Depression Persistence and Serotonin Transporter Genotype in Adolescents under Usual Care Conditions

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Abstract

Objectives: Polymorphisms of the serotonin transporter (SERT) gene have been shown to influence the risk for depression. The goal of this study was to investigate a possible effect of SERT polymorphisms on severity and course of depression symptoms in a community sample of adolescents.

Methods: Community-dwelling adolescents ($n = 192$) ages 13–17 years, who were at risk for depression, were followed for a period of 6 months. Subjects donated a saliva sample for genotyping of the 5-HTTLPR and STin2 VNTR polymorphisms of SERT.

Results: We found no associations between SERT genotype and severity of depressive symptoms at baseline. Depression symptom severity markedly decreased over time. For 5-HTTLPR, we observed a significant interaction between time and genotype, indicating the possibility that heterozygote genotype carriers (s/l) might experience a greater reduction in depression symptoms over time compared with adolescents with the 5-HTTLPR l/l genotype.

Conclusions: Our study shows that for most community-dwelling adolescents, depressive symptoms decrease over time. A possible interaction effect of time and SERT genotype will require confirmation in larger studies.

Introduction

DEPRESSION IN ADOLESCENTS IS ASSOCIATED WITH adverse health risk behaviors; impairment in social, familial, and school functioning; and risk of suicide (Jonsson et al. 2011). At the same time, depression symptoms in youth do not always persist, and control arms of treatment trials demonstrate high rates of spontaneous recovery and/or placebo response rates (Malone et al. 2006). The purpose of this study was to investigate whether genetic variants in the serotonin transporter (SERT) gene were associated with depression persistence in a community sample of adolescents, and, therefore, might be useful to identify youth at risk for persistent depression.

SERT is located on the presynaptic membrane, where it limits the intensity and duration of serotonin signaling through reuptake of the neurotransmitter (Torres et al. 2003). SERT has been extensively investigated as a candidate gene for depression, in particular a 43 base pair (bp) polymorphism in the SERT promoter region, 5-HTTLPR. The less well studied STin2 VNTR polymorphism is located in the second intron of the SERT gene and consists of 9, 10, or 12 copies of a 22–23 bp repeat (Battersby et al. 1996; Ogilvie et al. 1996). In adults, a small but significant increase in

depression susceptibility among s-allele carriers, particularly in response to chronic environmental stress such as childhood maltreatment or medical conditions, has repeatedly been shown (Clarke et al. 2010; Kiyohara and Yoshimasu 2010; Karg et al. 2011). In addition, l-allele carriers have better remission and response rates to selective serotonin reuptake inhibitor (SSRI) antidepressants (Porcelli et al. 2012). Despite this, two studies have indicated that adult subjects with the 5-HTTLPR l/l genotype might be at higher risk for experiencing chronic depression (Eaton et al. 2008; Myung et al. 2010). For STin2 VNTR, association of the 9- and 12- repeat alleles with depression have been shown (Ogilvie et al. 1996; Jarrett et al. 2007; Kohen et al. 2008). Far fewer studies on the effects of SERT genetic variants on adolescent depression have been published, and reports on the effect of these genetic polymorphisms on the time course of depression in youth are lacking. A family study of pediatric-onset depressive disorders did suggest an association between the 5-HTTLPR s-allele and depression (Nobile et al. 2004). However, several studies in adolescents have found no main effect of 5-HTTLPR on depressive symptoms (Chipman et al. 2007; Aslund et al. 2009; Benjet et al. 2010). An interaction of the 5-HTTLPR s-allele with environmental stress on youth depression prevalence is better supported

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(Benjet et al. 2010; Hankin et al. 2011; Petersen et al. 2012); however, several investigators have noted this effect only in girls, but not boys (Aslund et al. 2009; Hammen et al. 2010). The goals of our study were to determine whether the 5-HTTLPR and STin2 VNTR polymorphisms of SERT were associated, either alone or in combination, with greater severity of depression and greater persistence of depression symptoms in adolescents over time.

Materials and Methods

Subjects

Study subjects were participants in a longitudinal study of depressive symptoms in adolescents in primary care (the parent study), which has previously been described (Richardson et al. 2010a). In the parent study, depression questionnaires had previously been mailed to 4000 randomly selected community-dwelling adolescents, which led to the identification of 302 participants who 1) were considered at risk for major depression because they scored ≥ 2 on the two item Patient Health Questionnaire (PHQ)-2 depression screening tool, and 2) had completed their baseline assessment and were invited to participate in the longitudinal study. We have previously shown that the PHQ-2 has a sensitivity of 74% and specificity of 75% for detecting major depression among adolescents (Richardson et al. 2010b). Of the invited 302 subjects, 240 agreed to participate in genetic testing (which was an add-on study after the original longitudinal study had been completed) and 192 returned a usable saliva sample. Subjects received \$25 in cash for their participation (\$5 pre-incentive mailed with the saliva collection kit and \$20 mailed with a thank you letter after the kit was returned). All procedures were approved by the Group Health Research Institute Human Subjects Protection Committee.

