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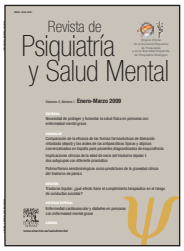

ORIGINAL

Role of serotonergic polymorphisms in the clinical severity of the panic disorder

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KEYWORDS
Genetic association;
Panic disorder;
Polymorphisms;
Serotonin transporter;
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Abstract
Introduction and objectives: To investigate the association between three serotonergic polymorphisms (A-1438G [rs6311] of the *HTR2A* gene, STin2 VNTR and 5-HTTLPR of the *SLC6A4* gene) and the severity of panic and depression symptomatology among mental health outpatients with diagnosis of panic disorder (PD).
Methods: 92 unrelated PD outpatients (DSM-IV criteria) from a homogeneous Spanish Caucasian population (mean age ± SD, 35.9 ± 12.4 years; 28 [30.4%] males) were assessed using the Panic and Agoraphobia Scale (PAS), and the Hamilton Depression Rating Scale (HDRS), and genotyped using standard methods.
Results: Age of onset of PD varied by STin2 VNTR genotype (F = 3.21; p = 0.045). On average, onset of PD occurred earlier for those with the 10/10 than for those with the 12/12 genotype (25.1 versus 33.3; p = 0.043). No relationship was found between A-1438G, 5-HTTLPR, and STin2 VNTR genotypes and PAS or HDRS total scores. Variation in scores on the HDRS Anxiety subscale by A-1438G genotype almost reached statistical significance (F = 3.03; p = 0.053). Post hoc pairwise comparisons showed higher anxiety levels among A/G than among A/A carriers (4.1 versus 2.9; p = 0.043). Finally, variation in scores on the Preoccupied with Health subscale of the PAS by 5-HTTLPR genotype approached statistical significance (F = 2.56; p = 0.083). Post hoc pairwise comparisons showed higher scores among L/S than among L/L carriers (2.4 versus 1.4; p = 0.078).

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Conclusions: Our data provide support of an involvement of the serotonin system, particularly, the *HTR2A* gene in the severity of PD.

PALABRAS CLAVE
Asociación genética;
Trastorno de pánico;
Polimorfismos;
Transportador de serotonina;
Receptor de serotonina 2A

Implicación de polimorfismos serotoninérgicos en la gravedad clínica del trastorno de pánico

Resumen
Introducción y objetivos: Investigar la relación entre tres polimorfismos serotoninérgicos (A-1438G [rs6311] del gen *HTR2A*, STin2 VNTR y 5-HTTLPR del gen *SLC6A4*) y la gravedad de la sintomatología de pánico y depresión en pacientes ambulatorios con un diagnóstico de trastorno de pánico (TP).
Métodos: Se evaluó a un total de 92 pacientes ambulatorios con TP (criterios diagnósticos del DSM-IV) no emparentados, de una población española caucásica homogénea (media de edad ± desviación estándar de 35,9 ± 12,4 años; 28 [30,4%] varones) con el empleo de las escalas Panic and Agoraphobia Scale (PAS) y Hamilton Depression Rating Scale (HDRS), y se determinó su genotipo con métodos estándar.
Resultados: La edad de inicio del TP variaba según el genotipo STin2 VNTR (F = 3,21; p = 0,045). En promedio, el inicio del TP se produjo antes en los pacientes con el genotipo 10/10 que en los que presentaban el genotipo 12/12 (25,1 frente a 33,3; p = 0,043). No se observó relación alguna entre los genotipos A-1438G, 5-HTTLPR y STin2 VNTR y las puntuaciones totales de las escalas PAS o HDRS. La variación en las puntuaciones de la subescala de Ansiedad de la HDRS en función del genotipo A-1438G alcanzaba casi la significación estadística (F = 3,03; p = 0,053). Las comparaciones post hoc de datos emparejados pusieron de manifiesto un grado de ansiedad mayor en los portadores del genotipo A/G que en los portadores del A/A (4,1 frente a 2,9; p = 0,043). Por último, la variación de las puntuaciones de la subescala de Preocupación por la Salud de la PAS, en función del genotipo 5-HTTLPR, se aproximaba a la significación estadística (F = 2,56; p = 0,083). Las comparaciones post hoc para datos emparejados mostraron unas puntuaciones más altas en los portadores del genotipo L/S que en los portadores del L/L (2,4 frente a 1,4; p = 0,078).
Conclusiones: Nuestros datos respaldan la participación del sistema serotoninérgico, y en especial del gen *HTR2A*, en la gravedad del TP.

