CLINICAL SCIENCE

Endophenotypes and serotonergic polymorphisms associated with treatment response in obsessive-compulsive disorder

Fábio M. Corregiari, Márcio Bernik, Quirino Cordeiro, Homero Vallada

Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Instituto de Psiquiatria, São Paulo/SP, Brazil.

OBJECTIVES: Approximately 40-60% of obsessive-compulsive disorder patients are nonresponsive to serotonin reuptake inhibitors. Genetic markers associated with treatment response remain largely unknown. We aimed (1) to investigate a possible association of serotonergic polymorphisms in obsessive-compulsive disorder patients and therapeutic response to selective serotonin reuptake inhibitors and (2) to examine the relationship between these polymorphisms and endocrine response to intravenous citalopram challenge in responders and non-responders to serotonin reuptake inhibitors and in healthy volunteers.

METHODS: Patients with obsessive-compulsive disorder were classified as either responders or non-responders after long-term treatment with serotonin reuptake inhibitors, and both groups were compared with a control group of healthy volunteers. The investigated genetic markers were the G861C polymorphism of the serotonin receptor 1Dβ gene and the T102C and C516T polymorphisms of the serotonin receptor subtype 2A gene.

RESULTS: The T allele of the serotonin receptor subtype 2A T102C polymorphism was more frequent among obsessive-compulsive disorder patients (responders and non-responders) than in the controls ($p<0.01$). The CC genotype of the serotonin receptor subtype 2A C516T polymorphism was more frequent among the non-responders than in the responders ($p<0.01$). The CC genotype of the serotonin receptor subtype 1Dβ G681C polymorphism was associated with higher cortisol and prolactin responses to citalopram ($p<0.01$ and $p<0.001$, respectively) and with a higher platelet-rich plasma serotonin concentration among the controls ($p<0.05$). However, this pattern was not observed in the non-responders with the same CC genotype after chronic treatment with serotonin reuptake inhibitors. This CC homozygosity was not observed in the responders.

KEYWORDS: Pharmacogenetics; Serotonin Receptor Subtype 1Dβ; Serotonin Receptor Subtype 2A; Citalopram; Challenge Test.

Corregiari FM, Bernik M, Cordeiro Q, Vallada H. Endophenotypes and serotonergic polymorphisms associated with treatment response in obsessive-compulsive disorder. Clinics. 2012;67(4):335-340.

Received for publication on December 3, 2011; **First review completed on** December 12, 2011; **Accepted for publication on** December 15, 2011

E-mail: marcio.bernik@uol.com.br

Tel.: 55 11 2661-6988

INTRODUCTION

Despite the efficacy of serotonin reuptake inhibitors (SRIs) in treating obsessive-compulsive disorder (OCD) (1), 40-60% of these patients are unresponsive to treatment (2). Individual genetic differences may play a role in determining both clinical responses to medications and adverse side effects. However, few genetic factors have been identified, and the mechanisms underlying clinical failure remain largely unknown (3).

One way to assess the brain serotonergic system is through the identification of endophenotypes with a variety of tests, such as the neuroendocrine challenge test. In a previous study (4), our group reported that cortisol response to citalopram in OCD patients who are responsive to SRIs was comparable to the response of the control subjects who had no mental disorders. In contrast, OCD patients who were therapeutically unresponsive after several long-term SRI trials demonstrated a blunted cortisol response to citalopram.

The goal of the present study was (1) to investigate whether there is an association of serotonergic polymorphisms and treatment response to SRIs in OCD patients and (2) to examine the association between the selected polymorphisms and endocrine responses to an acute intravenous (IV) citalopram challenge.

No potential conflict of interest was reported.

SUBJECTS AND METHODS

Samples

Outpatients who met the DSM-IV[5] diagnostic criteria for OCD and were between 18 and 65 years of age were classified according to their therapeutic response to long-term SRI

treatment (clomipramine, fluoxetine, fluvoxamine, citalopram, sertraline or paroxetine) as either non-responders (NRs) or responders (RPs) according to the following criteria:

- NR: no more than a 25% reduction in the Y-BOCS (6) score after at least: two different selective serotonin reuptake inhibitors (SSRIs) at a maximum or maximum tolerated dose for at least 8 weeks and (2) clomipramine at a maximum or maximum tolerated dose for at least 8 weeks (the NRs in this study have received an average of 13.27 ± 9.5 years of treatment).
- RP: a Y-BOCS score of less than 9 and a Sheehan Disability Scale (7) score of less than 10 after treatment with an SRI and stable for at least 2 months.