Measures

Subjects were assessed with the complete nine item Patient Health Questionnaire (PHQ-9) at baseline, after 6 weeks, and again after 6 months. We have previously shown that a PHQ-9 score of ≥ 11 had a sensitivity of 90% and specificity of 78% for detecting youth who met the *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed. criteria for major depression (American Psychiatric Association 1994; Richardson et al. 2010a). In addition, we reviewed group health administrative data about any antidepressant pharmacy fills during the follow-up period. The data indicated whether or not a subject had received at least one antidepressant pharmacy fill, without information about the type of antidepressant given, medication adherence, or the number of refills.

Genotyping

Subjects donated a saliva sample for genetic testing, using Oragene DNA OG-250 format mail-in collection kits (DNA Genotek Inc., Ontario, Canada); DNA was extracted from 0.5 mL saliva according to the manufacturer's protocol. Genotyping of 5-HTTLPR and STin2 VNTR was performed as previously described (Jarrett et al. 2007). Investigators performing DNA extraction and genotyping were blind to any subject information.

Data analysis

For statistical analysis, total PHQ-9 scores for depression were considered as the primary outcome variables and different genotypes of 5-HTTLPR and STin2 VNTR were the primary exposure variables. As the 9-repeat allele of STin2 VNTR is rare, and, like

the 12-repeat allele, is thought to be a risk allele for depression, those two alleles were grouped together in the analyses. Two sets of analyses were performed, one in which the two loci, 5-HTTLPR and STin2 VNTR were analyzed separately, and one in which they were grouped together. In the first set of analyses, we compared three genotype groups per variant. For 5-HTTLPR, the three genotype groups were l/l (no risk allele), s/l (one risk allele), and s/s (two risk alleles); for STin2 VNTR the three genotype groups were 10/10 (no risk allele), 9/10 or 10/12 (one risk allele), and 9/12 or 12/12 (two risk alleles; the genotype 9/9 was not observed in our sample). In the second analysis, we used an additive model with the number of risk alleles (5-HTTLPR s, STin2 VNTR 9-repeat or 12-repeat, total ranging from 0 to 4) as exposure variable. As PHQ-9 scores demonstrated a large deviation from normality, being bounded and skewed to the right, we chose more robust nonparametric tests such as Wilcoxon and Kruskal–Wallis tests to compare them across groups. We compared continuous variables between groups using Wilcoxon tests and categorical variables using χ^2 tests. Deviations from Hardy–Weinberg equilibrium in both genotypes were examined using χ^2 tests. Kruskal–Wallace tests were used to compare baseline depressive symptom score (PHQ-9) across genotype groups. In keeping with our prior findings, a PHQ-9 score of ≥ 11 was considered to indicate probable major depression (Richardson et al. 2010a). Genotype groups were compared by depression status using χ^2 tests. We conducted longitudinal analyses to study the genotype effect on PHQ-9 score over time. We ran regression models with PHQ-9 score as the dependent variable, with genotype, antidepressant pharmacy fills (yes/no), time, and interaction between time and genotype as predictors. To account for the within-subject correlation caused by repeated assessments of PHQ-9, we used a generalized estimating equation (GEE) approach. All analyses were done using the R statistical software version 2.12.2 (R Development Core Team 2010).

Results

The average age at entry into the study was 15.3 ± 1.3 years, and 32% ($n=62$) of our 192 subjects had probable major depression (PHQ-9 ≥ 11) at baseline. Female subjects ($n=109$) had higher baseline PHQ-9 scores (9.4 ± 5.4) than did males ($n=83$, 7.5 ± 5.3 , Wilcoxon test $p=0.003$) and were more likely to meet threshold criteria for probable major depression (39% of females vs. 23% of males, Pearson $\chi^2 p=0.015$).