Introduction

Panic disorder (PD) is accepted to be influenced by genetic factors, with a moderated heritability. To date, familial aggregation studies have estimated the risk of a mental disorder in relatives of PD patients to be 3.4-14.7 times greater than the risk in healthy control relatives.[1] Furthermore, the risk of PD in PD relatives is 14%[2-4] and the estimated heritability around 50%[5] Linkage studies performed in PD associated the disorder with different sites in chromosome 16,[6] chromosome 20,[7] and chromosome 15.[8] Nevertheless, other authors have not verified these data.[9,10]

Serotonin-related genes are good candidates for research in PD. However, serotonin gene transmission is a key point in the performance of the Selective Serotonin Reuptake Inhibitors (SSRI). Specifically, the serotonin transporter (5-HTT) plays a role in the action mechanism of SSRIs as well as the postsynaptic activation of the serotonin receptor 2A (HTR2A) in the patients' symptom improvement or remission.

The *HTR2A* gene is located on chromosome 13q14-q21. Two polymorphisms of this gene, T102C (rs6313) and A-1438G (rs6311), have been described as being in completed linkage disequilibrium in different populations.[11] However, association studies regarding the role of these polymorphisms in PD have produced conflicting results, with two positive findings,[12,13] and two negative results.[14,15] More recently, an association between polymorphic variants at the *HTR2A* gene (T102C, A-1438G, and rs2296972) and the severity of PD symptomatology has been suggested.[16,17]

The *5-HTT* gene (also known as SLC6A4 or SERT) is mapped on chromosome 17q11.1-q12. A functional polymorphism of this gene (5-HTTLPR) involving two

common L (44-base pair insertion) and S (deletion) alleles is related to the differential expression of 5-HTT binding sites in cell lines[18]. The S allele has been demonstrated to be less active, resulting in lower serotonin reuptake and in increased serotonin in the synaptic cleft. Most prior case-control association studies[13,15,17,19-27] and even meta-analytic evidences[28] have failed to find an association between the 5-HTTLPR and PD. On the other hand, more recently, Wachleski et al[29] have failed in the characterization of heritable 5-HTTLPR-related phenotypes associated with PD regarding personality traits. However, it has been suggested the possibility that the L allele could be related to the disorder,[13] as well as, with a better response to paroxetine in females.[30]

Another polymorphism of the SCL6A4, a 17 base pair (bp) variable number of tandem repeats (termed STin2 VNTR) involves different alleles that correspond to 12-, 10-, 9-, or 7-repeat units of 17 VNTR. The STin2.12 allele has been reported to be a transcriptional enhancer.[31] Studies looking for an association between this polymorphism and PD has reported negative findings.[13,15]

Most previous studies on the association between PD and serotonergic gene polymorphisms have been limited to differences in genotypic or allelic frequencies between cases and controls, with only two prior ones that focus on the association between serotonergic polymorphisms and symptom severity in PD.[16,17] The aim of this study was to investigate the association between three serotonergic polymorphisms (A-1438G (rs6311) of the *HTR2A* gene, and STin2 VNTR and 5-HTTLPR of the *SLC6A4* gene) and the severity of panic and depression symptomatology among mental health outpatients with a diagnosis of PD.

Methods

Patient population

The sample consisted of 92 unrelated outpatients of Spanish Caucasian origin, aged 17-82 years (mean age SD = 35.9 12.4 years); 28 (30.4%) male, 64 (69.6%) female), from the region of Asturias, Northern Spain (population: 1 million). All patients had a diagnosis of PD according to DSM-IV criteria. The age of onset was the age at which symptoms were of sufficient degree to meet the DSM-IV criteria for PD.

A signed informed consent form was obtained from all subjects included in the study. The study was subject to and in compliance with Spanish national legislation, was conducted according to the provisions of the World Medical Association Declaration of Helsinki, and received institutional approval.[32]

Assessment

In order to assess the severity of the patients' symptomatology, the Panic and Agoraphobia Scale (PAS)[33] and the 17-item version of the Hamilton Depression Rating Scale (HDRS)[34] were administered to all 92 subjects. The PAS is a 13-item scale that evaluates the severity of panic symptoms based on the reference period of the previous week. It produces an overall score (range = 0-52) and scores for 5 subscales: panic attacks (range = 0-12); agoraphobia (range = 0-12); anxiety (range = 0-8); disability (range = 0-12); and, preoccupation with health (range = 0-8).

