in PD.

The 5-HTTLPR genotype is also associated with the treatment of PD, depression, and AG. Studies on the responsiveness of drugs such as selective serotonin reuptake inhibitors have also been conducted according to the types of 5-HTTLPR genotype [39, 40]. Caucasians with depression and BPD have been reported to be more susceptible than Asians to remission with selective serotonin reuptake inhibitors if they have the L allele [24]. The distribution of the 5-HTTLPR genotype by ethnicity has also been reported. In a previous study on Japanese subjects, the S type tended to be dominant, as a much higher frequency of the S allele was found in East Asian (79%) than in European (42%) populations [41]. Our present study also revealed a dominant frequency of the S allele. The serotonin transporter is said to be involved in anxiety and fear [21], but there are racial differences in the 5-HTTLPR gene genotype, with the L type being common in Europe and the United States and the S type in Asia (especially in Japanese individuals). The prevalence of PD does not show significant racial-ethnic differences [42]. The genetic effect of the 5-HTTLPR polymorphism on PD remains to be clarified.

Recently, it was shown that the novel tri-allelic (S, La, Lg) 5-HTTLPR polymorphism which distinguishes between La and Lg alleles may increase the likelihood of determining the appropriate gene effect of the 5-HTTLPR polymorphism in psychiatric disease [23, 29]. An association between harm avoidance and the tri-allelic 5-HTTLPR polymorphism was reported in a clinical subgroup with depression and anxiety disorders [43]. A behavioral study found evidence that among the healthy subjects the S'S' group, in contrast to the L' carrier group, had more difficulty disengaging their attention from emotional stimuli [44]. Chang et al. [23] reported that female L' allele carriers had higher levels of neuroticism than women with the S'S' genotype. Since PD is more prevalent among females, the personality traits associated with PD, such as neuroticism, may be influenced by tri-allelic 5-HTTLPR polymorphisms.

Based on this background, it was considered appropriate to use personality traits to measure the genetic effects of 5-HTTLPR/rs25531 polymorphisms in the presence or absence of comorbidities with PD. An examination of the association between the presence or absence of depression and the genotype of the 5-HTTLPR gene revealed a significant interaction with the personality trait openness to experience in tri-allelic 5-HTTLPR genotypes even after Bonferroni correction. Openness to experience is associated with an increased risk for mood disorders. Midbrain binding of [^{11}C]DASB, a compound that binds to 5-HTT, was reported to be correlated negatively with scores for openness to experience, which showed a relationship between openness to experience and cerebral 5-HTT levels [45]. In a study about families with alcoholism, Stoltenberg et al. [46] reported that the openness scores of subjects with the S allele were higher than those of subjects with the LL genotype among adult men, but this effect was not statistically significant in women. On the other hand, in an adult Swedish cohort study, 5-HTTLPR was significantly associated with openness to experience, and the S'S' group was found to have lower levels of openness compared to the L' carrier group [47]. Our present study showed that the S'S' genotype group had higher openness score with comorbid depression, and the L' allele carriers had lower openness scores with comorbid depression. Further study of the association between openness to experience and tri-allelic 5-HTTLPR genotypes may clarify the genetic factor involved in the personality traits associated with PD.

Examination of the interaction between comorbid AG and the tri-allelic 5-HTTLPR genotype revealed that L' allele carriers were associated with higher trait anxiety than the S'S' genotype group in PD without AG. Demiralay et al. [48] reported that the anxiety-related scale showed lower scores in the S allele group than in the L allele group. In our study, the genetic effect of the 5-HTTLPR genotype on trait anxiety in PD patients without AG was strengthened by the tri-allelic 5-HTTLPR polymorphism. On the other hand, the results of the 5-HTTLPR meta-analysis with anxiety-related traits showed an association of the S'S' genotype with higher anxiety-related trait scores in Caucasians [43]. The S allele was reported to be associated more with anxiety [21]. AG is an anxiety disorder, and trait anxiety is closely related to AG [49]. AG is more related to other phobic disorders, which are also characterized by early disease onset [50, 51]. One possible interpretation of our study about trait anxiety in PD with comorbid AG is that the involvement of environmental factors such as life events may diminish the genetic effect of tri-allelic 5-HTTLPR polymorphisms, leading to the partial discrepancy between our results and those of previous studies. Our study showed that L' allele carriers had higher neuroticism and trait anxiety scores than subjects with the S'S' genotype among healthy controls (online suppl. Table 2). This tendency was also maintained in PD cases without AG, but not in those with comorbid AG (Fig. 3). This