We evaluated 239 OCD patients from our own service or referred to us for specialized treatment. From these, 60 patients met inclusion criteria. OCD patients who did not meet the criteria for either NR or RP were excluded from this study. Patients with severe depression (HAMD-21$>$25), affective bipolar disorder, schizophrenia, schizoaffective disorder, active substance abuse or dependence were excluded. Patients currently taking fluoxetine were excluded because of the drug's long half-life.

We selected 30 age- and gender-matched subjects who were free of mental and medical disorders as a control group (CN). The control subjects had never used psychotropic medications and were free from any medication for at least one month.

The clinical characteristics of patients and controls are presented elsewhere (4). All medications including SRIs (except for low-dose bedtime benzodiazepines, which were maintained at a consistent dose throughout the study) were gradually tapered off, and the patients were observed over a one-week washout period before the challenge test. Females were tested in the early follicular stage of their menstrual cycle (defined as the period between the third and tenth day of the cycle).

Challenge test procedure

Challenge test procedures began at 8:00 AM, after an overnight fast. After a peripheral intravenous insertion and 60 minutes of rest, blood sampling began at 9:00 AM. The samples were collected at 20-minutes intervals during 180 minutes. The citalopram infusion began 20 minutes after the first blood sample had been collected. Twenty milligrams of citalopram were diluted in 250 ml of saline and infused over 60 minutes. Subjects were tested in a reclining position and were kept awake.

Plasma cortisol, prolactin and growth hormone (GH) levels were quantified by standard immunoradiometric assays at -20, 0 (onset of the citalopram infusion), 20, 40, 60, 80, 100, 120, 140, and 160 minutes. The serotonin concentration in platelet-rich plasma was measured through high-performance liquid chromatography with a Shimadzu 10Vp system in conjunction with a fluorescence detector (Shimadzu RF-10A XL). The platelets were counted on a cell counter Ac T diff Coulter (Beckman Coulter) to normalize the results.

Investigated polymorphisms

The investigated polymorphisms included the G861C polymorphism (rs6296) of the serotonin receptor subtype 1Dβ (HTR1B) gene and T102C (rs6313) and C516T (rs6305) polymorphisms of the serotonin receptor subtype 2A (HTR2A) gene.

Genotyping

Genomic DNA was extracted using a salting out method (8). There were technical problems when genotyping three responders and three control subjects. Therefore, some of the gene variants were not analyzed for these samples. However, two resistant patients (excluded in the challenge test because of pairing issues) were included in the gene frequency analysis.

HTR1B (G861C). The G861C polymorphism of the gene coding for the serotonin receptor subtype 1Dβ was genotyped according to the procedures described by Mundo et al. (9). The 548-bp PCR fragment was digested with *Hinc II* restriction enzyme. The alleles were detected after separation on an agarose gel, and the G allele was the undigested fragment.

HTR2A (T102C). Genotyping of the T102C polymorphism of the gene coding for the serotonin receptor subtype 2A was performed using the primers and conditions described by Warren al. (10). After amplification, the PCR products were digested with *Msp I*. The fragment sizes were a single 342-bp band for the 102T allele and two bands (216 bp and 126 bp) for the 102C allele.

HTR2A (C516T). The primers and PCR conditions used for the analysis of the C516T polymorphism of the gene coding for the serotonin receptor subtype 2A were described by Arranz et al. (11). The amplified DNA products from the patient and control samples were digested with Sau 96I restriction enzyme (New England Biolabs Inc., cat. R0165S). The C516 allele (digested PCR product) showed 109-bp and 87-bp fragments, whereas the T516 allele remained uncut.

To confirm the genotyping results for all the investigated markers, we used a commercial 50–bp DNA ladder (GE Healthcare Life Sciences, product code: 27-4005-01). After the restriction enzyme cleavage reaction, the fragments of each marker were separated electrophoretically on an agarose gel and visualized under a UV light. All of the genotyping results were interpreted by two independently trained research technicians. The same person genotyped all samples. The genotyping process was repeated when a disagreement arose (e.g., the T516C genotyping of two heterozygous individuals was repeated during the analysis).

