

# Serotonin transporter-linked polymorphic region (5-HTTLPR) genotype and stressful life events interact to predict preschool-onset depression: a replication and developmental extension

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**Background:** Scientific enthusiasm about gene  $\times$  environment interactions, spurred by the 5-HTTLPR (serotonin transporter-linked polymorphic region)  $\times$  SLEs (stressful life events) interaction predicting depression, have recently been tempered by sober realizations of small effects and meta-analyses reaching opposing conclusions. These mixed findings highlight the need for further research. Converging evidence suggests that the effects of 5-HTTLPR genotype may be neurodevelopmental in origin, but we are not aware of empirical studies that have investigated whether the 5-HTTLPR genotype  $\times$  SLE interaction predicts preschool-onset depression (PO-MDD), the earliest validated form of depression. **Methods:** Children ( $n = 234$ ) aged 3–5 were recruited for a longitudinal study designed to examine PO-MDD. In a comprehensive baseline assessment, the child's primary caregivers completed questionnaires and were interviewed about their child's behaviors, psychiatric symptoms, and exposure to SLEs. **Results:** A 5-HTTLPR  $\times$  SLEs interaction emerged, such that children homozygous for the short allele were more susceptible to depression in the context of elevated SLE than long allele carriers. In contrast, at low SLE exposure, short allele homozygotes had fewer depressive symptoms. The data were best fit by a plasticity model with a substantial reduction in fit by diathesis-stress models. **Conclusions:** Extending studies in adult and adolescent populations, these data suggest that 5-HTTLPR genotype may provide plasticity to environmental influence, for better or worse. Specifically, children homozygous for the short allele were more susceptible to the depressogenic effects of SLEs but benefitted, in the form of reduced depressive symptoms, in the context of relatively benign environmental conditions (i.e. relatively low SLE exposure). These data highlight the importance of examining gene  $\times$  environment interactions across development, environment, and outcome but should be interpreted cautiously given the small sample size. **Keywords:** Depression, stress, 5-HTTLPR, serotonin, gene\*, interaction, plasticity, childhood, development, gene  $\times$  environment.

## Introduction

A small but growing body of literature suggests that clinically significant depressive symptoms can arise in children as young as 3 years old (Luby et al., 2002). Such early-onset depression occurs in 1–2% of children but relatively little is known about risk factors or whether there is etiologic convergence across depression based on age of onset (Egger & Angold, 2006; Wichstrom et al., 2012). Because preschool depression may reflect a more severe and chronic form, and treatment and prevention efforts may be more successful when applied early, it is important to identify individual differences that predict its development (Luby, 2010; Zisook et al., 2007). A logical starting place is to evaluate whether factors predicting adult- and adolescent-onset depression also predict preschool-onset childhood depression.

In addition to genetic variation (Sullivan, Neale & Kendler, 2000), little doubt remains that stress, particularly when occurring early in life, is among the strongest predictors of major depressive disorder

(MDD) (Hammen, 2005). Vulnerability to depression has predominantly been conceptualized within a diathesis-stress framework in which diatheses, or individual differences such as cognitive biases or genetic background, promote the development of depression in the context of stress. One of the earliest and arguably most controversial illustrations of the diathesis-stress model showed that serotonin transporter-linked polymorphic region (5-HTTLPR) genotype moderates the depressogenic effects of stressful life events (SLEs) (Caspi et al., 2003). Specifically, this report demonstrated that the short allele of the 5-HTTLPR [(which has been linked to relatively reduced serotonin-transporter expression as well as putative intermediate depressive phenotypes (Hariri et al., 2002; Lesch et al., 1996)] confers vulnerability to stress-related MDD relative to the long allele. The intuitive appeal of this hypothesis led to multiple replication attempts producing conflicting findings (Gillespie, Whitfield, Williams, Heath & Martin, 2005; Kendler, Kuhn, Vittum, Prescott & Riley, 2005). Recent meta-analyses have weighed heavily against evidence for the 5-HTTLPR  $\times$  SLEs interaction (Duncan & Keller, 2011; Munafò, Durrant, Lewis & Flint, 2009; Risch et al., 2009), although opinions continue

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to diverge (Caspi, Hariri, Holmes, Uher & Moffitt, 2010; Karg, Burmeister, Shedden & Sen, 2011), urging further research.

