

The association of serotonin transporter genotypes and selective serotonin reuptake inhibitor (SSRI)-associated sexual side effects: possible relationship to oral contraceptives[†]

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Objective To study the relationship between functional variants in the serotonin transporter gene (*SLC6A4*) and selective serotonin reuptake inhibitor (SSRI)-associated sexual dysfunction.

Methods One hundred fifteen subjects aged 18–40 years and currently being treated with an SSRI for depression were assessed for clinical variables known to affect sexual well-being. SSRI-associated sexual difficulties were assessed with the Changes in Sexual Functioning Questionnaire (CSFQ). Subjects were subsequently genotyped for the *SLC6A4* promoter region (5HTTLPR) insertion/deletion variant and a variable number of tandem repeats (VNTR) in the second intron.

Results The 5HTTLPR insertion/deletion variant was associated with sexual dysfunction in this study sample [odds ratio (OR) = 2.7; 95% confidence interval (CI) 1.2, 6.4; $p = 0.02$]. The relationship between promoter genotypes and sexual well-being differed in males and females and was related to whether females were taking an oral contraceptive (OC) medication. Females with the *ll* genotype were nearly eight times more likely to be categorized as having sexual dysfunction if they were taking OCs, while no relationship was observed in those not taking OCs.

Conclusions These results suggest that a functional variant in the serotonin transporter gene is associated with sexual difficulties in persons taking an SSRI for depression. This relationship may differ by sex and be dependent on OC status in females. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS — antidepressant; sexual dysfunction; serotonin transporter; polymorphism

INTRODUCTION

Sexual dysfunction is a common and disconcerting side effect of selective serotonin reuptake inhibitors (SSRIs) that influences a patient's desire to continue long-term antidepressant treatment (Bull *et al.*, 2002; Clayton *et al.*, 2002; Montejo *et al.*, 2001b). Studies specifically assessing changes in sexual well-being over time illustrate that the incidence of sexual side effects from SSRIs ranges from 20 to 70% depending on the characteristics of the study sample assessed (Clayton *et al.*, 2002; Montejo *et al.*, 1996, 2001b; Montejo-Gonzalez *et al.*, 1997). Sexual side effects

from antidepressants are of significant clinical importance, particularly in young, sexually active patients who do not tolerate changes in sexual functioning (Montejo *et al.*, 2001a). Developing strategies to predict who may be at the highest risk for adverse changes in their sexual well-being is an important step in improving the quality of life and treatment of patients who require antidepressant agents.

Several variables such as substance use/abuse, smoking, marital status, poor health, medication use, and other disease states have all been shown to have a relationship with sexual well-being in either depressed or euthymic individuals (Clayton *et al.*, 2002; Laumann *et al.*, 1999). In addition, variations in sex hormones such as testosterone, estrogen, or prolactin may influence sexual functioning. Generally speaking, these hormones affect the sexual experience independent from SSRIs, although relationships between estrogen and serotonin transporter expression and the binding affinity of serotonin receptors have been

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reported (Benmansour *et al.*, 2009; Cyr *et al.*, 1998; Pecins-Thompson *et al.*, 1998; Wihlback *et al.*, 2004).

The serotonergic actions of SSRIs have been implicated in the development of sexual side effects (Murray, 1998). Pharmacologically, these medications inhibit the serotonin transporter (5HTT) and increase the synaptic latency period of serotonin (5HT) molecules thereby facilitating the modulation of post-synaptic serotonin receptors (Stahl, 1998). Previous investigations indicate that genetic variability in the gene encoding the serotonin transporter (*SLC6A4*) is related to the general tolerability of SSRIs, but their specific relationship with sexual side effects has not been investigated (Popp *et al.*, 2006; Smits *et al.*, 2007). Therefore, characterizing the relationship(s) between *SLC6A4* and other genetic variations in the serotonin system with SSRI-associated sexual difficulties is an avenue of research that may allow us to develop prediction models of sexual side effects.

To our knowledge, only one other pharmacogenetic study of SSRI-associated sexual side effects has been reported (Bishop *et al.*, 2006). In that analysis, we discovered a common functional variation in the promoter region of the serotonin-2A receptor gene (*HTR2A*) with SSRI-associated sexual dysfunction. Therefore, the purpose of the present study was to assess a larger study sample to investigate two common functional variations in *SLC6A4* and their relationship to overall sexual well-being after controlling for other clinically relevant variables in a population of persons at low risk for other causes of sexual dysfunction.