Statistical analysis

The prolactin and cortisol responses to citalopram were measured as maximal percentage variation (max%Δ). Because of the null values observed at baseline, GH responses were measured as the maximal variation over baseline (maxΔ). The continuous variables were analyzed using an analysis of variance (ANOVA) or a paired t-test when appropriate. Friedman, Mann-Whitney or Wilcoxon matched tests were used when a non-parametric analysis was required. A general linear procedure was used to perform a two-way ANOVA and post-hoc analysis. Categorical data were analyzed using a chi-square test and a chi-square partition. The significance level was 5% and based on two-tailed tests. A standard deviation was calculated to indicate variability. A test for deviations from the Hardy-Weinberg equilibrium was performed using the

HWE program (12). The analysis was performed using EpiInfo version 6.0 and SPSS version 15.0.

ETHICS

The protocol was in accordance with Declaration of Helsinki for research with human subjects and approved by the Ethics Committee at the Hospital das Clínicas, University of São Paulo Medical School (CAPPesq). All subjects gave written, informed and free consent.

RESULTS

Hardy-Weinberg Equilibrium

The genotypic distributions of the HTR2A C516T, HTR2A T102C, and HTR1B G681C polymorphisms were in Hardy-Weinberg equilibrium (HWE) in the CN ($p>0.05$). The case group was in HWE for all polymorphisms except for the HTR2A T102C polymorphism ($p=0.0001$).

Frequency of Polymorphisms Distributions

The patient and control polymorphism frequency data are presented in Table 1. In the patients, the 102CC genotype is less frequent than in the CN ($p<0.001$ after a chi-square partition), and the heterozygosity frequency was higher ($p=0.02$), with no difference in the 102TT genotype. The frequency of the T allele was higher in the patients than in the CN ($p<0.01$). In Table 2, the allele frequency of each DNA marker in the OCD patients group was subdivided into responders versus non-responders for the SRIs. The 516CC genotype was more frequent, and consequently, the heterozygosity frequency was reduced because there were no patients with a 516TT genotype among the non-responders versus the responders ($p<0.01$). After a chi-square partition, CC homozygosity for the HTR1B G681C polymorphism was more frequent among non-responders versus responders ($p=0.018$).

Table 1 - Polymorphism frequency among patients and controls.

Genotypes	Patients (RP + NR) (%)	CN (%)	*p*-value
HTR2A C516T			
CC	48 (81.4)	26 (92.9)	0.16
CT	11 (18.6)	2 (7.1)	
TT	0 (0)	0 (0)	
HTR2A T102C			
TT	17 (28.3)	6 (20.0)	<0.001
TC	43* (71.7)	14* (46.7)	
CC	0** (0)	10** (33.3)	
HTR1B G681C			
CC	6 (10.2)	2 (6.9)	0.88
CG	24 (40.7)	12 (41.4)	
GG	29 (49.2)	15 (51.7)	
Alleles			
HTR2A C516T			0.18
C	107 (90.7)	54 (96.4)	
T	11 (9.3)	2 (3.6)	
HTR2A T102C			0.007
T	77 (64.2)	26 (43.3)	
C	43 (35.8)	34 (56.7)	
HTR1B G681C			0.69
C	36 (30.5)	16 (27.6)	
G	82 (69.5)	42 (72.4)	

Note: RP = Responders; NR = Non-responders; CN = Controls. *$p=0.02$ after a chi-square partition for heterozygosity; **$p<0.001$ after a chi-square partition for the CC genotype.

Table 2 - Polymorphism frequency among non-responders and responders.

Genotypes	NR (%)	RP (%)	*p*-value
HTR2A C516T			<0.01
CC	30 (93.8)	18 (66.7)	
CT*	2 (6.2)	9 (33.3)	
HTR2A T102C			0.54
TT	8 (25.0)	9 (32.1)	
TC*	24 (75.0)	19 (67.9)	
HTR1B G 681 C			0.054
CC	6** (18.8)	0** (0)	
CG	11 (34.4)	13 (48.1)	
GG	15 (46.9)	14 (51.9)	
HTR1B G 681 C			0.018
CC	6 (18.8)	0 (0)	
GC or GG	26 (81.2)	27 (100)	
Alleles			
HTR2A C516T			0.01
C	62 (96.9)	45 (83.3)	
T	2 (3.1)	9 (16.7)	
HTR2A T102C			0.68
T	40 (62.5)	37 (66.1)	
C	24 (37.5)	19 (33.9)	
HTR1B G681C			0.16
C	23 (35.9)	13 (24.1)	
G	41 (64.1)	41 (75.9)	

Note: RP = Responders; NR = Non-responders; CN = Control. *No TT genotype for HTR2A C516T and no CC genotype for HTR2A T102C were observed among patients; **$p<0.05$ for the CC genotype after a chi-square partition.