The HDRS assesses severity of current (or the past two days for some items) depressive symptoms. It provides an overall score (range = 0-34) as well as scores for 3 subscales: melancholy (range = 0-12), anxiety (range=0-8), and, sleep (range = 0-6).

Genotyping

Briefly, genomic DNA was extracted from peripheral white blood cells obtained from each participant, according to standard protocols.[35] *HTR2A* and *SLC6A4* gene polymorphisms were identified according to previously published methods.[15] The genotypes were determined by researchers who were blind to subject information.

Data analysis

Power calculations indicated that a sample size of 90 gave 80% power to identify as statistically significant, at the 5% level of significance, medium strength correlations (correlations coefficients > 0.3) and medium-sized between-group differences (Cohen's d = 0.6).

Observed genotype frequencies were compared to those expected according to the Hardy-Weinberg equilibrium through a chi-square (χ^2) test. Linkage disequilibrium between markers was assessed using measures obtained from the EMLD software package (http://epi.mdanderson.org/~qhuang/Software/pub.htm). The χ^2 test was used to examine associations between genotype frequencies and sex. Evidence of linear associations between current age, age of onset of PD, duration of illness and scores on the PAS and HDRS were assessed graphically and by Spearman's Rank Correlation Coefficient, rho. Student's t-test was used to assess gender differences in relation to current age, age of onset, duration of PD and scores on the PAS and HDRS.

One-way analysis of variance (ANOVA) was used to assess differences in these variables by A-1438G, 5-HTTLPR, and STin2 VNTR genotypes. In cases where Levene's statistic indicated that the equality of variances assumption was violated, the result of the ANOVA test was based on the Brown-Forsythe statistic. Tukey's 'honestly significant difference test' was the post hoc test used if the equality of variances assumption held. Otherwise, Dunnett's T3 was used. The statistical analyses were carried out using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA) and StatsDirect Statistical Software version 2.5.7 (http://www.statsdirect.com).

Results

Table 1 details the genotype frequencies of the A-1438G, 5-HTTLPR and STin2 VNTR polymorphisms. All were in Hardy-Weinberg equilibrium (A-1438G: $\chi^2 = 1.15$, $p = 0.283$; 5-HTTLPR: $\chi^2 = 0.39$, $p = 0.531$; STin2 VNTR: $\chi^2 = 2.18$, $p = 0.140$). Some degree of LD was found between 5-HTTLPR

and STin2 VNTR (Cramer's V = 0.300, p = 0.002, D' = 0.502, r^2 = 0.092).

No gender differences were evident in relation to genotype distribution of the three polymorphisms (table 1).

Age of onset of panic disorder

Age of onset ranged from 14 to 66 years (Mean [SD] = 31.0 (10.5) years; unknown for two subjects). There was a very strong, positive, linear association between age of onset and current age (Spearman's rho = 0.83, p<0.001). The older the individual, the later the onset of panic disorder. In addition, there was evidence that the older the patient, the longer the duration of their panic disorder (Spearman's rho = 0.38, p<0.001). There were no gender differences in relation to current age of subjects (male mean [SD] = 33.6 ±11.4 years; female mean [SD] = 36.8 (12.7) years), age of onset (Male mean ± SD = 28.2 (8.8) years; female mean ±SD = 32.2 (11.0) years) or duration of panic disorder (male mean ±SD = 4.8 (10.0) years; female mean SD = 4.1 (6.9) years).

Current age, age of onset and duration of panic disorder did not vary significantly by A-143 8G, 5-HTTLPR, and STin2 VNTR genotype, with one exception. There was evidence that age of onset of panic disorder varied by STin2 VNTR genotype (F = 3.21, df = 2, 86, p = 0.045). The mean ages at onset were 25.1, 30.1 and 33.3 years for subjects with the 10/10, 12/10 and 12/12 genotypes, respectively. The post hoc test indicated that the 10/10 and 12/12 groups differed significantly (p = 0.043). On average, onset of panic disorder occurred 8.2 years earlier for those with the 10/10 genotype than for those with the 12/12 genotype (95%CI = 0.2 to 16.2 years).