MATERIALS AND METHODS

Subjects

One hundred fifteen outpatients with depression being treated with an SSRI were recruited for this point prevalence study. Subjects were recruited through local advertisements for a study of "health and well-being" while being treated with any one of the following SSRIs: citalopram, escitalopram, fluoxetine, paroxetine, sertraline, or fluvoxamine for depression. Subject eligibility was determined during telephone screenings where they were also informed that the study would consist of an interview session to determine the efficacy of current antidepressant therapy, buccal cell sample for DNA analysis, and a questionnaire to assess changes in sexual well-being while being treated with an SSRI.

Subjects were included if they had been treated with medication for at least 6 weeks, were between the ages

of 18 and 40 years, and did not have problems with sexual desire or functioning before beginning medication. The age range of 18–40 years was chosen to minimize the confounds of age on the presence of sexual difficulties (Montejo *et al.*, 2001a). The presence or absence of impaired sexual desire or functioning prior to beginning current medication treatment was assessed during the screening interview and again during the study assessment period. Potential subjects were excluded if they were taking another medication for depression or any other medication known to affect sexual functioning (e.g., stimulants, hypertension medications, non-SSRI antidepressants, antipsychotics, non-contraceptive hormone therapies, nutritional supplements for sexual enhancement, phosphodiesterase-V inhibitors for the treatment of sexual difficulties, benzodiazepine use within the past 5 days, or other medication judged at the time of assessment to either positively or negatively influence sexual functioning), if they had any other documented primary axis I diagnosis, cardiovascular disease (i.e., hypertension, congestive heart failure, or coronary artery disease), neurological disorder, diabetes mellitus (type I or II), genitourinary disease, menstrual irregularities, had impaired sexual desire or functioning prior to medication initiation or reported frequent urinary tract infections. Medical charts were systematically reviewed by one of the investigators (JRB) to confirm that subjects met the above entry/inclusion criteria and had a diagnosis of depression.

Assessments

Subjects were assessed in person at the University of Iowa General Clinical Research Center where they were consented for the study, using an informed consent document approved by the University of Iowa Institutional Review Board (IRB). The procedures followed were in accordance with the ethical standards of the University of Iowa IRB and with the Helsinki Declaration of 1975, as revised in 1983. General assessments included vital signs, height, weight, current medications, and sociodemographic variables previously associated with sexual well-being. These included age, race/ethnicity, marital status, whether the participant had children, years of education, alcohol consumption (>five times in the past month), smoking status (any smoking within the past 6 weeks), or any reported illicit drug use. Assessments of depression status included the Hamilton Rating Scale for Depression 21 item version (HAM-D), Hamilton Rating

Scale for Anxiety (HAMA), specific SSRI used, dose, and duration of use. Dose categories were designed to evaluate the effect of medication dose on sexual dysfunction. For the purposes of this investigation, "higher" doses of SSRI medication were defined as ≥ 40 mg/day of citalopram, fluoxetine, or paroxetine, ≥ 100 mg/day of sertraline, and ≥ 20 mg/day escitalopram. Dosing characteristics for the study population are included in Table 1. Two of the authors (JRB and JM) conducted all of the assessments and interviews. A subset of the participants was simultaneously rated by both investigators during the initial stages of the study to generate interrater reliability estimates using interclass correlation coefficients which were 0.98 for both HAMD and HAMA assessments.

Sexual dysfunction was assessed with the self-administered Changes in Sexual Functioning Questionnaire (CSFQ) (Clayton *et al.*, 1997a). The CSFQ consists of separate assessments for males and females, and has been validated in healthy and depressed clinic populations, and used in both longitudinal and point prevalence studies of sexual well-being (Clayton, 2002; Clayton *et al.*, 1995, 1997b; Keller *et al.*, 2006). Questions on the CSFQ assess a variety of different causes of change in sexual functioning or desire (e.g., relationship changes, stress level, illness, medications, etc.). Sexual dysfunction is determined by falling below thresholds (47 for males and 41 females on a scale of 14–70), where lower scores are indicative of decreased sexual desire or functioning. The CSFQ also consists of subscales to assess specific aspects of sexual well-being. The pre-defined primary outcome variable for this study was sexual dysfunction in both males and females as defined by falling below the sex-specific total score thresholds mentioned above. Arousal and orgasm subscale measures were secondary outcomes assessed as continuous variables in males and females

separately. Results of the self-administered CSFQ allowed us to categorize whether a subject had sexual dysfunction associated with antidepressant treatment in 101 out of 115 (88%) of subjects, while arousal and orgasm score data were complete in 113 out of 115 (98%) and 114 out of 115 (99%) participants, respectively.