Relationship among genetic polymorphisms, study groups and endocrine/serotonin variables

Relationship with basal hormonal/serotonin levels

The basal levels of serotonin, prolactin, growth hormone (GH), and cortisol and the interactions with the investigated polymorphisms were compared among the three study groups (NR, RP, and CN). No associations were found among the groups; polymorphisms; and prolactin, GH or cortisol basal levels. The CN showed the highest platelet-rich plasma serotonin concentration ($p<0.001$). The only significant interaction was observed between the groups and the HTR1B G681C polymorphism ($p=0.022$; Table 3), with the highest serotonin level observed among the CC homozygous of the CN (558.01 ng/ml) and the lowest level observed among non-responders with the same genotype (74.68 ng/ml).

Relationship with endocrine response to intravenous citalopram

Prolactin, GH and cortisol responses to citalopram and interactions with the investigated polymorphisms were compared among the three groups (NR, RP, and CN).

As reported previously (4), the prolactin increase (as a percentage of the baseline) was greater in the CN than in the responder and the non-responder groups ($p=0.017$ for NR versus CN; $p=0.004$ for RP versus CN). However, there was no difference between the responder and the non-responder groups.

The prolactin response was not associated with any allele or genotype among the C516T HTR2A and T102C HTR2A polymorphisms. However, the subjects with CC homozygosity for the G681C HTR1B polymorphism showed a more intense prolactin response to citalopram ($p<0.01$ for both CC

Table 3 - Platelet-rich plasma serotonin levels in the three study groups, stratified by the G681C HTR1B polymorphism.

	G681C HTR1B Polymorphism Genotype	Mean (ng/ml) ± Standard Deviation (%)	N
NR	C C	74.68 ± 49.13	5
	C G	121.73 ± 79.37	11
	G G	184.39 ± 162.59	14
	Total	143.13 ± 127.12	30
RP	C G	163.26 ± 128.90	13
	G G	175.36 ± 124.81	12
	Total	169.07 ± 124.44	25
CN	C C	558.01 ± 80.12	2
	C G	540.52 ± 220.26	12
	G G	390.63 ± 117.03	15
	Total	464.20* ± 179.33	29
Total	C C	212.78 ± 241.45	7
	C G	276.32 ± 242.76	36
	G G	257.20 ± 167.96	41
	Total	261.69 ± 207.28	84

Note: RP = Responders; NR = Non-responders; CN = Control. The platelet-rich plasma level of serotonin was statistically higher in the CN (*$p<0.001$; $F = 14.63$) than in the other two groups. A significant interaction between the groups and the G681C HTR1B polymorphism was observed ($p = 0.022$; $F = 3.383$), with the highest serotonin level observed among the CC homozygous of the CN and the lowest among the NR group with the same genotype.

versus CG and CC versus GG genotype, Table 4). A significant group-genotype interaction was observed ($p<0.001$; Table 4) when the nine subgroups, corresponding to the combination group-genotype, were compared. The CC genotype was not detected among the responders. The control-CC genotype subgroup showed the highest prolactin response, even compared with the other two control subgroups (control-CG genotype and control-GG genotype; $p<0.001$ after a Bonferroni correction; Table 4).

The GH response to citalopram failed to show differences among the groups and was not associated with any of the studied polymorphisms.

As reported previously, the peak secretion of cortisol as measured through maximal percentage variation was less pronounced in the non-responders compared with the responders ($p = 0.015$) and was smaller compared to the CN ($p = 0.052$). There was no significant difference for the CN vs. the responder group ($p = 0.53$) (4). The cortisol maximal percentage variation was unrelated to the HTR2A C516T and T102C polymorphisms. A more intense cortisol response was observed in those subjects who were homozygous for the HTR1B G681C polymorphism ($p = 0.011$ for overall comparison). A significant group-genotype interaction was observed ($p = 0.001$). The control-CC subgroup showed the most intense cortisol response, higher than the other control group genotypes ($p \leq 0.003$ for all comparisons after a Bonferroni correction; Table 5).

