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Life stressors and 5-HTTLPR interaction in relation to mid-pregnancy depressive symptoms among African-American women

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Abstract

Objective—In previous analyses of non-Hispanic white women we found a stronger relation between abuse history and mid-pregnancy elevated depressive symptoms in women with the serotonin transporter (5-HTTLPR) S/S genotype. Here we focus on African-American women (N=698). Our inquiry is motivated by racial differences in depression diagnosis/treatment, stressors and frequency of major 5-HTTLPR alleles (S, L_A, L_G).

Methods—Stressful life events (lifetime) and depressive symptoms (current) were ascertained at 15–27 weeks gestation. A Center for Epidemiological Studies Depression score of ≥18 was considered "elevated". Life events were scored together and separated into six sub-constructs. 5-HTTLPR genotypes were grouped as follows: 1) L and S alleles, 2) S-L_G equivalence ("tri- to biallelic") and 3) L_A/L_A, all others, S/S ("high/intermediate/low"). Odds ratios (OR) for "elevated" depressive symptoms-life events (total and sub-constructs) relations were calculated for each genotype grouping.

Results—The prevalence of "elevated" depressive symptoms did not vary by genotype. The relation between stressful life events and "elevated" depressive symptoms was stronger in S/S compared to L_A/L_A genotype (interaction P=0.11). Of the six sub-constructs, only abuse showed a statistically significant gene-environment interaction. The OR for the abuse- "elevated" depressive symptoms association was greater for S/S vs. L_A/L_A (interaction P= 0.03) and in the "tri- to biallelic" grouping (interaction P=0.04). In the "high/intermediate/low" grouping, "low" (S/S) had a higher OR (5.5) than both "intermediate" and "high" (ORs≤2.3) (interaction P=0.10).

Conclusions—These results show the importance of examining racial groups, specific stressful events and different 5-HTTLPR genotype groupings when exploring gene-environment interactions in depression.

Keywords

5-HTTLPR; African American; depressive symptoms; gender; gene-environment interaction; stressful life events; pregnancy; women

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Introduction

Depressive disorders are some of the most common health problems, with a lifetime prevalence of 15–30%, serious morbidity (2004, Hudson, 2004, Thompson *et al.*, 2004) and mortality (Bertolote *et al.*, 2004, Conwell *et al.*, 1996). Women are twice as likely as men to experience depression (Blazer *et al.*, 1994, Robins and Regier, 1991), possibly because of differences in gonadotropic steroid milieu (Steiner *et al.*, 2003) and vulnerability related to interpersonal stressors (Gore *et al.*, 1993, Kendler *et al.*, 2001, Kessler and Mcleod, 1984). During the reproductive period, maternal depression can also affect child health. Maternal depression has been associated with lower birth weight, slower weight gain, higher risk of diarrhea (Rahman *et al.*, 2004) and a higher likelihood of childhood mood and behavior problems (Hammen and Brennan, 2003).

Depression has been correlated with dysregulation of serotonergic pathways (Maes *et al.*, 1995, Muck-Seler *et al.*, 2004, Stockmeier, 2003). A 44-base pair insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) results in a long ‘L’ variant and a short ‘S’ variant. The L variant is associated with higher serotonin transporter (5-HTT) expression (Greenberg *et al.*, 1999, Heils *et al.*, 1996) and higher serotonin (5-HT) uptake (Greenberg *et al.*, 1999, Heils *et al.*, 1996) compared to the S variant. In some case-control studies, the presence of an S allele in the 5-HTTLPR is associated with an increased risk of depression (Cervilla *et al.*, 2007, Hoefgen *et al.*, 2005, Nobile *et al.*, 2004), suicide/self-harm (Bellivier *et al.*, 2000, Courtet *et al.*, 2004, De Lara *et al.*, 2006, Mann *et al.*, 2000) and seasonal affective disorder (Johansson *et al.*, 2003, Rosenthal *et al.*, 1998), although results from other studies do not agree with these findings (Mendlewicz *et al.*, 2004, Pooley *et al.*, 2003, Willeit *et al.*, 2008).

Caspi *et al*. (2003) found that individuals with an S allele (S/S or S/L genotype) who had experienced stressful life events (SLE) were at a higher risk of major depression than those with the L/L genotype who had had similar exposure to stressful life events. Later studies have been mixed; some have shown interactions between the 5-HTTLPR genotype and environmental exposures in association with depression (Eley *et al.*, 2004, Grabe *et al.*, 2005, Kendler *et al.*, 2005, Wilhelm *et al.*, 2006), but at least two large-scale studies did not detect a 5-HTTLPR gene-environment interaction for Major Depressive Disorder (Gillespie *et al.*, 2004, Surtees *et al.*, 2006). Investigators are now beginning to explore the importance of type, severity, and timing of stressful life events when considering these interactions. In previous studies, exposure to specific life events (e.g., childhood maltreatment, unemployment, and chronic illness) differentially affected the strength of the association between depression and the 5-HTTLPR genotype (Caspi *et al.*, 2003, Grabe *et al.*, 2005, Kaufman *et al.*, 2004). In their recent review of relations among variation in the serotonin transporter gene, environment and depression, Caspi *et al*. (2010) summarize the positive and negative findings in human gene-environment interaction studies, advances in human neuroscience/imaging and data from rodent and primate studies and point to the importance of continued efforts to clarify the risk and protective factors underlying psychiatric disorders.