Genotyping

Genomic DNA was isolated from buccal cells collected with cheek brushes (Cyto-Pak, Medical Packaging Corp., Camarillo, CA, USA) with a previously described protocol (Richards *et al.*, 1993). Two common variations in the serotonin transporter (*SLC6A4*) gene were the focus of this investigation. These included an insertion/deletion variant in the promoter region (5HTTLPR) and a variable number of tandem repeats (VNTRs) in the second intron (STin2) known to be a regulator of gene transcription (Fiskerstrand *et al.*, 1999; Heils *et al.*, 1996; MacKenzie and Quinn, 1999). A previously described functional variant (rs6311 also known as -1438G/A) in the promoter region of the serotonin-2A receptor gene (*HTR2A*) was also assessed in these participants, as it has been previously associated with SSRI-associated sexual dysfunction (Bishop *et al.*, 2006). Polymerase chain reaction (PCR) primers and determination of the 5HTTLPR insertion/deletion variant in the *SLC6A4* promoter region were done with a previously described method (Heils *et al.*, 1996). STin2 VNTR genotypes were determined using previously described primers (Wendland *et al.*, 2006) with PCR conditions according to the following specifications: 95°C for 15 min, 94°C for 30 s, 80°C for 15 s, 70°C for 15 s, 60°C for 90 s, 80°C for 60 s, and a final extension at 80°C for 10 min with 500 nM concentration of each primer. PCR products were visualized by electrophoresis on 3.5% agarose gels stained with ethidium bromide. The genetic analysis for the *HTR2A* -1438G/A variant was done according to previously described method (Bishop *et al.*, 2006).

Data analysis

Differences in sociodemographic variables between medication categories and genotype groups were determined with Pearson's χ^2 or Student's *t*-test/one-way analysis of variance for dichotomous and normally distributed continuous variables, respectively. It was determined *a priori* that the primary genetic analyses would consist of a comparison of 5HTTLPR *ll* to

Table 1. Antidepressant dosing

Medication	Mean dose (SD)	Dose category ^a (% higher)	Dose range (mg/day)
Citalopram	31.4 (10.3)	57	20–40
Escitalopram	15.0 (6.9)	39	10–40
Fluoxetine	29.6 (17.4)	24	10–80
Paroxetine	22.5 (10.5)	18	10–45
Sertraline	77.6 (41.4)	41	50–200

^aDose categories were designed to evaluate the effect of medication dose on sexual dysfunction outcomes. For the purposes of this investigation, "higher" doses of SSRI medications were defined as ≥ 20 mg/day of escitalopram; ≥ 40 mg/day of citalopram, fluoxetine, or paroxetine; and ≥ 100 mg/day of sertraline.

ls + *ss* genotype groups. Comparisons of STin2.12/STin2.12 to STin2.10 or STin2.9 carriers were completed based on previous studies showing relatively higher 5HTT gene transcription in mice and cell lines containing the STin2.12 variant (Fiskerstrand *et al.*, 1999; MacKenzie and Quinn, 1999).

Spearman's correlation coefficients were calculated to identify potential confounders between clinical and demographic characteristics and sexual functioning outcome measures. Univariate logistic regression analyses and Wald χ^2 test statistics and 95% confidence intervals (CIs) were completed to determine the associations of clinical and genetic variables with sexual dysfunction. A multiple logistic regression analysis was then performed to examine the association of 5HTTLPR and STin2 VNTR genotype groups with sexual dysfunction after controlling for gender and HAMD scores, which were the other two participant characteristics significantly associated with sexual dysfunction outcome measures. An exploratory analysis of the relationship of SSRI-associated sexual side effects in females stratified by oral contraceptive (OC) status across 5HTTLPR genotype groups was then completed based on previously described relationships between serotonin function and menstrual hormones (Meston and Frohlich, 2000; Wihlback *et al.*, 2004).

A subset of the subjects included in this analysis was part of a previous study (Bishop *et al.*, 2006) where we reported an association of sexual dysfunction with the *HTR2A* promoter -1438G/A variant. Although the assessment of *HTR2A* genotypes was not the primary aim of this study, we conducted a *post hoc* analysis including *HTR2A* -1438 genotypes in a final prediction model along with 5HTTLPR genotypes to determine whether these two genetic factors are independent predictors of dysfunction in this study sample. Additional variables of the logistic regression analyses included gender and HAMD scores. The Wald χ^2 test statistic was used to determine whether a significant interaction between the two genetic variables was present in the context of a logistic model regression 5HTTLPR *ll*, *HTR2A* GG, and the interaction term of *ll* \times GG on sexual dysfunction.