As the center of a maelstrom of controversy, the overwhelming majority of depression studies have sought to replicate rather than extend the 5-HTTLPR  $\times$  SLEs interaction. For example, despite emerging evidence that the effects of 5-HTTLPR genotype on depression may be driven by its influence on neurodevelopment (Caspi et al., 2010; Hariri & Holmes, 2006), to our knowledge, no human research has evaluated the 5-HTTLPR  $\times$  SLEs interaction outside of adolescent- or adult-onset depression. The importance of examining the developmental trajectory of this relationship is further underscored in light of evidence that SLEs are a strong predictor of early depressive episodes, but are less predictive of its future recurrence (Kendler, Thornton & Gardner, 2000; Stroud, Davila & Moyer, 2008). This is broadly consistent with the kindling hypothesis which posits that the earliest episodes of depression might be more sensitive to environmental adversity whereas, via kindling, subsequent episodes are more greatly contingent on prior depressive episodes and less reliant on environmental provocation (Kendler et al., 2000; Post, 1992). These studies suggest a developmental rubric underpinning the interface of stress and diathesis for MDD, potentially with a more pronounced impact of their interaction earlier in development.

The goal of the present investigation was to examine whether 5-HTTLPR genotype moderates the association between SLEs and preschool-onset depression. Consistent with the literature, initially, we hypothesized that the short allele would be associated with increased risk for stress-related depression relative to the long allele. However, emergent theoretical work suggesting that diatheses may provide differential susceptibility to the environment, for better or worse, as opposed to vulnerability to negative outcomes in the context of adversity, has refined the diathesis-stress model and begun to accumulate empirical support (Belsky & Pluess, 2009; Belsky, Pluess & Widaman, in press; Hankin et al., 2011; Pluess, Belsky, Way & Taylor, 2010). In light of this evidence, we further hypothesized that the short allele of the 5-HTTLPR polymorphism might produce benefits with regard to depression when SLE exposure was low, providing plasticity to environmental influence, for better or worse, as opposed to vulnerability to adversity solely.

## Methods

### Participants

Children between the ages of 3.0 and 5.11 were recruited from community daycare, preschool and primary care sites throughout the Saint Louis area for

participation in a longitudinal study designed to examine preschool-onset depression. Recruitment sites were chosen at random using a geographically stratified method to best approximate the composition of the Saint Louis community. To recruit a large group of depressed children (see Luby, Si, Belden, Tandon & Spitznagel, 2009 for more detail regarding the recruitment of this sample), as well as smaller groups of healthy children and those with other psychiatric disorders for comparison, a validated screening checklist, The Preschool Feelings Checklist (PFC; Luby, Heffelfinger, Koenig-McNaught, Brown & Spitznagel, 2004) was completed by caregivers. Children with a PFC score of  $\geq 3$  were oversampled because a PFC score  $\geq 3$  has high sensitivity and specificity for the diagnosis of depression. Approximately 6,000 checklists were distributed to sites between May 2003 and March 2005; 1,474 were returned. Of these returned checklists, 899 children met all inclusion criteria and were contacted by phone for further screening. Preschoolers with chronic medical illness, marked speech and language delays, and/or neurologic or Autistic Spectrum Disorders were excluded. Those without exclusions ( $N = 416$ ) were invited for study participation and  $N = 305$  (i.e. 73%) agreed and completed the baseline wave of the study. The longitudinal study is currently ongoing and the data in the present manuscript reflect baseline data. Genetic data, via saliva samples, were collected at later study waves from 234 participants whom make up the sample of the present report (Table 1); additional genotyping for more subjects within the preschool depression study longitudinal sample is not presently planned. The study was approved by the Institutional Review Board at Washington University in St. Louis School of Medicine. Caregivers provided written informed consent and children provided verbal consent.

### Assessment of preschool-onset childhood depression and stressful life events

At baseline, children and their caregivers participated in a 3–4 hr comprehensive laboratory assessment during which primary caregivers (92% biological mothers) were interviewed about their child's behaviors, emotions, and psychiatric symptoms, as well as the child's exposure to stressful and traumatic life events using the Preschool Age Psychiatric Assessment (PAPA). The PAPA is an interviewer based diagnostic assessment with empirically established test retest reliability designed for use in caregivers of children aged 2.0–6.0 (Egger et al., 2006). Interviewers were trained to reliability administer the PAPA and audio was recorded for quality control. Details of reliability and quality control have been previously reported (Luby et al., 2009). In addition to generating categorical PO-MDD diagnosis using a DSM-IV computer algorithm which sets aside the duration criterion based on empirical data