Panic and depressive symptoms

Table 2 summarises the scale and subscale scores on the PAS and HDRS for the total sample and for men and women separately. Two significant sex differences were observed. On average, women recorded higher overall scores on the HDRS (female mean ±SD = 10.6 6.2, male mean ±SD = 8.1 ±3.7; t = 2.42, df = 81, p = 0.018; mean difference = 2.5 (95%CI = 0.5 to 4.6)) and on the Melancholy subscale of the HDRS (female mean ±SD = 4.3 [2.9], male mean SD = 2.9 ±2.3; t = 2.24, df = 90, p = 0.027; mean difference = 1.4 (95%CI = 0.2 to 2.7)).

There was some evidence of a linear association between scores on the Panic Attacks subscale and both current age (Spearman's rho = −0.32, p = 0.002) and age of onset (Spearman's rho = −0.24, p = 0.024) and between total scores on the PAS and current age (Spearman's rho = −0.25, p = 0.018). Diminishing severity of panic attacks was associated with older age or later age of onset. There was some limited evidence (Spearman's rho = 0.22, p = 0.042) to suggest that the greater the time since onset of panic disorder, the more severe the depressive symptoms related to sleep.

At the 0.05 level of statistical significance, overall and subscale scores on the PAS and HDRS did not vary significantly by A-1438G, 5-HTTLPR and STin2 VNTR genotypes (table 3).

Variation in scores on the HDRS Anxiety subscale by A-1438G genotype almost reached statistical significance (F = 3.03, df = 2, 89, p = 0.053) with a moderate effect size (η^2 = 0.064). Post hoc pairwise comparisons showed a significant difference between the A/G and A/A genotypes (p = 0.043) with the former associated with higher anxiety scores (A/G mean ±SD = 4.1 ±1.7, A/A mean SD = 2.9 ±1.6, mean difference = 1.19 (95%CI = 0.03 to 2.36)).

Variation in scores on the Preoccupation with Health subscale of the PAS by 5-HTTLPR genotype approached statistical significance (F = 2.56, df = 2, 89, p = 0.083, η^2 = 0.054). Post hoc pairwise comparisons showed that this concerned the L/S and L/L genotypes (p = 0.078). The L/S genotype was associated with somewhat higher scores (L/S mean [SD] = 2.4 [2.0], L/L mean = 1.4 [1.8], mean difference = 1.0 [95% CI = −0.1 to 2.1]).

Analyses examining interaction effects between pairs of polymorphisms on the PAS and HDRS showed no evidence

TABLE 1 **Genotype frequencies of the A-1438G, 5-HTTLPR and STin2 VNTR polymorphisms**

Genotype	All, n (%)	Men, n (%)	Women, n (%)	χ^2	df	*p*
A-1438G (rs6311)						
A/A	18 (19.6%)	4 (14.3%)	14 (21.9%)	1.35	2	0.510
A/G	51 (55.4%)	18 (64.3%)	33 (51.6%)			
G/G	23 (25.0%)	6 (21.4%)	17 (26.6%)			
5-HTTLPR						
L/L	32 (34.8%)	7 (25.0%)	25 (39.1%)	4.43	2	0.109
L/S	42 (45.7%)	12 (42.9%)	30 (46.9%)			
S/S	18 (19.6%)	9 (32.1%)	9 (14.1%)			
STin2 VNTR						
10/10	13 (14.1%)	3 (10.7%)	10 (15.6%)	1.07	3	0.787
12/10	34 (37.0%)	10 (35.7%)	24 (37.5%)			
12/12	44 (47.8%)	15 (53.6%)	29 (45.3%)			
12/9	1 (1.1%)	0	1 (1.6%)			

of interaction. However, the numbers involved in subgoups were limited and made positive findings unlikely. Similarly, a model including the main effects of the A-1438G, 5-HTTLPR and STin2 VNTR genotypes showed no significant findings.