Most investigations of stressful life events, the 5-HTTLPR genotype, and mental health outcomes have been conducted in predominantly white populations (Aguilera *et al.*, 2009, Bukh *et al.*, 2009, Caspi *et al.*, 2003, Eley *et al.*, 2004, Gillespie *et al.*, 2004, Kendler *et al.*, 2005, Laucht *et al.*, 2009, Lazary *et al.*, 2008, Nobile *et al.*, 2009, Ritchie *et al.*, 2009, Sjoberg *et al.*, 2005, Surtees *et al.*, 2006, Wilhelm *et al.*, 2006, Zalsman *et al.*, 2006). One study sampled African Americans, but only males, and the outcome of interest was suicide attempt (Roy *et al.*, 2007). Two other studies included African Americans in the total sample but did not report on race-stratified analyses (Cicchetti *et al.*, 2009, Kaufman *et al.*, 2004).

There is a paucity of data on differences in environmental and genetic risk factors for depression in African Americans (Carrington, 2006). Some studies have shown no significant difference between whites and African Americans in the prevalence of depressive disorders (Oquendo *et al.*, 2001, Oquendo *et al.*, 2004, Somervell *et al.*, 1989). However diagnosis and treatment of depression in African Americans lags far behind that of whites (Simpson *et al.*, 2007, Skaer *et al.*, 2000) and adds to the challenge of comparing race groups. In at least two studies of pregnant women, African Americans have reported higher levels of depressive symptoms compared with whites (Holzman *et al.*, 2006, Orr *et al.*, 2006). The context of depression in African-American women is understudied, yet as a group they are more frequently exposed to multiple chronic stressors such as poverty, racism and sexism (reviewed by Brondolo et al. 2009) which might contribute to depression.

Previously, we reported evidence of an interaction between the 5-HTTLPR genotype and one specific type of stressor, exposure to abuse, in relation to elevated (CES-D ≥ 18) depressive symptoms at mid-pregnancy. Our sample for those analyses included only the non-Hispanic white participants in the Pregnancy Outcomes and Community Health (POUCH) Study and we measured two alleles (L, S) in the 5-HTTLPR gene (Scheid *et al.*, 2007). In addition to the L and S alleles, a third functional allele characterized by an A to G polymorphism in the long allele (referred to as 'L_A' and 'L_G') has been described (Nakamura *et al.*, 2000). In lymphoblastoid cell lines from a clinic population the in vitro gene expression of the L_G allele was closer to that of the S allele than to that of the L_A allele (Hu *et al.*, 2006). The allelic frequency of L_G was higher in African Americans (0.24) compared to that of whites (0.15) (Hu *et al.*, 2006), suggesting it may be especially relevant to consider gene-environment interactions with these 5-HTTLPR allelic variations in African Americans.

In analyses presented here we focus on a sample of African-American women from the POUCH Study. We examine relations among the 5-HTTLPR genotype, stressful life events, and depressive symptoms at mid-pregnancy in a manner similar to our previous report in non-Hispanic white POUCH Study participants, and consider three 5-HTTLPR alleles, L_A, L_G and S using different grouping strategies to test genotype interactions.

Methods

Study Sample

A detailed description of the POUCH Study has been reported elsewhere (Holzman *et al.*, 2001). In brief, this cohort study was designed primarily to assess social and biologic factors along pathways to preterm delivery. Women from any of the 52 participating prenatal clinics in five Michigan communities were invited to participate when they presented for maternal serum alpha-fetoprotein (MSAFP) screening at 15–22 weeks of pregnancy. Additional eligibility criteria included English speaking, at least 15 years of age, study enrollment in the 15^{th} to 27^{th} week of pregnancy, a singleton pregnancy with no known congenital anomalies, and no history of prenatal diabetes mellitus. A sample of interested women was enrolled, including all eligible women with unexplained high MSAFP (7% of cohort), a biomarker of interest because of its link to increased risk of preterm delivery. Study protocols were approved by the Michigan State University Committee for Research on Human Subjects as well as institutional review boards from the nine participating hospitals.

At enrollment women completed in-person interviews with a study nurse and self-administered questionnaires which included self reports of race/ethnicity and detailed psychosocial measures. Blood was collected and stored. Of the 782 African-American women who were enrolled in the POUCH Study, four who carried rare variants of the 5-HTTLPR allele were excluded, one was missing information about depressive symptoms,

and nine were lost to follow-up. Interview data, blood collection, and 5-HTTLPR genotype was completed for 698 African-American POUCH Study participants.

Psychosocial Measures

Depressive symptoms in mid-pregnancy were assessed using the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977). A score of 16 or greater is often considered positive when this screening scale is used in clinical settings (Radloff, 1977). The average mid-pregnancy CES-D score among POUCH Study participants was 12.6 in non-Hispanic whites and 16.7 in African Americans. In our previous report in non-Hispanic white POUCH Study women, we used the top quartile scores of the CES-D (≥ 18) as evidence of 'elevated depressive symptoms' (Scheid *et al.*, 2007). For comparison purposes we chose the same cutoff for 'elevated depressive symptoms' in analyses of African-American POUCH Study women, though this threshold of ≥ 18 captured about 41% of African-American women in our sample.