Table 4 - A comparison of the prolactin maximal percent of variation among the eight subgroups based on the three groups of participants and the G681C HTR1B polymorphism genotypes.

Group	HTR1B G681C Polymorphism Genotype	Mean ± Standard Deviation (%)	N
NR	C C	20.53 ± 26.63	5
	C G	14.91 ± 28.43	11
	G G	18.25 ± 36.09	14
	Total	17.41 ± 31.06	30
RP	C G	23.96 ± 45.06	13
	G G	9.75 ± 15.79	12
	Total	17.14 ± 34.38	25
CN	C C	317.48*** ± 206.23	2
	C G	60.77 ± 101.17	12
	G G	33.21 ± 38.09	15
	Total	64.22* ± 106.62	29
Total	C C	105.38** ± 168.98	7
	C G	33.47 ± 67.39	36
	G G	21.23 ± 33.11	41
	Total	33.49 ± 70.84	84

Note: RP = Responders; NR = Non-responders; CN = Control. The CN showed a higher prolactin response overall (*$p<0.01$ for CN versus both RT and RP after a Bonferroni correction). Subjects with a CC homozygosity showed a higher prolactin response overall (**$p<0.01$ for both CC versus CG and CC versus GG comparisons yield significant results after Bonferroni correction). The interaction between group and genotype was significant ($p<0.001$). In the post-hoc analysis, the control-CC subgroup was the only significantly different group and showed a higher prolactin response than all other subgroups (***$p<0.001$ after a Bonferroni correction).

DISCUSSION

The present study investigates an association between DNA markers, the endocrine response to citalopram, peripheral serotonin concentration and the responsivity status in OCD patients and controls. As far as we know, this is the first study to examine these associations in OCD patients.

Few studies have investigated serotonergic polymorphisms and SRI treatment responses in OCD patients. Denys et al. (13) reported that in paroxetine-treated patients, the majority of the responders were homozygote for the G allele of the 1438 G/A polymorphism of the HTR2A gene. Cavallini et al. (14) found no association between the Cys23Ser polymorphism of the HTR2C gene and a therapeutic response to clomipramine.

An important finding in our study, which was previously unreported, was the absence of CC homozygosity of the

Table 5 - A comparison of the cortisol maximal percent of variation among the eight subgroups based on the three groups of subjects and the HTR1B G681C polymorphism genotypes.

Group	HTR1B G681C Polymorphism Genotype	Mean ± Standard Deviation (%)	N
NR	C C	-4.93 ± 8.76	5
	C G	20.06 ± 51.04	11
	G G	30.95 ± 71.75	14
	Total	20.98 ± 58.14	30
RP	C G	38.94 ± 66.79	13
	G G	50.61 ± 65.96	12
	Total	44.54 ± 65.26	25
CN	C C	261.59* ± 112.22	2
	C G	51.92 ± 86.62	12
	G G	48.83 ± 57.95	15
	Total	64.78 ± 89.72	29
Total	C C	71.21 ± 138.07	7
	C G	37.50 ± 69.28	36
	G G	43.24 ± 64.23	41
	Total	43.11 ± 73.96	84

Note: RP = Responders; NR = Non-responders; CN = Control. *$p \leq 0.003$ after a Bonferroni correction compared with all other groups.

HTR2A gene T102C polymorphism in both patient groups (responders and non-responders), whereas it was present in one-third of the CN ($p<0.001$). The T allele was more frequent among patients than among controls ($p<0.01$). These findings suggest that the presence of at least one T allele could be an OCD risk factor. The 102TT genotype was more frequent among patients than among controls (28.3% vs. 20.3%, respectively), but this difference was not statistically significant after a chi-square partition ($p = 0.39$). The study may be underpowered to confirm an excess of 102TT homozygous among the patients. The fact that the HTR2A T102C polymorphism was not in HWE in the patients group may be a concern. If deviations from HWE are present in the control population, then one or more of the model assumptions may have been violated or that a genotyping error has occurred. During our experiment, the genotyping process was repeated when a disagreement arose between the independently trained research technicians who interpreted the data. However, a known homozygous sample was not used as a control, which would have guaranteed the genotyping accuracy. Notably, the CN is in HWE ($p = 0.78$). Therefore, a genotyping error appears unlikely. In the present study, the patient samples may not need to be in HWE because the patients were not randomly selected from the population (15). In other words, a deviation from HWE in the case subjects may indicate an association of a locus with OCD (16,17). Another reason for the lack of HWE could be ethnic differences between the subjects and consequent sample stratification. As noted, this reason could be the case in the current study because we did not control for ethnic background. Interestingly, the HTR2A T102C polymorphism lacked HWE, and this polymorphism was associated with the disorder, suggesting that the association may be real.