A study sample size ≥ 96 was prospectively determined by calculating the total number of subjects needed to detect a 25% difference in the prevalence of sexual dysfunction between 5HTTLPR *ll* homozygotes and *ls/ss* genotype groups using a power of 0.80, and two-tailed alpha values of ≤ 0.05 were used as the threshold for statistical significance. SAS JMP version 5.01a (SAS Institute, Inc., Cary, NC, USA, 2002) was used for all statistical analyses.

RESULTS

One hundred fifteen subjects participated in this study. The prevalence of SSRI-associated sexual dysfunction in the entire study sample was 36% (36/101). Table 1 summarizes medication-dosing characteristics. Sexual dysfunction (as measured by failing to exceed the gender-specific threshold on the CSFQ Total score) associated with SSRI treatment was reported in 42% (32/77) of females and four out of 20 (17%) males ($\chi^2 = 4.9$, 1 df, $p = 0.03$). There were no differences in sexual dysfunction or any CSFQ subscale measure between SSRI medication groups in either the whole population or in gender subgroups. Seventy-three (63%) of the participants in this study were receiving a "lower" dose of SSRI. There were no differences in CSFQ outcomes between persons on "lower" or "higher" doses of SSRI. HAMD score was significantly associated with sexual dysfunction in the study sample [odds ratio (OR) = 19.9; 95% CI 1.9, 265; $p = 0.02$]. In females, there was no relationship with OC status and HAMD scores ($F = 0.03$, 1 df, $p = 0.86$) or the likelihood of being categorized as having sexual dysfunction ($\chi^2 = 1.0$, 1 df, $p = 0.31$).

Genotype counts (frequencies) for the 5HTTLPR variant were 47 *ll* (0.41), 46 *ls* (0.40), and 22 *ss* (0.19), which did not deviate significantly from values expected by Hardy-Weinberg Equilibrium (HWE) ($\chi^2 = 3.0$, 2 df, $p = 0.09$). For the STin2 VNTR, genotype counts were 2 (STin2.9/STin2.10), 34 (STin2.10/STin2.10), 3 (STin2.9/STin2.12), 38 (STin2.10/STin2.12), and 38 (STin2.12/STin2.12), which deviated from HWE ($\chi^2 = 10.6$, 3 df, $p = 0.014$). Subject characteristics did not differ significantly between *ll* and *ls/ss* genotype groups with the exception of gender, where the *ll* genotype group contained a higher proportion of females than the *ls/ss* group (see Table 2). No statistically significant differences in these variables were observed between VNTR genotype groups (see Table 3).

The *ll* genotype was significantly associated with the presence of sexual dysfunction in this study sample (OR = 2.7; 95% CI 1.2, 6.4; $p = 0.02$), while there was no association of STin2.12/STin2.12 genotype with this outcome (OR = 1.3; 95% CI 0.53, 3.0; $p = 0.59$). After controlling for sex and HAMD scores, subjects carrying the *ll* genotype remained significantly more likely to be categorized as having sexual dysfunction than persons with a *ls* or *ss* genotype (OR = 2.8; 95% CI 1.1, 7.2; $p = 0.03$). Adjusting for these variables did not influence the significance of the VNTR variant on the likelihood of being categorized as having sexual dysfunction (OR = 1.4; 95% CI 0.5, 3.4; $p = 0.50$).

Table 2. Subject characteristics stratified by serotonin transporter promoter insertion/deletion genotype group