**Table 1** Demographic and diagnostic characteristics of the sample

	S/S (N = 56)		S/L (N = 117)		L/L (N = 61)		$\chi^2$	p
	%	N	%	N	%	N		
T1 age								
3 years	39.3	22	23.9	28	29.5	18	5.47	0.2426
4 years	37.5	21	47.9	56	37.7	23		
5 years	23.2	13	28.2	33	32.8	20		
Gender								
Male	44.6	25	55.6	65	60.7	37	3.17	0.2048
Female	55.4	31	44.4	52	39.3	24		
Ethnicity								
Caucasian	50.0	28	58.1	68	57.4	35	4.69	0.3202
African American	35.7	20	26.5	31	36.1	22		
Other	14.3	8	15.4	18	6.6	4		
T1 total family income								
<\$20,000	24.5	14	20.6	22	19.6	11	4.59	0.5974
\$20,001-\$40,000	16.4	9	19.6	21	26.8	15		
\$40,001-\$60,000	23.6	13	15.9	17	14.3	8		
>\$60,000	34.5	19	43.9	47	39.3	22		
T1 parental education								
High school or less	21.4	12	11.1	13	18.0	11	4.24	0.6445
Some college	37.5	21	43.6	51	37.7	23		
College degree	14.3	8	19.7	23	19.7	12		
Graduate education	26.8	15	25.6	30	24.6	15		
T1 psychiatric disorders								
MDD	17.9	10	30.8	36	26.2	16	3.25	0.1974
ADHD	21.4	12	14.5	17	13.1	8	1.80	0.4056
Oppositional defiant	26.8	15	25.6	30	23.0	14	0.25	0.8823
Conduct	12.5	7	12.8	15	9.8	6	0.36	0.8357
Generalized anxiety	7.1	4	6.8	8	8.2	5	F.E.	0.9460
Separation anxiety	10.7	6	19.7	23	19.7	12	2.36	0.3072
PTSD	1.8	1	1.7	2	3.3	2	F.E.	0.8413
Mania	7.1	4	9.4	11	11.5	7	0.64	0.7249
	Mean	SD	Mean	SD	Mean	SD	F	p
N T1core MDD symptoms	2.29	1.71	2.66	1.89	2.48	1.70	0.84	0.4344

ADHD, Attention-deficit/hyperactivity disorder; F.E., Fisher's Exact test; MDD, Major depressive disorder; PTSD, Post-traumatic stress disorder.

suggesting it may not be applicable in young children (Gaffrey, Belden & Luby, 2011), the number of depressive symptoms was also summed to provide a continuous measure of depression severity (Gaffrey, Luby et al., 2011; Luby, Mrakotsky, Heffelfinger, Brown & Spitznagel, 2004). Traumatic and stressful life events were also measured by the PAPA which has established reliability for these assessments (Costello, Angold, March & Fairbank, 1998). At baseline assessment primary caregivers were asked about the preschooler's experience of stressful and traumatic events over their lifetime. Life events were categorized into 15 categories and the total frequency of events was used for analyses (Table 2).

### Genotyping

5-HTTLPR and the 5-HTT SNP at rs25531 were genotyped according to established procedures. Saliva was collected using oragene collection kits from which DNA was extracted using the manufacturer's protocol (www.dnagenotek.com). A PCR protocol (Forward primer: 5'-TGAATGCCAGCACCTAACCC-3', Reverse primer: 5'-GTGCCACCTAGACGCCAGG-3')

**Table 2** Stressful life events and their occurrence in the sample

Stressful life events	%	N
Change in daycare/school	43.6	102
Death of a pet	22.2	52
Lives/attends daycare/ school in unsafe environment	1.3	3
Loss of home without family separation	1.7	4
Lost significant person through moving	14.5	34
Marital family conflict	3.0	7
Moving house	56.4	132
New child in home	56.4	132
New parental figure	15.8	37
Parental arrest	8.1	19
Parental divorce	6.8	16
Parental hospitalization	42.7	100
Parental separation	23.5	55
Reduction in standard of living	6.0	14
Separation from parent (1 week or more)	32.9	77

was used to amplify a 400 (S) or 444 bp (L) region. The amplification was followed by a restriction enzyme digestion with MspI (New England Biolabs, Beverly, MA) to determine the Sa, Sg, La and Lg alleles.

The digestion products were electrophoresed on a 2.5% agarose gel, stained with ethidium bromide and visualized under UV light. Participants were grouped as S homozygotes (i.e. SS), heterozygotes (i.e. SL), or L homozygotes (i.e. LL; Table 1). When the L allele was present alongside the G allele of the rs25531 SNP, it was re-categorized as an S allele due to functional and clinical evidence that the presence of the G allele at the SNP within the long allele results in S-like function (Wendland, Martin, Kruse, Lesch & Murphy, 2006; Zalsman et al., 2006). Sixty individuals in the present sample were recategorized in this manner. Alleles were within HWE for the 5-HTTLPR and rs25531 within long alleles ( $\chi^2 < 2.27$ ;  $ps > .13$ ).