Most (189) of our participants were successfully genotyped for 5-HTTLPR and 190 for STin2 VNTR. In the remaining subjects, genotyping failed because of insufficient DNA extracted from saliva as a result of sample collection errors. The majority of our successfully genotyped participants identified themselves as white ($n=146$), followed by Asian ($n=8$), African American ($n=5$), or multiracial/other ($n=30$); ethnicity data from one participant was missing. Differences in baseline PHQ-9 and prevalence of probable major depression in white versus nonwhite subjects were not significant. Genotype distributions for 5-HTTLPR and STin2 VNTR were in Hardy–Weinberg equilibrium, and did not differ significantly by race (not shown). However, as population differences in genotype distribution have been previously described for 5-HTTLPR and STin2 VNTR (Gelernter et al. 1997; Noskova et al. 2008), we conducted our analyses twice, once for all subjects, and once for those identifying themselves as white only, to control for the effects of population stratification.

There were no significant associations between 5-HTTLPR and baseline PHQ-9 score (Kruskal–Wallis $p=0.56$) or depression

status (Pearson χ^2 $p=0.25$). Likewise, there were no significant associations between STin2 VNTR and baseline PHQ-9 (Kruskal–Wallis $p=0.3$) or depression status (Pearson χ^2 $p=0.66$). The total number of combined 5-HTTLPR and STin2 VNTR risk alleles was also not significantly associated with either baseline PHQ-9 (Kruskal–Wallis $p=0.93$) or depression status (Pearson χ^2 $p=0.48$). Results remained nonsignificant when only white subjects were analyzed (not shown).

Sixteen subjects had at least one antidepressant drug fill during our follow-up period. There were no significant associations between either 5-HTTLPR, STin2 VNTR, or the number of risk alleles and antidepressant drug fills (not shown).

We observed a decreasing severity of PHQ-9 scores over time when all subjects were considered (Fig. 1) as well as when considering only those who met study criteria for depression at baseline. We conducted separate generalized estimation equation models for 5-HTTLPR, for STin2VNTR, and for the number of 5-HTTLPR + STin2 VNTR combined risk alleles (Table 1). For 5-HTTLPR, we observed a significant modification by time for the s/l genotype compared with the l/l genotype that served as reference category ($p=0.0035$ for all subjects, $p=0.015$ for white subjects only). When only subjects who were depressed at baseline were

analyzed, the modification by time for the s/l genotype compared with the l/l genotype was no longer significant ($p=0.071$ for all subjects, $p=0.2$ for white subjects only). We did not find any significant effect modification of time on PHQ-9 scores by STin2 VNTR genotype groups, or total number of risk alleles, regardless of whether the entire group of adolescents was investigated or only those meeting depression criteria at baseline. Results remained essentially the same when only white subjects were analyzed (not shown).

Discussion

In this study, we investigated the effect of two SERT gene polymorphisms, 5-HTTLPR and STin2 VNTR, on depressive symptoms in adolescents sampled in a primary care setting. Subjects were community-dwelling adolescents judged to be at risk for depression based on a screening test (PHQ-2), but only a minority of subjects (62 of 192) had clinically significant depression symptoms. Only a small fraction of our subjects (16 of 192) received antidepressant drug treatment during our follow-up period, in keeping with prior reports that only a fraction of depressed subjects in primary care receive antidepressant pharmacotherapy

FIG. 1. Mean PHQ-9 scores at each time point (baseline, 6, and 24 weeks) for genotype groups at both SERT polymorphisms (upper: 5-HTTLPR; lower: STin2 VNTR), with vertical bars representing 95% CIs.