Another novel finding was that an excess of CC homozygosity and the C allele of C516T polymorphism of the HTR2A gene was observed among the non-responders. This finding may indicate that having at least one T allele could be a factor that indicates SRI responsiveness during OCD treatment.

Notably, our previous study found no differences in the allele and genotype frequencies between OCD patients and the control groups for the T102C HTR2A gene polymorphism (18). Those results suggested that the C516T variant of the HTR2A gene may be a genetic risk factor for OCD. Another study by our group found no association between the 5HTTLPR and STin2 polymorphisms in the promoter region of the serotonin transporter gene (SLC6A4), the G861C polymorphism (rs6296) of HTR1B, the T102C (rs6113) and C516T (rs6305) polymorphisms of HTR2A and the clomipramine response in OCD patients (19). In the present study, these divergences may be caused by differences in sample characteristics with the refractory patients in the RT group and the highly responsive patients in the RP group.

Finally, CC homozygosity for the HTR1B gene G681C polymorphism showed another relevant finding regarding the relationship between the endocrine response to citalopram and the investigated groups, despite a low tendency toward gene frequency discrepancies between responders and non-responders ($p = 0.054$). This genotype was present in only 6 of the 32 non-responders and was absent from the responders. The effect of this genotype on the hormonal response to IV citalopram, an indirect measure of serotonin functioning, is notable. The subgroup of the controls with the CC genotype (n = 2) showed the highest prolactin and cortisol response after a citalopram infusion and presented the highest platelet-rich plasma serotonin concentration. In contrast, OCD patients with the same genotype (n = 6), all of whom were non-responders, presented the lowest cortisol responses and serotonin concentrations (not statistically significant with regard to other subgroups except for the Controls-CC genotype). The difference in magnitude is surprising (prolactin response: CN-CC = $558.01 \pm 80.12\%$; NR-CC = $74.68 \pm 49.13\%$; cortisol response: CN-CC = $317.48 \pm 206.23\%$; NR-CC = $20.53 \pm 26.63\%$; $p<0.001$ after a Bonferroni correction for both comparisons). Interestingly, when we analyzed the frequency of the HTR1B CC homozygosity alone in the OCD group, there was an excess of this genotype among the non-responders ($p = 0.018$; 6 of 32 NRs; 0 of 27 RPs; see Table 2). These data should be interpreted cautiously because of the small subgroup size.

Nevertheless, we hypothesized what could account for this genotype-group interaction and lead to a robust cortisol response to citalopram in the CC-Control subgroup and response in the CC-non responders subgroup. The 5-HT1Dβ receptor is localized chiefly in the 5-HT terminals in which its stimulation inhibits the release of 5-HT. Therefore, the 5-HT1Dβ receptor may be less active in the CC homozygous members of the CN, allowing a more intense post-synaptic serotonergic stimulation after citalopram infusion. The 5-HT1Dβ receptor is also expressed, however, in the terminals of nonserotonergic neurons, exerting heteroregulation of neurotransmitter release and a postsynaptic receptor. The latter occurs mainly in the basal ganglia. Chronic SRI administration desensitizes the 5-HT1Dβ receptors (20). This phenomenon, together with the desensitization of the somatodendritic 5-HT1A receptor (21), leads to progressive enhancement of 5-HT neurotransmission, correlating with a therapeutic response (22). Therefore, in CC homozygous patients, the observed blunted response could be related to the chronic SRI exposure. We infer that a higher acute serotonin response after SRI treatment could lead to more intense adaptation after chronic SRI exposure and consequently to a blunted cortisol response after a single dose of citalopram. Accordingly, disrupted 5-HT1Dβ acute regulation may cause other mechanisms to overcome an increased serotonin transmission (e.g., post-synaptic receptor down-regulation or decreased serotonin synthesis), which may lead to an over-adaptation to chronic SRI exposure and decreased SRI effectiveness. These hypotheses could be evaluated in future studies.