discrepancy between the two genotype groups tended to be smaller than that in healthy controls, PD patients without AG, and PD patients with AG. These results suggest that genetic factors may be involved in the severity of the disease. Recently, it was reported that severity and comorbidity are higher in individuals meeting the DSM-5 AG criteria compared with individuals meeting the DSM-IV AG criteria [51]. Identifying the susceptibility genes in early life could provide the foundation for lifestyle interventions to prevent the development of comorbidities [50].

It is suggested that the 5-HTTLPR gene genotype may have a genetic effect on disease characteristics such as personality and anxiety traits, depending on the presence or absence of comorbid disease in PD [43]. The present study showed that the effects of interactions between comorbidities and the 5-HTTLPR genotype on some anxiety and personality traits could be mediated by the gene effect of tri-allelic 5-HTTLPR polymorphisms. Our results suggest that there are genetic effects between tri-allelic 5-HTTLPR genotypes in cases of PD with comorbidity. There are few association studies involving the tri-allelic 5-HTTLPR genotypes, especially studies of PD and comorbidities. Our present study showed the possibility that some personality traits could be determined by the tri-allelic 5-HTTLPR genotypes. We believe that further study of tri-allelic 5-HTTLPR genotypes may enhance our understanding of the pathogenesis of PD and related diseases.

The present study has several limitations. First, only one representative functional SNP within the 5-HTTLPR gene genotype was examined. It would have been desirable to study all tagging SNPs within the 5-HTTLPR gene genotype as well as other variants associated with serotonergic neurotransmission. Second, a self-reported questionnaire assessment measure was used to evaluate personality-related PD symptoms. Additional assessments by clinicians may be more accurate and/or reliable. The personality inventory scores may reflect the effects of the symptoms, and the STAI state may correspond to the severity of the symptoms. Further data on the severity of the symptoms of patients with PD are needed. For replication studies, it is desirable to collect more detailed information and a homogeneous group of subjects with larger sample sizes that could lead to more specific evidence about anxiety disorders such as PD. The younger average age of our subjects of 38 years entails a risk that the healthy controls could develop PD in the future. In addition, comorbidities of PD might occur during the course of the illness. Therefore, in the future, when comparing samples from PD patients with those from healthy subjects, it is desirable to target older age groups.

Conclusion

We found that the 5-HTTLPR genotype may affect an individual's pathological anxious state depending on the presence or absence of comorbidities in PD cases. The comorbidity may influence some personality traits of PD due to the 5-HTTLPR genotype. Our results suggest that there are distinct genetic effects among tri-allelic 5-HTTLPR genotypes in cases of PD with comorbidity. By adding new tri-allelic 5-HTTLPR genotype analysis to conventional bi-allelic analysis, future studies might clarify the factors that influence the pathogenesis of PD.

Acknowledgments

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Statement of Ethics

This study was performed according to the World Medical Association's Declaration of Helsinki and was approved by the institutional ethics committees of Mie University Graduate School of Medicine (No. 192). Prior to the study, explanations were given to all subjects and written consent was obtained.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S. Tanahashi executed the experimental protocols and statistical analyses and wrote the first draft of the manuscript. S. Tanahashi, Y. Konishi, and H. Tanii were responsible for the study concept and design. Y. Konishi, T. Otowa, T. Sasaki, M. Tochigi, H. Kaiya, and Y. Okazaki contributed to the acquisition of clinical data. S. Tanahashi and Y. Konishi conducted the data analysis. T. Otowa, T. Sasaki, and M. Okada interpreted the findings and provided critical revisions of the manuscript.