Stressful life circumstances were queried using an adaptation of the Turner, Wheaton and Lloyd Checklist (Turner *et al.*, 1995). We refer to these circumstances as stressful life events (SLE) though in fact many are chronic stressors. For these analyses we evaluated women's reports of the presence/absence of 14 SLE for three separate periods: childhood, adulthood, and within the previous six months (Scheid *et al.*, 2007). The scores for the SLE construct ranged from 0 to 13 with a mean of 4.7 (s.d.=3.3). We considered any exposure during the lifetime as a 'positive' response. An overall SLE construct score was calculated by adding all 'yes' responses across questions as has been done in other studies of SLE and 5-HTTLPR gene - environment interactions (Caspi *et al.*, 2003, Gillespie *et al.*, 2004). SLE questions were also grouped *a priori* into 'sub-constructs' that were subjected to confirmatory factor analysis that showed adequate fit for six sub-constructs, 'economic', 'abuse', 'substance use', 'legal', 'violence' and 'loss' (Holzman *et al.*, 2006). Women were considered 'exposed' if they answered yes to any of the questions for the sub-construct.

DNA extraction and genotyping

Genomic DNA was prepared from venous samples using the Gentra Systems (Minneapolis, MN) Puregene kit. Genotypes for the 5-HTTLPR S/L polymorphism were determined using the method described by Lesch *et al.* (1996) with the following modification. The primers for Polymerase Chain Reaction (PCR) amplification were 5′-GGT TGC CGC TCT GAA TGC CA and 5′-CAC TGA GCT GGA CAA CCA CG and the cycling conditions were: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denature at 95°C for 30 seconds, anneal at 64°C for 30 seconds and extend at 72°C for 45 seconds with a final extension at 72°C for 4 minutes. The PCR products were separated by 2% agarose gel electrophoresis and the size of the PCR products (L allele – 523 base pair fragment, S allele – 479 base pair fragment) were recorded for each sample. Qiagen Taq DNA Polymerase kit was used due to G-C rich conditions.

Samples containing an L allele were re-amplified as above and digested with the restriction enzyme MSP I (NEB) at 37°C for 2–3 hour and separated by 2% agarose gel electrophoresis yielding the following products: L_A – 340, 120 and 64 base pair fragments; L_G – 174, 166, 120 and 64 base pair fragments plus the 479 base pair S allele when present.

Analytic methods

The 5-HTTLPR genotype was modeled using three approaches: 1) the bi-allelic grouping; 2) a tri-allelic to bi-allelic reclassification in which the expression of the L_G allele is considered equivalent to that of the S allele (Hu *et al.*, 2006, Neumeister *et al.*, 2006, Parsey *et al.*, 2006, Zalsman *et al.*, 2006); and 3) a novel reclassification approach in which S/S

homozygotes are defined as 'low expression', L_A/L_A homozygotes are defined as 'high expression' and the remaining genotype combinations (L_A/L_G, L_A/S, L_G/S) are considered to be 'intermediate expression'. All analyses described below were conducted with each of the three genotype grouping approaches. Within the third grouping approach we also compared just the extremes, i.e. the S/S with the L_A/L_A.

Chi-square tests were used to determine if the prevalence of elevated depressive symptoms differed by 5-HTTLPR genotype groupings, and/or by exposure to SLE. To examine if 5-HTTLPR genotype modified the relationship between SLE and elevated depressive symptoms, logistic regression models (SAS 9.1 Proc Logistic) related the odds of elevated depressive symptoms to the 5-HTTLPR genotype groupings, SLE construct (total score or sub-construct scores) and their interaction. Probabilities of elevated depressive symptoms for a given number of SLE were estimated from these models and plotted to display the differences across genotypes.

Odds ratios were calculated to assess relations among the SLE sub-constructs and elevated depressive symptoms for each 5-HTTLPR genotype, and the Breslow-Day test for homogeneity of the odds ratios was used to determine if there was statistically significant effect modification by genotype. Since all hypotheses were generated a priori in order to replicate the study of white women in the POUCH Study published earlier (Scheid *et al.*, 2007), no adjustments for multiple tests were applied. All p-values are reported in the tables.

In our previous POUCH Study analyses of the 5-HTTLPR genotype in non-Hispanic white participants, 13% of women reported use of sleeping pills, tranquilizers, sedatives and other psychotropic medications during pregnancy, all of which were considered potential treatment for depression/anxiety. In that study we repeated our analyses of gene-environment interactions after removing this small subset and our results were strengthened. In this study of African-American POUCH Study participants, only 42 (6%) women reported use of psychotropic medications during pregnancy. Results from analyses conducted with and without these women were similar and therefore this subset was not removed from the final models presented here.