Discussion

In the present study, we found an association between the STin2 VNTR polymorphism of the *SLC6A4* gene and the age of onset of PD, with patients carrying the 10/10 genotype having an earlier onset that those with the 12/12 genotype. On the other hand, we found an inverse relationship between the severity of panic symptoms with the current age and the age of onset of the PD. No relationship was found between A-1438G, 5-HTTLPR, and STin2 VNTR genotypes and PAS or HDRS total scores. However, we found some evidences for a possible association between the A-1438G polymorphism and HDRS anxiety scores with patients with the A/G genotype having higher anxiety scores than A/A carriers. Finally, we found a tendency for

TABLE 2 **Summary statistics of the Panic and Agoraphobia Scale (PAS) and Hamilton Depression Rating Scale (HDRS)**

Scale/subscale	All mean (SD)	Men mean (SD)	Women mean (SD)	t	df	*p*
Total PAS	17.9 ± 8.7	17.1 ± 8.6	18.3 ± 8.8	0.61	90	0.542
Panic attacks	3.9 ± 3.2	3.9 ± 3.2	3.9 ± 3.2	0.06	90	0.951
Agoraphobia	5.2 ± 3.0	4.6 ± 3.3	5.4 ± 2.9	1.19	90	0.239
Anxiety	3.1 ± 2.2	3.3 ± 2.0	3.0 ± 2.3	0.54	90	0.591
Disability	3.7 ± 3.0	3.4 ± 2.8	3.8 ± 3.1	0.66	90	0.509
Preoccupation with health	2.0 ± 2.0	1.9 ± 2.4	2.1 ± 1.8	0.36	90	0.720
Total HDRS	9.8 ± 5.6	8.1 ± 3.7	10.6 ± 6.2	2.42	81	0.018
Meloncholy	3.8 ± 2.8	2.9 ± 2.3	4.3 ± 2.9	2.24	90	0.027
Anxiety	3.9 ± 1.8	3.4 ± 1.7	4.1 ± 1.8	1.64	90	0.105
Sleep	1.5 ± 1.6	1.2 ± 1.2	1.6 ± 1.7	1.29	70	0.203

SD: standard deviation.

TABLE 3 **Summary statistics of the Panic and Agoraphobia Scale (PAS) and Hamilton Depression Rating Scale (HDRS) by genotype of the A-1438G, 5-HTTLPR and STin2 VNTR polymorphisms**

Genotype	Total PAS	Panic attacks	Agora-phobia	Anxiety	Disability	Preoccu-pation with health	Total HDRS	Melon-choly	Anxiety	Sleep
A-1438G (rs6311)										
A/A	15.8 ± 7.0	3.7 ± 2.5	4.9 ± 2.8	2.9 ± 1.9	2.5 ± 2.8	1.8 ± 1.9	9.2 ± 5.9	3.8 ± 3.1	2.9 ± 1.6 [a]	1.3 ± 1.3
A/G	18.1 ± 8.7	3.6 ± 3.3	5.2 ± 3.2	3.1 ± 2.3	3.9 ± 2.9	2.4 ± 2.2	10.1 ± 5.5	3.8 ± 2.8	4.1 ± 1.7	1.6 ± 1.7
G/G	19.2 ± 9.9	4.9 ± 3.5	5.2 ± 3.1	3.3 ± 2.2	4.3 ± 3.2	1.5 ± 1.6	9.7 ± 6.0	3.9 ± 2.8	4.0 ± 2.1	1.3 ± 1.4
5-HTTLPR										
L/L	16.9 ± 9.1	3.6 ± 3.3	5.3 ± 3.3	3.2 ± 2.3	3.5 ± 3.2	1.4 ± 1.8 [b]	10.1 ± 5.7	4.1 ± 2.8	3.8 ± 1.8	1.6 ± 1.6
L/S	18.2 ± 8.8	4.2 ± 3.3	5.0 ± 3.1	3.1 ± 2.1	3.5 ± 3.1	2.4 ± 2.0	9.7 ± 6.2	3.6 ± 3.1	3.7 ± 1.8	1.4 ± 1.6
S/S	19.0 ± 8.1	3.8 ± 3.1	5.3 ± 2.4	2.9 ± 2.3	4.7 ± 2.2	2.3 ± 2.2	9.5 ± 4.1	3.9 ± 2.1	4.4 ± 1.9	1.3 ± 1.3
STin2 VNTR*										
10/10	19.2 ± 8.8	4.5 ± 3.5	5.3 ± 3.1	2.5 ± 2.2	5.1 ± 3.5	1.8 ± 2.1	12.3 ± 6.5	4.7 ± 3.4	4.2 ± 1.2	2.2 ± 1.6
12/10	16.9 ± 7.4	4.0 ± 3.0	4.9 ± 3.1	3.0 ± 2.3	3.0 ± 2.4	2.1 ± 2.2	9.3 ± 5.3	3.6 ± 2.6	3.5 ± 1.9	1.3 ± 1.3
12/12	18.1 ± 9.6	3.6 ± 3.3	5.3 ± 3.0	3.3 ± 2.1	3.8 ± 3.1	2.1 ± 1.9	9.4 ± 5.6	3.7 ± 2.9	4.0 ± 1.9	1.3 ± 1.6