### Statistical analysis

Prior to all data analyses, SLE outliers were winsorized; specifically, values that were greater than 3 standard deviations (SDs) above or below the mean were set to the closest observed value within 3 SDs of the mean. In an effort to diminish potential artifactual scaling effects (Eaves, 2006), the number of MDD symptoms was natural log transformed (after adding 1 to all subjects to account for those who endorsed 0 symptoms) because of its positively skewed distribution. Linear and logistic regression were used to assess the main effects of 5-HTTLPR genotype and stressful life events, as well as the 5-HTTLPR genotype  $\times$  stressful life event interaction, on the number of log-transformed depressive symptoms and a diagnosis of preschool-onset depression, respectively. The PROCESS macro was used for analyses and predictor variables were mean centered prior to the computation of interaction terms and analyses (Hayes, 2013). Age, gender, and ethnicity (i.e. European-American/not European-American; African-American/not African-American) were included as covariates in analyses. Post-hoc testing for significant interactions was conducted by using simple-slope testing and Johnson–Neyman tests, which identify the values on the continuum of a moderator (in this case, stressful life events) at which point the effect of a predictor variable (i.e. in this case, 5-HTTLPR genotype) transitions between statistical significance in either direction along this continuum (Roisman et al., 2012).

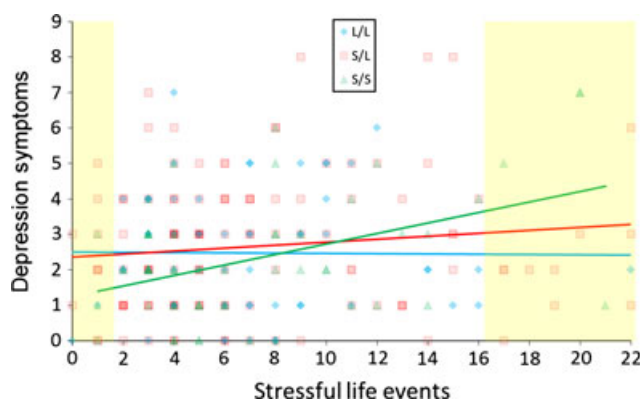
### Results

There was no evidence for main effects of 5-HTTLPR genotype on number of depression symptoms ( $B = -0.03$ ,  $SE = 0.05$   $p = .63$ ), MDD diagnosis ( $B = -0.03$ ,  $SE = 0.41$   $p = .41$ ) or the number of stressful life events ( $B = 0.13$ ,  $SE = 0.34$   $p = .69$ ). Number of stressful life events was associated with depressive symptoms at a trend level ( $B = 0.02$ ,  $SE = 0.01$   $p = .09$ ), but only in the absence of the interaction term. Including the 5-HTTLPR genotype  $\times$  SLE interaction in the models produced significant

interactions for both the number of depression symptoms,  $\Delta F(1,226) = 4.80$ ,  $\Delta r^2 = .02$   $p < .03$ , and MDD diagnosis,  $Z = 2.56$ ,  $p < .02$  (Figure 1).

Post-hoc simple-slope tests revealed a positive association between number of stressful life events and number of depressive symptoms in short (SS) homozygotes ( $t = 2.79$ ,  $p < .006$ ) while this association was absent in (SL) heterozygotes ( $t = 1.56$ ,  $p < .12$ ) and long (LL) homozygotes ( $t = -0.61$ ,  $p > .53$ ). Moreover, Johnson–Neyman tests revealed that the *short allele* was associated with *increased* depressive symptoms with *increasing* exposure to SLEs (i.e.  $>16.15$  SLEs, all  $ps < .05$ ) but *decreased* depressive symptoms with *decreasing* exposure to SLEs (i.e.  $<1.35$  SLEs; all  $ps < .05$ ). Post hoc analyses revealed the same pattern for PO-MDD diagnosis; there was a positive association between stressful life events in short homozygotes ( $z = 2.51$ ,  $p < .02$ ) that was absent in heterozygotes ( $z = 0.56$ ;  $p < .57$ ) and long homozygotes ( $z = -1.55$ ,  $p < .13$ ). Moreover, Johnson–Neyman tests revealed a similar pattern; at high levels of stressful life events (i.e.  $>11.54$  SLEs; all  $ps < .05$ ), the short allele was associated with increased depression; however, at low levels of stressful life events (i.e.  $<3.37$  SLEs; all  $ps < .05$ ), it was associated with decreased depression. Additional analyses across different genotype groupings (i.e. S carriers vs. LL and L carriers vs. SS) and within European American and African American subsamples are reported on in the Appendix S1. Briefly, these results suggest that the present effects are driven by S homozygotes (see also Figure 1). Analyses in ethnic subsamples produce significant or trending results for each in a direction consistent with the entire sample.