Results

Sample demographics

In our sample of pregnant African Americans 68% were between 20 and 34 years of age; a similar percentage had 12 or fewer years of education (Table 1). The vast majority of women (80%) had received public health care insurance (Medicaid) at some point and 60% were multiparous. To evaluate the representativeness of our sample, we conducted race-stratified comparisons of POUCH Study data with maternal data recorded on birth certificates from the study communities in the year 2000. We found that the distributions of maternal characteristics (i.e. maternal age, parity, education level, Medicaid insurance use, preterm delivery, previous stillbirth, previous preterm infant, previous low birth weight infant) were very similar, with the exception that the percentage of African-American women over 30 years of age was lower in the POUCH Study sample (14%) than in the communities (21%).

5-HTTLPR genotype, elevated depressive symptoms, and stressful life events

Allele frequencies for the triallelic ($S/L_A/L_G$) 5-HTTLPR genotype were 2.8:5.0:1.9, consistent with that previously published for the African-American population (Hu *et al.*, 2006). The proportion of women with elevated depressive symptoms did not differ significantly by genotype regardless of the approach used to group genotypes (Table 2). The relation between elevated depressive symptoms and SLE score was not significantly

modified by the 5-HTTLPR genotype using the biallelic L, S approach to grouping (P-value for interaction=0.29), the triallelic to biallelic approach (P-value for interaction=0.24), or the grouping approach that assumes those containing the L_G allele have intermediate expression (P-value for interaction=0.27). We compared the genotypes that might represent the extremes of 5-HTTLPR expression, i.e. S/S vs. L_A/L_A, and found that the S/S genotype had a greater risk of elevated depressive symptoms in relation to a higher SLE score, although the interaction was not significant (P-value for interaction=0.11 Figure 1).

5-HTTLPR genotype, elevated depressive symptoms, and stressful life events sub-constructs

Scores for five of the six SLE sub-constructs ('economic', 'abuse', 'substance use', 'legal' and 'violence') were associated with significantly increased odds of elevated depressive symptoms in mid-pregnancy (OR ranging from 1.8 to 2.8, P<0.001 data not shown). The 5-HTTLPR genotype did not appear to modify the risk of depression in association with the 'economic', 'substance use', 'legal', 'violence', or 'loss' SLE sub-constructs. This lack of effect modification was noted in all analyses of these sub-constructs using each of the genotype grouping approaches. The P-value for the test of homogeneity across genotype ranged from 0.22 to 0.94. Results from the 5-HTTLPR triallelic to biallelic grouping example are provided in Table 3. For the association between elevated depressive symptoms and abuse, the odds ratio for the S/S genotype was larger (OR= 5.5, 95% CI 2.0, 14.9) than that found in other genotypes (OR ≤2.3) (Table 4). Heterogeneity of ORs by genotype was statistically significant for the triallelic to biallelic genotype grouping approach (P-value for interaction=0.04), and for the comparison between extremes of 5-HTTLPR expression, i.e. S/S versus L_A/L_A (P-value for interaction=0.03).

Discussion

This study was undertaken to examine relations among polymorphisms of the 5-HTTLPR, stressful life events, and depressive symptoms at mid-pregnancy in African-American women. Many types of stressful life events were associated with elevated depressive symptoms at mid-pregnancy, but only the abuse-elevated depressive symptoms association was modified by the 5-HTTLPR genotype. There was a strong link between reported history of abuse and mid-pregnancy elevated depressive symptoms among women with the S/S genotype, but among women with the L_A/L_A genotype this link was weak and not statistically significant. The use of the triallelic to biallelic genotype grouping resulted in the creation of the L_A/L_A homozygote (highest expression) as the referent group, and led to the detection of a gene-environment interaction that might have been missed had we used only the biallelic 5-HTTLPR grouping.

A comparison of gene-environment interactions across race groups is facilitated when the samples are drawn from the same study. Our earlier study of non-Hispanic white participants from the POUCH Study (Scheid *et al.*, 2007) showed that the association between abuse and elevated levels of depressive symptoms in the subgroup with the S/S genotype was of a similar magnitude (OR=5.3, 95% CI 1.9, 15.2) to that reported here in the African-American POUCH Study participants (OR= 5.5, 95% CI 2.0, 14.9). In the non-Hispanic whites the magnitude of this association increased dramatically (OR=24.5) after removing women who used psychotropic medications, raising the possibility that chance and small numbers inflated this estimate. In contrast, the odds ratio for the abuse-depression association was not altered in African-American women after removing those who used psychotropic medications. Taken together the results from both race groups support our hypothesis about gene-environment interactions in relation to depressive symptoms at mid-pregnancy.

To draw meaningful conclusions regarding the relations between abuse and depression in African-American women it is important that the instrument used to measure depressive symptoms is valid in this population. The screening tool we used to measuring depressive symptoms, the CES-D, has been widely used in samples from different race/ethnic groups. We have conducted our own validity study by comparing CES-D item endorsement and factor structure in non-Hispanic white vs. African-American POUCH Study participants and found that the CES-D measurement properties were comparable across the two race/ethnic groups (Canady *et al.*, 2009). Another study came to a similar conclusion using a nationally representative sample (Nguyen *et al.*, 2004).