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Supplementary Material

Supplementary Table 1. Effects of tri-allelic 5-HTTLPR genotypes on anxiety sensitivity scores and NEO-PI-R scores in the PD patient group

Tri-Allelic 5-HTTLPR	S'S' genotype	L' allele carriers	t	P
Male/Female	108/273	40/94	※0.110	0.741
Mean Age	38.906±0.536	38.201±0.833	0.683	0.495
Age of Onset	29.361±0.502	29.104±0.789	0.265	0.791
SDS	43.424±0.792	46.042±1.278	1.702	0.090
STAI-S	45.539±0.880	46.792±1.429	0.732	0.465
STAI-T	50.000±0.909	53.310±1.622	1.822	0.070
ASI	42.819±0.840	44.208±1.373	0.838	0.403
Neuroticism	112.265±1.653	117.443±2.750	1.579	0.115
Extraversion	94.232±1.429	94.143±2.850	0.030	0.976
Openness to Experience	107.009±1.094	108.086±1.891	0.492	0.623
Agreeableness	111.028±1.015	110.486±1.872	0.263	0.793
Conscientiousness	100.787±1.403	101.400 ±2.777	0.210	0.833

Data are the means ± standard errors of the mean. Nominal *p < 0.05; **p < 0.01 ※ χ2value
Abbreviations: SDS, Self-rating Depression Scale; STAI-S, State Anxiety; STAI-T, Trait Anxiety

Supplementary Table 2. Effects of tri-allelic 5-HTTLPR genotypes on anxiety sensitivity scores and NEO-PI-R scores in the healthy control group

Tri-Allelic 5-HTTLPR	S'S' genotype	L' allele carriers	t	P
Male/Female	112/213	34/81	※0.918	0.388
Mean Age	37.702±0.5816	38.183±1.101	-0.409	0.683
STAI-S	38.232±0.458	39.619±0.902	-1.471	0.142
STAI-T	40.901±0.535	43.683±1.093	-2.286	*0.024
Neuroticism	94.670±1.157	102.460±2.152	-3.318	**0.001
Extraversion	100.970±0.984	98.320±1.727	1.349	0.178
Openness to Experience	111.250±0.858	110.380±1.461	0.511	0.610
Agreeableness	114.220±0.792	114.730±1.183	-0.358	0.721
Conscientiousness	103.740±1.062	103.220±1.808	0.249	0.803

Data are the means ± standard errors of the mean. Nominal *p < 0.05; **p < 0.01 ※ χ2value
P-values that survived the Bonferroni correction are marked in bold.
Abbreviations: SDS, Self-rating Depression Scale; STAI-S, State Anxiety; STAI-T, Trait Anxiety

Supplementary Table 3. Effects of tri-allelic 5-HTTLPR genotypes with comorbid depression on anxiety sensitivity scores and NEO-PI-R scores in the PD patient group

Group	w/o Depression		with Depression		ANCOVA	
Tri-Allelic 5-HTTLPR	S'S' genotype	L' allele carriers	S'S' genotype	L' allele carriers	F	P
Male/Female	83/202	25/71	29/71	11/23	※0.093	0.761
Mean Age	39.081±0.617	38.680±0.963	38.385±1.089	36.794±1.665	0.252	0.616
Age of Onset	29.246±0.577	29.080±0.951	29.698±1.020	29.176±1.387	0.493	0.483
SDS	42.776±0.866	42.851±1.539	45.574±1.840	52.292±1.688	2.035	0.155
STAI-S	45.321±0.982	44.229±1.763	46.277±1.976	51.917±2.122	2.254	0.135
STAI-T	48.981±1.014	50.000±2.059	53.522±1.969	59.792±2.081	1.374	0.242
ASI	42.117±0.929	42.640±1.708	44.981±1.872	47.773±2.127	0.362	0.548
Neuroticism	111.427±1.845	112.298 ±3.372	115.191±3.695	127.957±4.016	0.464	0.496
Extraversion	94.695±1.637	98.532±3.167	92.617±2.944	85.174±5.405	3.160	0.077
Openness to Experience	106.256±1.245	109.894±2.032	109.638±2.271	104.391±3.946	8.256	**0.004
Agreeableness	110.091±1.098	108.830±2.406	114.298±2.429	113.870±2.820	0.441	0.507
Conscientiousness	101.262±1.636	106.234±3.271	99.128±2.680	91.522±4.613	1.981	0.161

Data are adjusted for the covariate of age and listed as means ± standard errors. Nominal *p < 0.05; **p < 0.01. ※χ2value
P-values that survived the Bonferroni correction are marked in bold.
Abbreviations: SDS, Self-rating Depression Scale; STAI-S, State Anxiety; STAI-T, Trait Anxiety; ASI, Anxiety Sensitivity Index; ANCOVA, analysis of covariance