To empirically test whether a diathesis-stress or plasticity (differential susceptibility) model better explained the observed data, we employed recently developed model-fitting procedures (Widaman et al., 2012). Specifically, we compared strong and weak versions of plasticity and diathesis stress (Belsky



**Figure 1** Number of MDD Core Symptoms by 5HTT Genotype and Stressful Life Events Frequency. Depression symptoms represent raw, untransformed data. SLEs represent winsorized data. Shaded areas represent regions of significance

et al., in press). The strong version of each model presumes that L carrier children are not affected by early life stressful events (thus constraining their slope to 0), whereas the weaker models propose that L carriers are affected by these events (hence their slope parameter is estimated), but to a lesser degree than S homozygotes. Both models assume that S homozygotes are affected by SLEs. In the diathesis-stress model, the crossover point (i.e. the point at which the slope of L carriers and S homozygotes intersects) was constrained to be the lowest observed frequency of stressful life events in the dataset, testing the assumption that increasing stressful events would be associated with a greater number of depressive symptoms in S homozygotes and that group lines would intersect at the lowest observed SLE value. Conversely, in the plasticity model, this crossover point parameter was allowed to vary freely, testing the assumption that SLEs would be significantly related at both ends of the continuum.

Model fitting analyses suggest these data best fit plasticity (i.e., differential susceptibility) models ( $r^2 = 0.49\text{--}0.53$ ; AIC =  $-272.11$  to  $-270.94$ ); diathesis stress models resulted in a substantial reduction in fit ( $r^2 = 0.007\text{--}0.02$ ; AIC =  $-263.98$  to  $-266.05$ ; all  $ps < .009$ ; Table 3). Thus, the best-fitting model was a strong plasticity model, indicating that S homozygotes were susceptible to increased and decreased depressive symptoms at both the high and low ends of SLE exposure, respectively, and that L carriers were relatively unaffected by SLE.

## Discussion

That SLEs promote onset and exacerbate course of depression is unequivocal (Hammen, 2005). The mechanism linking SLEs to depression, however, remains an area of active research. One etiologic mechanism that is frequently explored is the extent to which genetic liability to depression modifies this relationship. For instance, in an early seminal study using data on identical and fraternal twins, Kendler and colleagues (Kendler et al., 1995) found that the risk for depression onset was elevated in individuals whose identical cotwin also had a history of depression, but only if they had themselves been exposed to a severe SLE. Almost a decade later, an initial report demonstrated that 5-HTTLPR genotype moderated the well-documented depressogenic effects of stressful life events. This influential study by Caspi and colleagues (Caspi et al., 2003) has generated numerous replication attempts that have yielded conflicting findings. Indeed, even meta-analyses have reached opposing conclusions; one recent meta-analysis that adopted an inclusive approach suggests that this effect is replicable when broadly conceptualizing SLEs (Karg et al., 2011), while another using more conservative inclusion criteria for SLE measurement, concludes it is a false positive that has been

propagated by publication bias (Duncan & Keller, 2011).

Much like the original report (Caspi et al., 2003) and several replication studies in adults (Karg et al., 2011), we found that preschool-aged children homozygous for the 5-HTTLPR short allele are more susceptible to the depressogenic effects of stress relative to individuals with the long allele. Consistent with emergent data (Hankin et al., 2011; Pluess et al., 2010), post hoc and model-fitting analyses provided support for a plasticity model wherein short homozygotes might benefit from benign environments and experience less depression, relative to long allele carriers, in the context of low SLE exposure. These data allude to the significance of considering the developmental framework in which this GxE interplay may operate and suggest that some nonreplications may potentially arise from attenuation of the 5-HTTLPR  $\times$  SLE interaction effects in later episodes of depression, possibly due to kindling effects observed for main effects of SLEs.