Sociodemographic characteristics of women in the POUCH Study varied by race/ethnicity, and these may be important considerations when examining gene-environment interactions. Compared to non-Hispanic white women, African-American women as a group had more risk factors for depression including younger age during pregnancy, lower education level, and lower socioeconomic status (indicated here by enrollment in the Medicaid Insurance program). In addition, African-American women in our study reported a greater number of stressful life events, and were more likely to report stressors from multiple stressor sub-constructs. It is particularly noteworthy that in this African-American sample with greater exposure to adversity we were still able to detect gene-environment interactions between the 5-HTT polymorphism, a history of abuse and elevated depressive symptoms.

Similar to some (Anguelova *et al.*, 2003a, De Lara *et al.*, 2006, Kraft *et al.*, 2007, Mendlewicz *et al.*, 2004, Pooley *et al.*, 2003, Wichers *et al.*, 2008, Willeit *et al.*, 2008, Willis-Owen *et al.*, 2005) but not all studies (Anguelova *et al.*, 2003b, Barton *et al.*, 2008, Nobile *et al.*, 2004) on mental health and the 5-HTTLPR genotype, we found that there was no direct association between genotype and depressive symptoms in our sample of African-American pregnant women. These null results were consistent with those from our earlier study of non-Hispanic white pregnant women. In another recent study of pregnant women, the 5-HTTLPR genotype was unrelated to depression measured in late pregnancy (> 30 weeks) but s allele carriers were at increased risk of depression at 1–8 weeks post partum (Binder *et al.* 2010). Our study may not be directly comparable to studies that sampled non-pregnant populations (Anguelova *et al.*, 2003a, Anguelova *et al.*, 2003b, Barton *et al.*, 2008, De Lara *et al.*, 2006, Kraft *et al.*, 2007, Mendlewicz *et al.*, 2004, Nobile *et al.*, 2004, Pooley *et al.*, 2003, Wichers *et al.*, 2008, Willeit *et al.*, 2008, Willis-Owen *et al.*, 2005) or studies focused on depression diagnosed in clinical populations (Anguelova *et al.*, 2003a, Anguelova *et al.*, 2003b, Barton *et al.*, 2008, De Lara *et al.*, 2006, Kraft *et al.*, 2007, Mendlewicz *et al.*, 2004, Nobile *et al.*, 2004, Pooley *et al.*, 2003, Willeit *et al.*, 2008). Because the CES-D is a screening tool, we could not use our data to infer gene and gene-environment associations with specific depressive diagnoses, or severity of depression.

We first assessed stressful life events by using a measure similar, though not identical to that used by Caspi *et al.* (2003), namely a summing of self-reported stressful life events occurring over the lifespan. In our study of African-American pregnant women, as the number of stressful life events increased the probability of elevated depressive symptoms also increased. The slope of this increase was greatest among women with the S/S genotype, but the gene-environment interaction was not statistically significant. It is possible that this interaction becomes most notable when the number of stressful life events is very high, or the stressful conditions are extreme, or the time from stressor exposure to measurement of depressive symptoms is short. The number of pregnant women within our community sample who met these latter conditions might have been too few to detect a statistically significant, though more modest, gene-environment interaction using a total life events score. Similar challenges related to sample heterogeneity and statistical analysis have been discussed in Caspi *et al.* (2010).

Our use of stressful life event sub-constructs in the POUCH Study has been guided by the hypothesis that relations between stressors and depressive symptoms may vary by the type of stressor, not just the number of stressors. Numerous investigators have reported on the long-term psychiatric sequelae of childhood abuse (reviewed by Heim and Nemeroff, 2001), which may be mediated by neuroanatomical and neurochemical modification (reviewed by Kaufman and Charney, 2001). In our earlier work we noted that self-reports of abuse, particularly during childhood and adulthood, stood out as the exposure most strongly associated with elevated depressive symptoms at mid-pregnancy (Holzman *et al.*, 2006). In addition we have found that among the various stressors, only abuse interacted with the 5-HTTLPR genotype in models of exposures related to elevated depressive symptoms, both in African-American women (results presented here) and in non-Hispanic white women (Scheid *et al.*, 2007). The interaction between 5-HTT polymorphism and abuse, and not the other stressor sub-constructs in relation to depressive symptoms may be related to the traumatic nature of the abuse. Evidence to support this is provided by a recent study of depressive symptoms in an Asian population (Goldman *et al.*, 2010). In that study, the 5-HTTLPR genotype interacted specifically with a measure of traumatic events, and not with a measure of stressful life events. It is possible that among women of reproductive age, such as the POUCH study sample, past or current abuse might be the most common 'traumatic' stressor. It is likely that women who report abuse are a mixed group with varying levels of abuse, some traumatic and some not, but we did not pursue detailed questions that would have captured these distinctions. Similar gene-environment interactions have been shown for 5–HTTLPR, abuse exposure and post traumatic stress disorder for European Americans and African Americans sampled from the general community (Xie *et al.* 2009).