Measure	Entire study sample N = 115 Mean (SD)	5HTTLPR promoter insertion/deletion genotypes (<i>l</i> = long, <i>s</i> = short)		Test statistic
		<i>ll</i> (N = 47) Mean (SD)	<i>ls/ss</i> (N = 68) Mean (SD)	
Age	29.2 (5.8)	26.0 (5.4)	26.3 (6.1)	($F = 0.05$, 1 df, $p = 0.82$)
HAMD	5.9 (3.2)	5.6 (3.3)	6.0 (3.1)	($F = 0.35$, 1 df, $p = 0.55$)
HAMA	5.7 (3.0)	5.5 (3.3)	5.9 (2.8)	($F = 0.50$, 1 df, $p = 0.48$)
Treatment duration (years)	1.5 (2.2)	1.5 (2.3)	1.6 (2.0)	($F = 0.03$, 1 df, $p = 0.87$)
Measure	N (%)	N (%)	N (%)	
Sex				
Female	87 (76)	41 (87)	46 (68)	($\chi^2 = 5.8$, 1 df, $p = 0.03$)
Race				
Caucasian	106 (92)	44 (94)	62 (91)	($\chi^2 = 2.3$, 5 df, $p = 0.81$)
Hispanic	4 (3)	2 (4)	2 (3)	
Asian	2 (2)	1 (2)	1 (1)	
African-American	1 (1)	0 (0)	1 (1)	
Other	2 (2)	0 (0)	2 (3)	
Marital status				
Single	85 (74)	35 (74)	50 (74)	($\chi^2 = 0.013$, 1 df, $p = 0.91$)
Children				
≥ 1 child	30 (15)	10 (21)	6 (9)	($\chi^2 = 3.6$, 1 df, $p = 0.06$)
Smoker (any smoking within past 6 weeks)				
Yes	15 (13)	8 (17)	7 (10)	($\chi^2 = 1.1$, 1 df, $p = 0.40$)
Dose category				
Low	73 (63)	30 (64)	43 (64)	($\chi^2 = 0.004$, 1 df, $p = 0.95$)
Medication				
Citalopram	14 (12)	5 (11)	9 (13)	($\chi^2 = 1.9$, 4 df, $p = 0.76$)
Escitalopram	36 (31)	12 (26)	24 (35)	
Fluoxetine	25 (22)	11 (23)	14 (21)	
Paroxetine	11 (10)	5 (11)	6 (9)	
Sertraline	29 (25)	14 (30)	15 (22)	
Alcohol use ($>5\times$ in past month)				
Yes	30 (26)	13 (28)	17 (25)	($\chi^2 = 0.10$, 1 df, $p = 0.75$)
Oral contraceptive status				
Yes	48 (55)	25 (61)	23 (50)	($\chi^2 = 1.1$, 1 df, $p = 0.39$)

HAMA, Hamilton Rating Scale for Anxiety; HAMD, Hamilton Rating Scale for Depression 21 item version; 5HTTLPR, Serotonin transporter gene promoter region.

CSFQ arousal scores were then compared across genotype groups in males and females. Mean (SD) scores, out of a maximum of 15, were 9.5 (2.5) and 9.6 (2.4) for females in *ll* and *ls/ss* genotype groups, respectively ($F = 0.04$, 1 df, $p = 0.85$). For males, arousal scores were 13.3 (1.6) and 11.8 (2.0) for those in the *ll* and *ls/ss* genotype groups, respectively ($F = 2.9$, 1 df, $p = 0.1$).

Mean scores on the orgasm subscale of the CSFQ (out of a maximum of 15) were 8.5 (3.4) and 10.2 (2.9) for the *ll* and *ls/ss* genotype groups in females ($F = 6.7$, 1 df, $p = 0.01$), respectively. For males, scores averaged 12.8 (0.8) and 10.4 (1.9) for the *ll* and *ls/ss* groups ($F = 8.8$, 1 df, $p = 0.007$), respectively. There were no significant differences in arousal or orgasm scores across STin2 VNTR genotype groups. Arousal scores in females averaged 9.7 (2.2) and 9.5 (2.5) for STin2.12/STin2.12 homozygotes and non-STin2.12 carriers, respectively ($F = 0.16$, 1 df, $p = 0.69$). In males, mean arousal scores were 11.8 (2.3) and 12.3

(1.8) for STin2.12/STin2.12 homozygotes and non-STin2.12 carriers, respectively ($F = 0.43$, 1 df, $p = 0.52$). Orgasm scores in females averaged 9.6 (2.8) and 9.3 (3.5) for STin2.12/STin2.12 homozygotes and non-STin2.12 carriers, respectively ($F = 0.08$, 1 df, $p = 0.78$). In males, mean orgasm scores were 10.1 (2.1) and 11.3 (1.9) for STin2.12/STin2.12 homozygotes and non-STin2.12 carriers, respectively ($F = 2.3$, 1 df, $p = 0.14$).