## Similarities and Differences with the Extant Literature

The results of this study are broadly consistent with literature suggesting that the short 5-HTTLPR allele provides plasticity to the environment; however, there are some key differences in the results reported here. First, studies have predominantly reported a graded relationship in which heterozygotes (i.e. 'SL' genotypes) are intermediate in their vulnerability to depression upon exposure to SLEs between homozygote groups (SS and LL) or a relationship in which short allele carriers (SS and SL) are more susceptible to depression than long homozygotes (LL) in the context of stress. The results of our study are driven by differences between short homozygotes (SS) and long allele carriers (SL and LL) (see also Appendix S1). While this direction of effect has been previously reported (Hankin et al., 2011), it is not the predominant pattern noted in the literature. Importantly, however, when analyzing the data across 3 genotype groups the graded pattern, wherein heterozygotes are intermediate is observed, though not at a statistically significant level, perhaps due to the relatively small sample that comprises this study. Second, there was a main effect of genotype (when comparing long allele carriers against short allele homozygotes) in our sample wherein long allele carriers were more susceptible to depression diagnosis regardless of SLE exposure. Of note, there was no significant main effect when analyzing the data as three different genotype groups (SS, SL and LL) or considering depressive symptoms. Critically, this sample has a large African American population that may contribute to these differences. For example, Gelernter and colleagues (Gelernter, Kranzler, Coccaro, Siever & New, 1998) have found that, contrary to the relationship

**Table 3** Model fitting of plasticity and diathesis stress models

	Strong Plasticity (Model 1A)	Weak Plasticity (Model 1B)	Stong DS (Model 2A)	Weak DS (Model 2B)
rSS	70.635	70.358	73.783	72.487
Parameters	3	4	2	3
$B_0$	1.135 (0.041)	1.148 (0.052)	1.094 (0.040)	1.002 (0.061)
$B_1$	0.00 (-)	0.010 (0.011)	0.00 (-)	0.021 (0.010)
$C$	1.146 (1.206)	1.316 (1.438)	-4.79 (-)	-4.79 (-)
$B_2$	0.073 (0.022)	-0.073 (0.022)		0.031 (0.015)
$R^2$	0.049	0.053	0.007	0.024
$F$ vs. 1A		0.91	10.30	
$F$ vs. 1A $df$		1,230	1,231	
$F$ vs. 1A $p$		0.342	0.002	
$F$ vs. 1B			5.60	6.96
$F$ vs. 1B $df$			2,230	1,230
$F$ vs. 1B $p$			0.0042	0.0089
$F$ vs. 2A				4.13
$F$ vs. 2A $df$				1,231
$F$ vs. 2A $p$				0.0433
AICc	-272.11	-270.94	-263.98	-266.05

rSS, residual sum of squares; Parameters, No. of parameters estimated in the model;  $B_0$ , the estimated y value (i.e. log-transformed depressive symptoms) at which point the two groups crossover or converge;  $B_1$ , = the slope for the  $x$  value (i.e. mean-centered stressful life event frequency) for the long allele carrier group;  $C$ , the estimated  $x$  value (i.e. mean-centered stressful life event frequency) at which point the two groups crossover or converge;  $B_2$ , the slope for the  $x$  value (i.e. mean-centered stressful life event frequency) for the short homozygote group;  $R^2$ , estimated amount of variance in log-transformed depressive symptoms explained by the model;  $F$  vs. 1A, an  $F$  test of the difference in  $R^2$  for the specified model in contrast to Model 1A (i.e. Strong Plasticity);  $F$  vs. 1B, an  $F$  test of the difference in  $R^2$  for the specified model in contrast to Model 1B (i.e. Weak Plasticity);  $F$  vs. 2A, an  $F$  test of the difference in  $R^2$  for the specified model in contrast to Model 2A (i.e. Strong Diathesis Stress). Values in () represent *SE*. (-) in *SE* represents a fixed parameter. AICc, Akaike's Information Criteria with a correction for sample size.

observed in European-American individuals (Lesch et al., 1996), African Americans with the long allele have elevated neuroticism. Despite accounting for ethnicity as a covariate, the ethnic diversity of the sample may have contributed to the genotype main effect. However, secondary analyses conducted separately in Caucasian and African American subjects revealed interaction effects in the same direction as the entire sample.

Interestingly, the results of this study suggest a crossover interaction wherein individuals with the short allele appear more sensitive to SLE exposure, for better or worse. Specifically, at high SLE exposure, short homozygotes (SS) are more vulnerable to depression but at low SLE exposure they appear to benefit from a benign environment and experience relatively reduced rates of depression. These findings are consistent with theoretical work, supported by empirical data, suggesting that many genotypes, including the short allele of the 5-HTTLPR polymorphism, originally conceptualized as conferring vulnerability to adversity may be better conceptualized as conferring variability in susceptibility to environmental influence, in both positive and negative directions (Belsky & Pluess, 2009). Critically, the crossover interaction we observed was present for a continuous measure of depressive symptoms as well as a dichotomous diagnosis of depression. The convergence of findings across a continuous and dichotomous phenotype suggests that the observed interaction may not be a spurious finding resulting from scaling effects as has been observed with

simulated data (Eaves, 2006). However, some caution is required with interpreting the crossover interaction – while intuitively appealing, simulation data suggest that smaller samples, like the present one, are more likely to produce false-positive crossover interaction effects (Sher, personal communication).