As acknowledged, it may be premature to determine the validity of the association of therapeutic response and genetic polymorphisms because, as an alternative interpretation, they may be in linkage disequilibrium with other proximal polymorphisms that could affect the therapeutic response.

Another limitation of this study was that the groups were unmatched for ethnic origin. In populations of highly mixed ethnicity, such as the Brazilian population, phenotyping expressions of ethnicity, such as skin, hair and eye pigmentation, is not viable (23,24). The sample size may be the main methodological limitation of this study. Because this was an exploratory study, we did not calculate the sample power a priori and instead studied all of the available subjects. It is worthwhile to note the unique features of this sample. OCD patients were selected by their long-term therapeutic responses; some of the resistant patients have been in treatment for decades. Moreover, in

this study, the evaluated groups were extreme. Non-responders were unresponsive to at least three different SRIs and remained symptomatic; responders met tight remission criteria; and controls (healthy volunteers) who neither mental health nor major medical problems. Many putative patients and volunteers were excluded. These strict criteria may have increased the study power but decreased the external validity.

Because the data can be interpreted as false positive (at the 0.01 level of significance), further investigations using a larger sample size are necessary to confirm our results. Prospective studies with larger sample sizes and using selection criteria according to the subjects' genotype would be interesting. In addition, the examined polymorphisms may be in linkage disequilibrium with yet to be identified markers or genes that contribute to OCD. Additionally, further research should be performed to evaluate whether the endocrine response to citalopram can be considered an endophenotype.

CONCLUSIONS

The conclusions of this study are as follows:

(1) The T allele of the 5-HTR2A T102C polymorphism was more frequent among OCD patients.

(2) The CC genotype of the HTR2A C516T polymorphism was more frequent among OCD patients who were nonresponsive to several SRI trials than among the SRI-responsive patients.

(3) The CC genotype of the HTR1B G681C polymorphism was associated with higher cortisol and prolactin responses to citalopram and a higher platelet-rich plasma serotonin concentration among health volunteers. However, this pattern was not observed among nonresponsive OCD patients with the same genotype after chronic SRI treatment.

ACKNOWLEDGMENTS

This research was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) grant number 99/00170-4. We thank Elisabete Miracca for technical support.

AUTHOR CONTRIBUTIONS

Corregiari FM took part in the project design, clinical evaluation, challenge tests execution and analysis, statistical analysis and contributed to the discussion and manuscript. Bernik M took part in the project design, clinical evaluation, challenge tests analysis, statistical analysis and contributed to the discussion and manuscript. Cordeiro Q took part in the project design, was responsible for the genotypings and contributed to the discussion and manuscript. Vallada H took part in the project design, was responsible for the genotypings and contributed to the discussion and manuscript.