Forty-eight of the females with completed CSFQ data in this study were taking OC medications, while 39 were not. These were predominantly estrogen or estrogen/progestin formulations, with three subjects taking depo-progestin-only formulations. The relationship of the 5HTTLPR genotype with sexual dysfunction in these two groups is summarized in Table 4. Briefly, in women taking OCs, those with the *ll* genotype were nearly eight times more likely to be categorized as having sexual dysfunction ($p = 0.007$), while no significant relationship was observed in those

Table 3. Subject characteristics stratified by serotonin transporter intron 2 variable number of tandem repeats genotype group

Measure	STin2 VNTR genotype group (STin2.9, STin2.10 = carriers of the 9 or 10 repeat alleles, STin2.12/STin2.12 = homozygous for 12 repeats)		Test statistic
	STin2.9, STin2.10 (N = 77) Mean (SD)	STin2.12/STin2.12 (N = 38) Mean (SD)	
Age	26.9 (5.9)	24.7 (5.2)	($F = 3.9$, 1 df, $p = 0.05$)
HAMD	5.9 (3.1)	5.7 (3.5)	($F = 0.21$, 1 df, $p = 0.65$)
HAMA	5.9 (3.1)	5.3 (2.9)	($F = 0.94$, 1 df, $p = 0.33$)
Treatment duration (years)	1.6 (2.4)	1.3 (1.6)	($F = 0.53$, 1 df, $p = 0.47$)
Measure	N (%)	N (%)	
Sex			
Female	58 (75)	29 (76)	($\chi^2 = 0.014$, 1 df, $p = 0.91$)
Race			
Caucasian	73 (95)	33 (87)	($\chi^2 = 8.9$, 5 df, $p = 0.11$)
Hispanic	3 (4)	1 (3)	
Asian	0 (0)	3 (5)	
African-American	1 (1)	0 (0)	
Other	0 (0)	2 (5)	
Marital status			
Single	55 (71)	30 (79)	($\chi^2 = 0.75$, 1 df, $p = 0.68$)
Children			
≥ 1 child	10 (21)	6 (9)	($\chi^2 = 3.6$, 1 df, $p = 0.06$)
Smoker (any smoking within past 6 weeks)			
Yes	10 (13)	5 (13)	($\chi^2 = 0.001$, 1 df, $p = 0.098$)
Dose category			
Low	51 (66)	22 (58)	($\chi^2 = 0.76$, 1 df, $p = 0.38$)
Medication			
Citalopram	10 (13)	4 (11)	($\chi^2 = 1.8$, 4 df, $p = 0.78$)
Escitalopram	26 (34)	10 (26)	
Fluoxetine	16 (21)	9 (24)	
Paroxetine	8 (10)	3 (8)	
Sertraline	17 (22)	12 (32)	
Alcohol use ($>5\times$ in past month)			
Yes	21 (27)	9 (24)	($\chi^2 = 0.17$, 1 df, $p = 0.68$)
Oral contraceptive status			
Yes	29 (50)	10 (34)	($\chi^2 = 1.9$, 1 df, $p = 0.25$)

HAMA, Hamilton Rating Scale for Anxiety; HAMD, Hamilton Rating Scale for Depression 21 item version; 5HTTLPR, serotonin transporter gene promoter region.

not taking OCs ($p = 0.42$). When the CSFQ arousal and orgasm subscale measures were assessed, scores did not differ based on OC status (both $ps > 0.30$). The relationship of these subscale outcomes based on OC status was then stratified by 5HTTLPR genotype group. For females taking OCs, arousal scores averaged 9.2 (2.8) and 9.4 (2.2) in the *ll* and *ls/ss* groups, respectively ($F = 0.14$, 1 df, $p = 0.71$). Arousal scores in females not taking OCs averaged 10 (2.1) and 9.7 (2.5) for *ll* and *ls/ss* groups ($F = 0.11$, 1 df,

$p = 0.74$), respectively. Orgasm scores averaged 8.0 (2.7) and 10.3 (2.9) for *ll* and *ls/ss* groups ($F = 8.4$, 1 df, $p = 0.006$), respectively. Orgasm scores in those not taking OCs averaged 9.3 (4.3) and 10.1 (2.9) for *ll* and *ls/ss* groups ($F = 0.59$, 1 df, $p = 0.45$).

In a previous publication, we reported a positive association of a functional variant in the serotonin-2A receptor (*HTR2A*) gene with sexual dysfunction (Bishop *et al.*, 2006). Therefore, in a *post hoc* assessment of subjects from this study, we conducted a logistic regression analysis including both 5HTTLPR and *HTR2A* -1438 genotypes while controlling for gender and HAMD scores. In this context, the 5HTTLPR *ll* and *HTR2A* -1438 GG genotypes were both significantly associated with sexual dysfunction in this study sample (OR = 3.2; 95% CI 1.3, 8.8; $p = 0.02$) and (OR = 3.2; 95% CI 1.1, 10.0; $p = 0.04$), respectively. A subsequent model did not indicate that a significant interaction between these two genetic predictors was present (Wald $\chi^2 = 0.80$, 1 df, $p = 0.40$).