We considered different approaches for grouping the 5-HTTLPR genotypes because of the evolving work on allelic variation and its impact on expression levels within this gene. Historically, studies have used the biallelic S/L genotype of the 5-HTTLPR polymorphism to examine interactions with stressors in relation to mental health. Additional functional allelic variations in 5-HTTLPR (Hu *et al.*, 2006) invite new strategies for examining gene-environment interactions with the 5-HTTLPR genotype. Hu and colleagues found that variants of the L allele resulted in different levels of 5-HTT mRNA expression with L_A similar to L and L_G similar to S (Hu *et al.*, 2006). These findings motivated both our grouping of the six tri-allelic genotypes by levels of expression and reconfiguration to produce a new three-level bi-allelic grouping. The findings by Hu and colleagues (Hu *et al.*, 2006) also suggest that studies using the biallelic S/L genotype of the 5-HTTLPR polymorphism contain some misclassification because they combine lower-expression (L_G) and higher expression (L_A) individuals into one group (L). The extent of misclassification will depend on the sample prevalence of the L_G allele. In our African-American sample, where the allele ratio was calculated as S:L_A:L_G 2.8: 5.0:1.9, similar to that reported by Hu et al. (2006), the presence of the L_G allele is of sufficient frequency to warrant separate grouping of the L_A andL_G alleles. We would not have detected a significant 5-HTTLPR polymorphism-abuse interaction in this African-American sample if we had focused only on the bi-allelic genotype grouping.

Some investigators have chosen to group the L_G and S alleles together (Zalsman *et al.*, 2006), based on Hu's study using genotype-specific mRNA expression in lymphoblast cell lines that showed a statistical equivalence in the basal levels of expression of the S and L_G alleles (Hu *et al.*, 2006). However, in the same report, expression under conditions designed to stimulate or reduce transcription, S and L_G alleles behaved differently (Hu *et al.*, 2006). Another study of the enhancer/silencer function of the polymorphic L_A and L_G alleles showed these to be equivalent (Sakai *et al.*, 2002). These studies suggest that grouping S and L_G alleles together may not be the best approach. We found a larger effect size for the abuse-elevated depressive symptoms when the at-risk genotype was the homozygote S/S

without any (L_G) individuals. We also found the lowest risk genotype to be the homozygote L_A/L_A without any (L_G) individuals. These results may support the concept of intermediate expression by the L_G allele or response differences in specific cells in the brain. Ultimately, understanding serotonin transporter gene-environment interactions may require measurement of mRNA and protein levels or measurement of serotonin transporter activity. Furthermore, more recent data showing gene-gene-environment interactions involving 5-HTTLPR, corticotropin releasing hormone (CRH), childhood abuse and depressive symptoms (Ressler *et al.* 2009) show the importance of considering a multitude of interacting factors for psychiatric disorders.

The assessment of gene-environment interactions and mental health outcomes is fraught with methodological complexities as discussed in multiple reviews (Brown and Harris, 2008, Monroe and Reid, 2008, Uher and Mcguffin, 2008). In this study we inferred expression levels based on the 5-HTTLPR genotype but we were not able to determine circulating or tissue/organ-specific expression levels. Animal models investigating HTTLPR genotype expression levels in the central nervous system may help to fill in the gaps from human studies, especially models that consider baseline expression levels and situational variability in signaling. We measured depressive symptoms only once, and at the same time as self-reports of stressful life events; therefore we could not establish a clear temporal relation between exposure and outcome. While individuals with elevated depressive symptoms may be more likely to recall stressful life events, we do not expect this recall to vary systematically by genotype and explain the gene-environment interaction we observed. Investigators (Kendler *et al.*, 1998, Wainwright and Surtees, 2002) have asserted that recent exposures to stressful life events present a higher risk for developing a depressive episode than do distant events. Ideally models of depression risk would take into account many factors including recent and distant stressful events, personality characteristics, prior history of depression and gender (Kendler *et al.*, 2002, Kendler *et al.*, 2006).

A major strength of this study includes the focus on African-American women. The majority of investigations on relations among stressful life events, the 5-HTTLPR genotype, and mental health have been conducted with Caucasian samples (Bukh *et al.*, 2009, Caspi *et al.*, 2003, Eley *et al.*, 2004, Gillespie *et al.*, 2004, Kendler *et al.*, 2005, Laucht *et al.*, 2009, Lazary *et al.*, 2008, Zalsman *et al.*, 2006). In addition our sample was from multiple communities representing a diversity of social class, thereby enhancing the generalizability of the findings. We demonstrated that gene-environment interactions related to a specific mental health outcome, elevated depressive symptoms, can be detected during a period of atypical hormonal milieu, i.e. pregnancy. The cohort design included those with a full spectrum of depressive symptoms and avoided selection bias that can accompany studies of individuals seeking treatment for depression. Finally, we examined different allelic grouping approaches for the 5-HTTLPR polymorphism; this strengthened our inferences about gene-environment interactions based on expression levels.

Overall our results highlight the importance of examining different race groups, subtypes of stressful life events, and variations in allelic grouping of the 5-HTTLPR genotype when exploring gene-environment interactions related to mental health outcomes.