#### Possible contributors to mixed results in the literature

SLEs are amongst the strongest predictors of initial depression onset, but their predictive power is diminished in subsequent recurrent depressive episodes (Kendler et al., 2000; Post, 1992; Stroud et al., 2008). This is consistent with the kindling theory of depression which posits that SLEs are associated with early episodes of depression while later episodes of depression are predicted by previous episodes but not SLEs (Kendler et al., 2000; Post, 1992). Implicit in this hypothesis is that depressive episodes induce psychological changes that set the stage for subsequent episodes (i.e., these individuals are kindled) which are then less tightly linked to environmental experiences. Importantly, however, research suggests that while SLEs are less linked to recurrent episodes, minor stressors, such as daily hassles, which would not be detected as an SLE, may provoke a depressive episode in a kindled individual (Monroe & Harkness, 2005). As such, it may be that conflicting findings associating the 5-HTTLPR genotype  $\times$  SLE interaction with

depression are affected by kindling. Some data suggesting this interaction is driven by more low-level stressors in adults is consistent with this possible interpretation (Kendler et al., 2005). Critically, however, while the present data demonstrate that 5-HTTLPR  $\times$  SLE predicts preschool-onset depression, they do not directly assess the kindling hypothesis. It will be important for future longitudinal studies to assess whether the 5-HTTLPR  $\times$  SLE interaction is diminished or disappears in recurrent depressive episodes.

Another feature of the present study worth noting is the assessment of SLEs. A wealth of literature suggests that mode of measurement of SLEs can have a profound impact on their reported occurrence and predictive utility (Monroe & Reid, 2008). Collectively, research suggests that interview-based assessments provide more accurate data relative to self-report checklists. However, in many studies, particularly those involving large samples, self-report checklists are used (Monroe & Reid, 2008). In this study we used an interview-based assessment of SLEs, which may provide a better phenotyping of SLEs than the self-report measures used in many gene  $\times$  environment interaction studies. Additionally, because children were relatively young during this assessment, the retrospective recall was for a short duration and concerned early childhood, a period in which parents are highly aware and attentive to stress.

### *The importance of neural development*

In light of the present results and meta-analytic data suggesting that early life stress, as opposed to stress that occurs later in life, is a particularly potent predictor of depression in short allele carriers, it is important to consider emerging neuroscience data suggesting that 5-HTTLPR genotype and early life stress may act through shared neurodevelopmental consequences to influence depression vulnerability (Caspi et al., 2010; Hariri & Holmes, 2006; Karg et al., 2011). First, supporting the role of 5-HTT disruption in the etiology of depressed mood, pharmacologic blockade of the 5-HTT in early life has been shown to result in anxiety- and depression-like behaviors in rodents (Ansorge, Zhou, Lira, Hen & Gingrich, 2004; Homberg, Schubert & Gaspar, 2010). Second, there is also evidence from human studies indicating that the effects of the 5-HTTLPR polymorphism on amygdala function is driven by brain structure, not current 5-HTT availability; this suggests that differences conferred by 5-HTT genotype arise from indirect neurodevelopmental consequences on brain structure as opposed to its direct effects on serotonin transporter function (Kobiella et al., 2011). Perhaps most importantly, there is growing support for the idea that both 5-HTTLPR and early life stress may have downstream consequences on serotonin receptor function. Research

across species suggests that the short allele is associated with diminished 5-HT<sub>1A</sub> binding, which may reflect downstream consequences of elevated serotonergic tone (Christian et al., 2013; David et al., 2005). A similar effect is seen as a consequence of early life stress exposure in rodents, nonhuman primates, and humans (Goodfellow, Benekareddy, Vaidya & Lambe, 2009; Jovanovic, Perski, Berglund & Savic, 2011; Spinelli et al., 2010), clearly implicating shared neurobiological pathways. In fact, this reduced 5-HT<sub>1A</sub> binding is associated with differences in cortical neurodevelopment that may confer vulnerability to depression and anxiety through disrupted prefrontal regulation of subcortical circuits, including the amygdala (Goodfellow et al., 2009; Jovanovic et al., 2011; Pezawas et al., 2005). Collectively, these results suggest that 5-HTTLPR genotype and early life stress may confer depression vulnerability through potentially additive effects on serotonergic receptor regulation during early life.