TABLE 1. GENERALIZED ESTIMATION EQUATION MODELS
LONGITUDINAL ANALYSIS

	<i>All subjects</i>			
	<i>Coefficient</i>	<i>SE</i>	<i>p value</i>	<i>Effect size</i>
5-HTTLPR:				
l/l	Ref			
s/l	0.91	0.80	0.26	1.13
s/s	1.21	1.06	0.25	1.15
Time	-0.05	0.02	0.01	-2.51
Any drug fills	2.15	1.34	0.11	1.60
Genotype $\times$ Time:				
s/l $\times$ Time	-0.07	0.03	<0.01	-2.92
s/s $\times$ Time	-0.03	0.04	0.52	-0.65
STin2 VNTR:				
10/10	Ref			
9/10 or 10/12	-0.15	1.10	0.89	-0.14
9/12 or 12/12	-1.48	1.05	0.16	-1.41
Time	-0.10	0.03	<0.001	-4.04
Any drug fills	2.11	1.33	0.11	1.59
Genotype $\times$ Time:				
9/10 or 10/12 $\times$ Time	0.00	0.03	0.88	0.15
9/12 or 12/12 $\times$ Time	0.03	0.03	0.30	1.04
Number of risk alleles:	-0.07	0.31	0.82	-0.23
Time	-0.08	0.02	<0.001	-3.46
Any drug fills	2.03	1.36	0.14	1.48
Number of risk alleles $\times$ Time	0.00	0.01	0.88	-0.16
<i>Depressed at baseline</i>				
	<i>Coefficient</i>	<i>SE</i>	<i>p value</i>	<i>Effect size</i>
5-HTTLPR:				
l/l	Ref			
s/l	0.63	1.44	0.66	0.44
s/s	1.08	1.51	0.47	0.72
Time	-0.09	0.06	0.14	-1.46
Any drug fills	1.22	1.72	0.48	0.71
Genotype $\times$ Time:				
s/l $\times$ Time	-0.12	0.07	0.07	-1.80
s/s $\times$ Time	-0.08	0.09	0.39	-0.86
STin2 VNTR:				
10/10	Ref			
9/10 or 10/12	1.13	1.41	0.42	0.80
9/12 or 12/12	-1.14	1.22	0.35	-0.93
Time	-0.15	0.06	0.01	-2.68
Any drug fills	1.29	1.71	0.45	0.76
Genotype $\times$ Time:				
9/10 or 10/12 $\times$ Time	-0.05	0.07	0.52	-0.65
9/12 or 12/12 $\times$ Time	0.01	0.07	0.93	0.09
Number of risk alleles:	-0.19	0.45	0.68	-0.42
Time	-0.14	0.07	0.05	-1.97
Any drug fills	1.20	1.71	0.48	0.70
Number of risk alleles $\times$ Time	-0.01	0.03	0.64	-0.47

Analysis includes subjects of all ethnicities (white only not shown). Effect sizes are indicated by the standardized coefficient.

(Simon et al. 2004). The longitudinal follow-up of a community sample of adolescents receiving usual care, that is, only limited intervention, is a strength of this study, however, as it allowed us to observe the natural trend of spontaneous recovery in mostly antidepressant drug-free subjects.

We found no associations between SERT genotype and severity of depressive symptoms at baseline. Depression symptoms strongly declined over a 6 month follow-up period. Our results indicate that, relative to the reference category of l/l genotype carriers, the PHQ-9 score dropped at a higher rate for those with the s/l genotype. Given our small sample size, this finding must be interpreted with great caution. Subjects who met depression criteria at baseline (PHQ-9 ≥ 11 , $n = 62$) showed a similar trend, but, possibly because of the small size of this subgroup, the effect was no longer significant. Our finding is in keeping with prior studies on depression persistence in adults, in whom the 5-HTTLPR l/l genotype has been associated with higher rates of depression chronicity (Eaton et al. 2008; Myung et al. 2010). We found no association between the STin2 VNTR genotype and level of symptoms over a 6 month follow-up period. We did not genotype the rs25531 variant of SERT, a single nucleotide polymorphism (SNP) located immediately upstream of 5-HTTLPR, because it would have been difficult to show an association in our small sample, given the low minor allele frequency of $\sim 7\%$ for this variant (Wendland et al. 2006).

Taken together, our results indicate that the relationship between SERT genotype and depression in adolescent subjects is complex, whereby a genotype that might protect against the onset of depressive symptoms (5-HTTLPR l/l) could also simultaneously lead to higher persistence of depressive symptoms, once they have developed, when compared with heterozygous genotype carriers (s/l). Hence, a global concept of SERT risk alleles for depression might not be justified, as different genotypes potentially confer different vulnerability to different aspects of the disease. In keeping with this, our tally of 5-HTTLPR + STin2 VNTR alleles previously associated with depression prevalence in adults did not show any significant association with depression symptoms in our youth sample.

Limitations

A major weakness of this study was its small sample size. Moreover, subjects were adolescents at risk for major depression, but only about one third of our participants met criteria for clinically significant depression at baseline. Also, depressive symptoms were assessed based on questionnaire data as opposed to a diagnostic interview. Another weakness in our study design was the relatively short follow-up period of only 6 months, with only two assessments (at 6 weeks and 6 months) made during that time period. In addition, our data on antidepressant treatment during the follow-up period were limited in that they only showed whether a subject had received at least one pharmacy fill, without information about the duration of treatment, subject compliance, or other modes of treatment such as psychotherapy. The rs25531 variant of SERT was not genotyped in this study. Statistical analyses were performed without correction for multiple testing. In addition, we did not explicitly test for population stratification using principal component analysis or logistic regression.

Conclusions

Our study shows that for most community-dwelling adolescents, depressive symptoms tend to decrease significantly over a period of 6 months. Our results suggest the possibility that 5-HTTLPR heterozygote genotype carriers (s/l) might experience a greater reduction in depression symptoms over time compared with adolescents with the l/l genotype; however, given our small sample size, this preliminary finding must be interpreted with great caution.

Disclosures

No competing financial interests exist.

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