Table 4. Relationship of serotonin transporter genotypes with sexual dysfunction in females taking oral contraceptives

Genotype	Taking oral contraceptives (n = 41)		p-value
	OR	95% CI	
5HTTLPR <i>ll</i>	7.8	1.9, 41.0	0.007
Genotype	Not taking oral contraceptives (n = 36)		p-value
	OR	95% CI	
5HTTLPR <i>ll</i>	1.7	0.45, 7.0	0.42

DISCUSSION

The primary finding of this investigation is that the serotonin transporter promoter insertion/deletion genotype is associated with sexual dysfunction in this study sample of subjects aged 18–40 years without other significant risk factors such as other major psychiatric illnesses, medical diagnoses, or medications affecting this outcome. This was assessed with the use of the CSFQ, a tool validated in a similar population as the one assessed here in similar study designs. This relationship between genotypes and sexual dysfunction was still present after controlling for other important variables such as sex and depression symptoms as assessed with the HAMD. No relationships between medication, medication dose category, smoking history, substance use, or other sociodemographic variables were observed. Nor were significant associations between the STin2 VNTR of the serotonin transporter gene and sexual dysfunction outcomes. On further analysis, it appears that the relationship between promoter region genotypes and sexual well-being in the context of SSRI treatment may differ in males and females. Arousal and orgasm scores in males were higher (better) in those with the *ll* genotype, while in females, those with the *ll* genotype had significantly lower (worse) scores. Our ability to characterize the exact nature of this relationship in males is limited by the smaller number of males who volunteered to participate in this study and should be the subject of future investigations.

We previously published findings highlighting the relationship of the *HTR2A* -1438 GG genotype with SSRI-associated sexual dysfunction (Bishop *et al.*, 2006). Therefore, we conducted a *post hoc* analysis of the *HTR2A* variant in this larger sample to determine if this relationship was still present and further if there was any interaction with the serotonin transporter variants that were the primary focus of the current investigation. When *HTR2A* genotypes were added to the logistic regression model including gender, HAMD, and 5HTTLPR genotypes, both *HTR2A* GG and 5HTTLPR *ll* genotypes were independent predictors of SSRI-associated sexual dysfunction in this study sample.

Previous investigations of the relationship of 5HTTLPR genotypes with response and side effect outcomes in patients taking SSRIs have largely associated the *ll* genotype with both better response and fewer side effects in Caucasian study samples (Serretti *et al.*, 2007; Smits *et al.*, 2007). Our primary results, particularly in females as a whole, are therefore contrary to these findings. Interestingly, ovarian

hormones may influence serotonergic transmission in the body. Important in the context of the population targeted by our recruitment strategies are previous studies showing that estrogen and progesterone changes throughout the menstrual cycle affect serotonin reuptake and 5HT_{2A} binding (Wihlback *et al.*, 2004). Recent reports suggest that 5HTT expression may differ between males and females based on methylation differences (Philibert *et al.*, 2007) and furthermore that ovarian hormones may affect the binding of SSRIs to 5HTT (Benmansour *et al.*, 2009).

We found no relationship between OC status and mood or sexual well-being in the absence of stratification by 5HTTLPR genotypes. However, the relationship of 5HTTLPR genotype status to SSRI-associated sexual dysfunction in females appears to differ based on whether females were taking an OC medication. Specifically, there was no genotype–sexual dysfunction relationship in females not taking OCs, while those taking OCs were nearly eight times more likely to be categorized as having SSRI-associated sexual dysfunction if they were homozygous 5HTTLPR *ll*. Further, this appears to be driven by differences observed in the CSFQ orgasm subscale, where significant differences were observed across 5HTTLPR genotype groups in females taking OCs. A difference in arousal scale scores across genotype groups was not observed in women when they were stratified by OC status. The reasons for this are unclear. However, serotonin functioning has been related to orgasm difficulties in both males and females and is deleteriously affected by SSRIs in some patients (Clayton *et al.*, 2006; Meston and Frohlich, 2000). In non-human primates, treatment with OC formulations has been shown to reduce the expression of serotonin transporter mRNA (Bethea *et al.*, 1998). Perhaps the effect of serotonin transporter expression by OCs differs based on 5HTTLPR genotype, which has also been shown to affect gene expression (Bradley *et al.*, 2005; Philibert *et al.*, 2007). Our ability to further examine this relationship and its underlying mechanisms are limited in the context of the present investigation.