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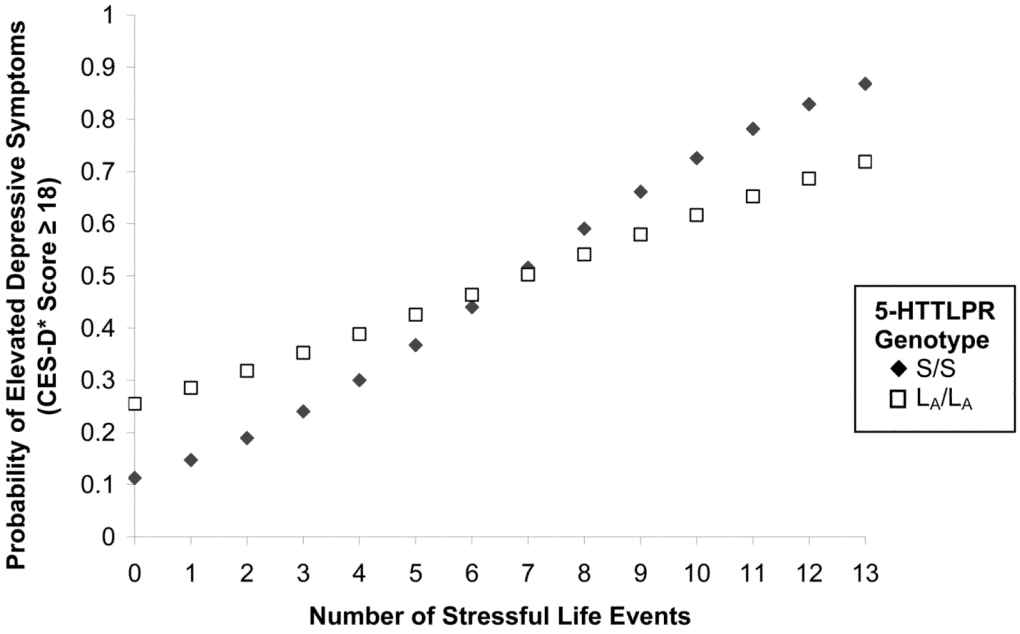

Figure 1.
Probability of elevated depressive symptoms among African-American women in relation to mid-pregnancy lifetime exposure to stressful life events and 5-HTTLPR genotype.
*CES-D = Center for Epidemiologic Studies Depression Scale

Table 1

Maternal characteristics in a sample of POUCH Study African-American women (N=698)

	N	%
Maternal Age (years)		
<20	195	28
20–34	478	68
≥35	25	4
Education (years)		
<12	252	36
12	225	32
>12	221	32
Parity		
Primiparous	280	40
Multiparous	418	60
Medicaid Use *		
Never	136	20
Ever	560	80
Preterm Birth		
≥37 Weeks of Pregnancy	595	85
<37 Weeks of Pregnancy	103	15
CES-D Score		
< 18	407	58
≥18	291	42
5-HTTLPR Genotype		
L_A/L_A	200	28
S/L_A	181	26
A/L_G	141	20
S/L_G	75	11
L_G/L_G	26	4
S/S	75	11

CES-D = Center for Epidemiologic Studies Depression Scale

5-HTTLPR - serotonin transporter gene

* Missing data on 2 women

Table 2

The 5-HTTLPR genotype in relation to elevated depressive symptoms at mid-pregnancy in African-American women (N=698)

5-HTTLPR Genotype Grouping*	CES-D Score		Chi-Square Test (genotype by CES-D)
	≥18 % (N)	< 18 % (N)	
Biallelic			
L/L	41 (150)	59 (218)	
S/L	43 (110)	57 (145)	
S/S	41 (31)	59 (44)	
			P=0.84
Triallelic to Biallelic grouping			
L_A/L_A	42 (85)	58 (115)	
S/L_A, L_G/L_A	41 (131)	59 (191)	
S/S, S/L_G, L_G/L_G	43 (75)	57 (101)	
			P=0.88
Expression			
'high' L_A/L_A	42 (85)	58 (115)	
'intermediate' L_G/L_G, S/L_G, L_A/L_G, S/L_A, L_A/L_A	41 (175)	59 (248)	
'low' S/S	41 (31)	59 (44)	
			P=0.96
Extremes of expression			
'high' L_A/L_A	42 (85)	58 (115)	
'low' S/S	41 (31)	59 (44)	
			P=.86

CES-D = Center for Epidemiologic Studies Depression Scale

5-HTTLPR - serotonin transporter gene

*The 'S' allele has been associated with lower gene expression and with depression in some but not all previous studies.

Table 3

Odds ratios of five life stressor subconstructs in relation to elevated depressive symptoms at mid-pregnancy by 5-HTTLPR genotype (triallelic to biallelic grouping) in African-American women (N=698)