Supplementary Table 4. Effects of tri-allelic 5-HTTLPR genotypes with comorbid BPD on anxiety sensitivity scores and NEO-PI-R scores in the PD patient group

Group	w/o BPD		with BPD		ANCOVA	
Tri-Allelic 5-HTTLPR	S'S' genotype	L' allele carriers	S'S' genotype	L' allele carriers	F	P
Male/Female	94/235	34/82	14/38	6/12	※0.001	0.974
Mean Age	39.188±0.583	38.690±0.907	37.115±1.351	35.056±1.978	0.269	0.605
Age of Onset	29.663±0.544	29.388±0.844	27.412±1.265	27.278±2.233	0.001	0.979
SDS	42.738±0.867	45.559±1.369	46.714±1.872	48.417±3.502	0.010	0.919
STAI-S	44.427±0.958	46.533±1.535	50.971±2.004	48.083±3.965	0.843	0.359
STAI-T	48.865±0.986	52.831±1.742	55.514±2.129	55.667±4.447	0.239	0.625
ASI	42.011±0.890	43.733±1.454	46.861±2.274	46.583±3.963	0.019	0.889
Neuroticism	109.546±1.743	115.661±2.955	125.054±4.093	127.000±7.048	0.275	0.601
Extraversion	94.448±1.591	92.186±2.963	93.216±3.273	104.636±8.389	2.992	0.085
Openness to Experience	106.862±1.169	107.542±2.153	107.703±2.981	111.000±3.440	0.169	0.682
Agreeableness	111.454±1.055	112.932±1.811	109.027±2.994	97.364±5.606	6.255	*0.013
Conscientiousness	101.362±1.496	101.542±3.054	98.081±3.833	100.636±6.951	0.001	0.978

Data are adjusted for the covariate of age and listed as means ± standard errors. Nominal *p < 0.05; **p < 0.01. ※χ2value
Abbreviations: SDS, Self-rating Depression Scale; STAI-S, State Anxiety; STAI-T, Trait Anxiety; ASI, Anxiety Sensitivity Index; ANCOVA, analysis of covariance

Supplementary Table 5. Effects of tri-allelic 5-HTTLPR genotypes with comorbid AG on anxiety sensitivity scores and NEO-PI-R scores in the PD patient group

Group	w/o AG		with AG		ANCOVA	
Tri-Allelic 5-HTTLPR	S'S' genotype	L' allele carriers	S'S' genotype	L' allele carriers	F	P
Male/Female	45/94	16/32	63/179	24/62	※2.161	0.142
Mean Age	39.727±1.002	38.188±1.496	38.434±0.618	38.209±1.001	0.374	0.541
Age of Onset	30.978±0.890	32.625±1.516	28.438±0.595	27.140±0.827	1.970	0.162
SDS	44.099±1.358	49.870±2.173	42.975±0.964	44.208±1.522	2.252	0.135
STAI-S	46.000±1.359	50.833±2.627	45.228±1.157	44.771±1.637	1.523	0.218
STAI-T	49.458±1.482	58.500±2.671	50.369±1.152	50.660±1.943	6.677	*0.010
ASI	43.391±1.346	45.913±2.315	42.434±1.077	43.408±1.704	0.606	0.437
Neuroticism	110.628±2.572	124.913±5.440	113.392±2.160	113.787±3.014	2.488	0.116
Extraversion	92.756±2.300	89.652±4.320	95.248±1.824	96.340±3.668	0.671	0.413
Openness to Experience	105.384±1.779	111.739±3.452	108.128±1.380	106.298±2.234	1.322	0.251
Agreeableness	110.953±1.592	109.000±3.596	111.080±1.322	111.213±2.184	0.210	0.647
Conscientiousness	101.186±2.026	99.217±5.675	100.512±1.921	102.468±3.100	0.100	0.752

Data are adjusted for the covariate of age and listed as means ± standard errors. Nominal *p < 0.05; **p < 0.01. ※χ2value
Abbreviations: SDS, Self-rating Depression Scale; STAI-S, State Anxiety; STAI-T, Trait Anxiety; ASI, Anxiety Sensitivity Index; ANCOVA, analysis of covariance