The enrollment of female subjects was done without inquiry of time of menstrual cycle, although irregular menses was an exclusion criterion. While we did not prospectively match females based on OC status, there were not significant differences in variables influencing SSRI-associated sexual difficulties such as HAMD scores or other sociodemographic variables examined with the exception of age. Women not taking OCs were on average about 3 years older than those who were taking OCs (24.4 vs. 27.7 years) which while

statistically different ($p=0.004$) is not likely to be clinically significant in the context of this study. Age was not a significant predictor of SSRI-associated sexual difficulties in the present sample although it is known that individuals older than 40 years are more likely to have sexual difficulties than younger individuals for a variety of physiological, medical, and environmental reasons (Clayton *et al.*, 2002), which does not explain the relationship seen here. It is possible that some unknown or uncollected characteristics of women taking OCs and those who were not may explain these results, but we feel that this is unlikely, particularly in the context of this study of persons at low risk for sexual difficulties other than taking an antidepressant. Additionally, the documented relationship between serotonin and ovarian hormones is consistent with our findings (Benmansour *et al.*, 2009; Pecins-Thompson *et al.*, 1998; Wihlback *et al.*, 2004).

When interpreting the results of this study, there are other important aspects that warrant consideration. We attempted to minimize the potential confounding effects of depressive symptoms on sexual functioning, by enrolling subjects who had been on SSRIs for at least 6 weeks, in order for them to have an adequate chance to respond to medication. While this was obviously achieved in our study (mean HAMD scores = 5.9), we may have missed subjects who discontinued an SSRI before 6 weeks because of sexual side effects. It is our opinion that missing these subjects is more likely to have diluted the effect that was seen in this sample than result in a spuriously positive outcome. It should also be noted that while we required a minimum of 6 weeks of SSRI treatment for study entry, the treatment duration was variable in our population. It is possible that some of the subjects may have had a gradual improvement in their sexual functioning over time, which could have also lowered the point prevalence sexual dysfunction values observed and possibly minimized the pharmacogenetic relationship seen. However, sexual difficulties from SSRIs are known to be persistent, with most patients not experiencing improvement or alleviation of this unfortunate side effect over time (Montejo *et al.*, 2001a; Montejo-Gonzalez *et al.*, 1997). Finally, the STin2 VNTR marker assessed in this study sample deviated slightly from HWE, which may be viewed as a source of population stratification. Because our study sample was predominantly composed of Caucasian participants, and the 5HTTLPR variant did not deviate from HWE, it is our feeling that this is not likely due to stratification. One explanation of the presence of more

of the variant alleles than are normally observed is that we selected our study participants based on MDD status, and previous reports have suggested that rare VNTR alleles are overrepresented in some depressed populations (Battersby *et al.*, 1996; Ogilvie *et al.*, 1996).

Sexual dysfunction is arguably the most clinically significant and most common SSRI-related adverse event in persons taking these medications. Therefore, identifying who may be at an increased risk for this disconcerting outcome is extremely important. In the present study, we are the first to describe the relationship of 5HTTLPR variants specifically with this outcome as assessed by a validated measure, in a model that also holds when including the previously described relationship with the *HTR2A* -1438 variant. Moreover, this is the first report to indicate that SSRI-associated outcomes may differ by genotype differently in the context of other commonly used medications like OCs. We utilized a previously validated assessment to determine not only the overall measure of sexual health in these subjects, but also the different aspects of sexual well-being. Our subject population was almost completely devoid of confounding medical and pharmacological confounders, which is extremely rare for this type of investigation. Many studies analyzing sexual dysfunction related to antidepressant therapy do not exclude persons based on concurrent medications or other diagnoses. Although that strategy increases the generalizability of a study, employing that methodology in the current study would have made it extremely difficult to determine the relative effects of genetics and medical variables that play a role in a person's overall state of sexual well-being.

In conclusion, we report here that a common genetic variant in the promoter region of the serotonin transporter is associated with SSRI-associated sexual dysfunction. This relationship appears to differ in males and females and may depend on whether a female is taking an OC medication. These results warrant replication in larger study samples but exemplify how pharmacogenetics may eventually allow us to predict important clinically significant adverse events in patients taking SSRI medications.

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