		CES-D Score			
5-HTTLPR Genotype* (triallelic to biallelic grouping)	**Stressor Subconstruct†**	**≥ 18 % (N)**	**< 18 % (N)**	**Odds Ratio (95 % CI)**	**Test for Homogeneity of Odds Ratios across Genotypes**
Economic					
L_A/L_A	Exposed	49 (63)	51 (66)	2.1 (1.1, 3.9)	
	Not Exposed	31 (22)	69 (49)		
S/L_A, L_G/L_A	Exposed	51 (95)	49 (93)	2.8 (1.7, 4.5)	P=0.44
	Not Exposed	27 (36)	73 (98)		
S/S, S/L_G, L_G/L_G	Exposed	56 (56)	44 (44)	3.8 (2.0, 7.3)	
	Not Exposed	25 (19)	75 (57)		
Substance Use					
L_A/L_A	Exposed	50 (39)	50 (39)	1.7 (0.9, 2.9)	
	Not Exposed	38 (46)	62 (76)		
S/L_A, L_G/L_A	Exposed	52 (71)	48 (66)	2.2 (1.4, 3.5)	P=0.72
	Not Exposed	32 (60)	68 (125)		
S/S, S/L_G, L_G/L_G	Exposed	53 (39)	47 (35)	2.0 (1.1, 3.8)	
	Not Exposed	35 (36)	65 (66)		
Legal					
L_A/L_A	Exposed	56 (19)	44 (15)	1.9 (0.9, 4.0)	
	Not Exposed	40 (66)	60 (100)		
S/L_A, L_G/L_A	Exposed	49 (32)	51 (34)	1.5 (0.9, 2.6)	P=0.33
	Not Exposed	39 (99)	61 (157)		
S/S, S/L_G, L_G/L_G	Exposed	65 (22)	35 (12)	3.1 (1.4, 6.7)	
	Not Exposed	37 (53)	63 (89)		
Violence					
L_A/L_A	Exposed	47 (35)	53 (40)	1.3 (0.7, 2.3)	
	Not Exposed	40 (50)	60 (75)		
S/L_A, L_G/L_A	Exposed	51 (63)	49 (60)	2.0 (1.3, 3.2)	P=0.47

5-HTTLPR Genotype* (triallelic to biallelic grouping)	Stressor Subconstruct†	CES-D Score ≥ 18 % (N)	CES-D Score < 18 % (N)	Odds Ratio (95 % CI)	Test for Homogeneity of Odds Ratios across Genotypes
	Not Exposed	34 (68)	66 (131)		
S/S, S/L_G, L_G/L_G	Exposed	55 (30)	45 (25)	2.0 (1.0, 3.9)	
	Not Exposed	37 (45)	63 (76)		
Loss					
L_A/L_A	Exposed	43 (71)	57 (96)	1.0 (0.5, 2.1)	
	Not Exposed	42 (14)	58 (19)		
S/L_A, L_G/L_A	Exposed	42 (109)	58 (153)	1.2 (0.7, 2.1)	P=0.91
	Not Exposed	37 (22)	63 (38)		
S/S, S/L_G, L_G/L_G	Exposed	43 (62)	57 (81)	1.2 (0.5, 2.6)	
	Not Exposed	39 (13)	61 (20)		

CES-D = Center for Epidemiologic Studies Depression Scale; CI = Confidence Interval

5-HTTLPR - serotonin transporter gene

*The 'S' allele has been associated with lower gene expression and with depression in some but not all previous studies.

†Stressor subconstructs: exposure as a child or adult or both.

Table 4

Odds ratios of abuse in relation to elevated depressive symptoms at mid-pregnancy by 5-HTTLPR genotype groupings in African-American women (N=698)

5-HTTLPR Genotype Grouping*	Abuse†	CES-D Score ≥18 % (N)	CES-D Score < 18 % (N)	Odds Ratio (95 % CI)	Test for Homogeneity of Odds Ratios across Genotypes
Biallelic					
L/L	Exposed	52 (73)	48 (68)	2.1 (1.4, 3.2)	
	Not Exposed	34 (77)	66 (150)		
S/L	Exposed	53 (54)	47 (48)	1.9 (1.2, 3.2)	P=.18
	Not Exposed	37 (56)	63 (97)		
S/S	Exposed	65 (20)	35 (11)		
	Not Exposed	25 (11)	75 (33)	5.5 (2.0, 14.9)	
Triallelic to biallelic grouping					
L_A/L_A	Exposed	49 (41)	51 (43)		
	Not Exposed	38 (44)	62 (72)	1.6 (0.9, 2.8)	
S/L_A, L_G/L_A	Exposed	51 (63)	49 (61)		
	Not Exposed	34 (68)	66 (130)	2.0 (1.2, 3.1)	P=0.04
S/S, S/L_G, L_G/L_G	Exposed	65 (43)	35 (23)		
	Not Exposed	29 (32)	71 (78)	4.6 (2.4, 8.8)	
Expression					
'high' L_A/L_A	Exposed	49 (41)	51 (43)		
	Not Exposed	38 (44)	62 (72)	1.6 (0.9, 2.8)	
'intermediate' L_G/L_G, S/L_G, L_A/L_G, S/L_A, L_A/L_A	Exposed	54 (86)	46 (73)		
	Not Exposed	34 (89)	66 (175)	2.3 (1.5, 3.5)	P=.10
'low' S/S	Exposed	65 (20)	35 (11)		
	Not Exposed	25 (11)	75 (33)	5.5 (2.0, 14.9)	
Extremes of expression					
'high' L_A/L_A	Exposed	49 (41)	51 (43)		
	Not Exposed	38 (44)	62 (72)	1.6 (0.9, 2.8)	
'low' S/S	Exposed	65 (20)	35 (11)		P=.03
	Not Exposed	25 (11)	75 (33)	5.5 (2.0, 14.9)	

CES-D = Center for Epidemiologic Studies Depression Scale; CI = Confidence Interval

5-HTTLPR - serotonin transporter gene

*The 'S' allele has been associated with lower gene expression and with depression in some but not all previous studies.

†Abuse: exposure as a child or adult or both.