Excluding the one subject with a 12/9 genotype.

[a] $1F = 3.03$, df = 2, 89, $p = 0.053$, $\eta^2 = 0.064$.

[b] $2F = 2.56$, df = 2, 89, $p = 0.083$, $\eta^2 = 0.054$.

an association between the 5-HTTLPR polymorphism and the scores on the Preoccupied with Health subscale of the PAS. In other words, the L/S genotype was associated with higher scores on this subscale than the L/L genotype.

Association case-control studies looking for an association between PD and serotonergic polymorphisms have produced conflicting results due, at least in part, to the small sample size of studies,[28] ethnicity differences,[17] and to the lack of a clear phenotype definition. However, DSM-IV diagnoses may be heterogeneous constructs that combine elements with distinct genetic influences.[36] Nevertheless, recent data suggest that genetic variants of serotonergic genes, of a minor individual effect, may contribute to the susceptibility to PD. However, that genetic variability may have a distinctive influence on pure and comorbid phenotypes of PD.[37]

In spite of the suggested implications of the serotonergic system in the aetiology and therapeutic mechanism of PD, only two prior studies focus on the association between serotonergic polymorphisms and symptom severity in PD.[16,17] Unschuld et al,[16] suggested an association between a polymorphic variant (rs2296972) of the *HTR2A* gene and PAS severity scores. However, PD patients who reported more severe symptoms, tended to have the less frequent allele suggesting a gene-dose effect. On the other hand, somewhat similar to our data, they did not report any association between A-1438G or T102C polymorphisms and total PAS scores. Moreover, these authors,[16] suggested strongest single-locus associations between the intronic SNPs rs2770304, T102C, and A-1438G and the personality trait Reward Dependence. More recently, Yoon et al[17] found that API (Acute Panic Inventory) scores were significantly higher among patients with the 102C/C, or −1438G/G genotypes of the *HTR2A* gene. Differences between the results of Yoon et al[17] and those presented here may be due to the fact that the type of psychometric tool used to measure severity plays a crucial role in detecting associations between polymorphic variants and PD severity. On the other hand, it is important to keep in mind that in our sample, the A/G genotype was associated with higher HDRS anxiety scores.

No prior studies have been conducted looking for an involvement of the *SCL6A4* gene in PD severity. On the other hand, regarding the 5-HTTLPR polymorphism, most prior case-control association studies in PD have been conducted thinking of this polymorphism as biallelic. Nevertheless, Hu et al38 have reported that the 5-HTTLPR polymorphism is functionally triallelic (resulting from an A→G substitution in the L allele), and the LG allele is similar to the S allele in its effect on gene expression, whereas the LA allele is the highest expressing allele. However, it is possible that unrecognized LG alleles in LL and LS genotypes could minimize differences between groups and lead us to negative results.

There are some limitations in the present study. The reduced statistical power of the study may have contributed to the lack of highly statistically significant findings. Variation in scores on the Anxiety subscale of PAS by A-1438G genotype almost reached statistically significant at the 0.05 level, yet the effect size, as measured by η^2, was not small. The power of the study to identify the observed association as statistically significant was 57%, lower than the level of 80% that is preferred in order to avoid type II error. Therefore, the study was vulnerable to type II error and may have failed to identify genuinely significant associations. The study may also have been prone to type I error as we did not adjust for multiple comparisons. It should be borne in mind that a number of the study findings would not have reached statistical significance had we applied a Bonferroni adjustment.

The findings presented in this study might support the involvement of the serotonin system, particularly, the *HTR2A* gene in the severity of PD. However, more replication studies are needed to confirm or reject present data.

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Competing interests

The author(s) declare that they have no competing interests.

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