REFERENCES

1. Stein DJ. Obsessive-compulsive disorder. Lancet. 2002 Aug 3;360(9330):397-405, http://dx.doi.org/10.1016/S0140-6736(02)09620-4.
2. Pigott TA, Seay SM. A review of the efficacy of selective serotonin reuptake inhibitors in obsessive-compulsive disorder. J Clin Psychiatry 1999;60(2):101-6.
3. Collier DA. Pharmacogenetics in psychosis. Drug News Perspect. 2003;16(3):159-65, http://dx.doi.org/10.1358/dnp.2003.16.3.737958.
4. Corregiari FM, Gattaz WF, Bernik M. Acute hormonal changes after IV citalopram and treatment response in OCD. Psychopharmacology (Berl). 2007 Sep;193(4):487-94, http://dx.doi.org/10.1007/s00213-007-0793-0.
5. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC: American Psychiatric Association; 1994.
6. Kim SW, Dysken MW, Kuskowski M. The Yale-Brown Obsessive-Compulsive Scale: a reliability and validity study. Psychiatry Res. 1990;34(1):99-106, http://dx.doi.org/10.1016/0165-1781(90)90061-9.
7. Leon AC, Shear MK, Portera L, Klerman GL. Assessing impairment in patients with panic disorder: the Sheehan Disability Scale. Soc Psychiatry Psychiatr Epidemiol. 1992;27(2):78-82, http://dx.doi.org/10.1007/BF00788510.
8. Miller SA, Dykes DD, Polesky HF. A single salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215, http://dx.doi.org/10.1093/nar/16.3.1215.
9. Mundo E, Richter MA, Zai G, Sam F, McBride J, Macciardi F, et al. 5HT1Dβ Receptor gene implicated in the pathogenesis of Obsessive-Compulsive Disorder: further evidence from a family-based association study. Mol Psychiatry. 2002;7(7):805-9, http://dx.doi.org/10.1038/sj.mp.4001059.
10. Warren JT Jr, Peacock ML, Rodriguez LC, Fink JK. An MspI polymorphism in the human serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis. Hum Mol Genet. 1993;2(3):338, http://dx.doi.org/10.1093/hmg/2.3.338.
11. Association between clozapine response and allelic variation in 5-HT2A receptor gene. Lancet. 1995;346(8970):281-2.
12. Ott J. Utility Programs for Analysis of Genetic Linkage. 1988.
13. Denys D, Van Nieuwerburgh F, Deforce D, Westenberg HG. Prediction of response to paroxetine and venlafaxine by serotonin-related genes in obsessive-compulsive disorder in a randomized, double-blind trial. J Clin Psychiatry. 2007;68(5):747-53, http://dx.doi.org/10.4088/JCP.v68n0512.
14. Cavallini MC, Di Bella D, Pasquale L, Henin M, Bellodi L. 5HT2C CYS23/SER23 polymorphism is not associated with obsessive-compulsive disorder. Psychiatry Res. 1998;9;77(2):97-104, http://dx.doi.org/10.1016/S0165-1781(97)00151-0.
15. Ziegler A, Van Steen K, Wellek S. Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis. Breast Cancer Res Treat. 2011;128(1):197-201, http://dx.doi.org/10.1007/s10549-010-1295-z.
16. Szeszko JS, Howson JM, Cooper JD, Walker NM, Twells RC, Stevens HE, et al. Analysis of polymorphisms of the interleukin-18 gene in type 1 diabetes and Hardy-Weinberg equilibrium testing. Diabetes. 2006;55(2):559-62, http://dx.doi.org/10.2337/diabetes.55.02.06.db05-0826.
17. Hounie AG, Cappi C, Cordeiro Q, Sampaio AS, Moraes I, Rosário MC, et al. TNF-alpha polymorphisms are associated with obsessive-compulsive disorder. Neurosci Lett. 2008;442(2):86-90, http://dx.doi.org/10.1016/j.neulet.2008.07.022.
18. Meira-Lima I, Shavitt RG, Miguita K, Ikenaga E, Miguel EC, Vallada H. Association analysis of the catechol-o-methyltransferase (COMT), serotonin transporter (5-HTT) and serotonin 2A receptor (5HT2A) gene polymorphisms with obsessive-compulsive disorder. Genes Brain Behav. 2004;3(2):75-9, http://dx.doi.org/10.1046/j.1601-1848.2003.0042.x.
19. Miguita K, Cordeiro Q, Shavitt RG, Miguel EC, Vallada H. Association study between genetic monoaminergic polymorphisms and OCD response to clomipramine treatment. Arq Neuropsiquiatr. 2011;69(2B):283-7, http://dx.doi.org/10.1590/S0004-282X2011000300003.
20. Chopin P, Moret C, Briley M. Neuropharmacology of 5-hydroxytryptamine1B/D receptor ligands. Pharmacol Ther. 1994;62(3):385-405, http://dx.doi.org/10.1016/0163-7258(94)90051-5.
21. Blier P, de Montigny C, Chaput Y. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. J Clin Psychopharmacol. 1987;7(6 Suppl):24S-35S.
22. Mongeau R, Marsden CA. Effect of imipramine treatments on the 5-HT1A-receptor-mediated inhibition of panic-like behaviours in rats. Psychopharmacology (Berl). 1997;131(4):321-8, http://dx.doi.org/10.1007/s002130050299.
23. Silva MA, Cordeiro Q, Miracca EC, Guindalini C, Vallada H. Distribution of alleles of the VNTR polymorphism in the 3'-untranslated region of the DAT1 gene (SLC6A3) in São Paulo/Brazil and its importance to genetic studies of neuropsychiatric disorders in ethically admixed populations. Rev Med Chil. 2005;133(11):1392-3.
24. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A. 2003;100(1):177-82, http://dx.doi.org/10.1073/pnas.0126614100.