### *Limitations and future directions*

While this study has several notable strengths including a sample of preschool-aged children with depression (aged 3–5) and an interview-based assessment of SLEs, it is not without limitations. First and foremost, while the sample size is relatively large for an investigation of preschool-onset depression, it is relatively small for a study of gene  $\times$  environment interaction; it is clearly underpowered to detect the small effect that meta-analyses suggest this interaction may confer (Duncan & Keller, 2011). Moreover, given the unique population (i.e., preschool-aged children with depression) we were unable to obtain a replication sample as has been suggested for G $\times$ E in light of false positive concerns (Duncan & Keller, 2011). As such, it will be important for further research to replicate this interaction at this developmental stage. We are only aware of one study (the Generation R study) in a similarly-aged sample. This recent report showed that a relatively large sample ( $n = 2,136$ ) of similarly-aged young children with the short 5-HTTLPR allele are more susceptible to emotional problems if their mother experienced postnatal anxiety or depression symptoms (Tiemeier et al., 2012). However, this study did not report on SLEs or depression more specifically. Perhaps, most importantly, it is unclear exactly how maternal postnatal depression and anxiety symptoms translate to risk for the young child. These maternal symptoms likely reflect both a genetic loading for mood and anxiety disorders as well as a potentially stressful environment. As such, the present study and the Generation R study have broadly consistent and potentially generalizable effects but may not represent replication in a more direct sense. Nevertheless, the generalizable and convergent result can provide more confidence in the findings reported here (Karg et al., 2011).

Duncan & Keller (Duncan & Keller, 2011), as well as others, have also argued that low confidence in priors (i.e., the biological rationale for the selection of 5-HTT as a candidate gene) and subsequent lack of identification of this genotype at genomewide levels of statistical significance ( $5E-8$ ) in genomewide association studies (GWAS), coupled with low power (discussed above) contribute to an inordinately high false discovery rate. Despite replicating the broad results of the Generation R study, we cannot exclude the possibility that our finding constitutes a false positive. However, it is also worth noting that there is a robust literature that implicates 5-HTT in the etiology of depression (Caspi et al., 2010). Whether investigators consider this burden of evidence to sufficiently categorize 5-HTT as a putative candidate is, largely, a matter of opinion. There are also several reasons why GWAS may not implicate the 5-HTTLPR polymorphism. The 5-HTTLPR may be imputed with accuracy (~93–95%) in Caucasians using multiple SNPs contained on some GWAS arrays (Knodt, 2012; Lu et al., 2012). However, we are unaware of any GWAS reporting use of such methodology for imputation. Furthermore, the utility of such imputation procedures in ethnicities other than Caucasians requires additional attention (Knodt, 2012). Moreover, GWAS have largely examined the main effects of genotype on depression and related constructs. Given that main effects of 5-HTTLPR on psychopathology are uncommon (although noted in the present study), it would not be entirely surprising that they are not found. In the absence of systematic genomewide G $\times$ E efforts (which are emerging) with careful assessment of SLEs, as well as imputation allowing for 5-HTTLPR typing, this criticism of the putative role of 5-HTTLPR may be premature.

While we speculate that kindling effects may diminish 5-HTTLPR  $\times$  SLE interaction effects in later depressive episodes, we were unable to directly test this hypothesis with the current baseline data. It will be important for longitudinal studies (including longitudinal data from the current study that is now being collected) to directly test this hypothesis as has been carried out for the main effects of SLEs on depression (Kendler et al., 2000). In this sample, the 5-HTTLPR  $\times$  SLEs interaction predicted depression. However, this study did not probe the neurobiological mechanisms through which this effect may emerge. Human neuroimaging and nonhuman animal models suggest several pathways including individual differences in threat-related amygdala reactivity, 5-HT<sub>1A</sub> availability, HPA axis function, reward processing, conflict monitoring, and neurodevelopment (Caspi et al., 2010). It will be important for future multimodal research to interrogate this interaction while simultaneously collecting measures of biological function to assess potentially mediating roles.

Lastly, PO-MDD is just beginning to be characterized and its clinical significance is not yet well understood (Luby et al., 2009). However, it has shown longitudinal stability with MDD in later childhood and has been detected in numerous independent study samples (Bufferd, Dougherty, Carlson, Rose & Klein, 2012; Luby et al., 2009; Wichstrom et al., 2012). Further, there is a rapidly emerging literature demonstrating biological correlates similar to those known in adult MDD (Barch, Gaffrey, Botteron, Belden & Luby, 2012; Gaffrey, Luby, Botteron, Repovs & Barch, 2012; Gaffrey, Luby et al., 2011; Pagliaccio et al., 2012). Collectively, these new findings are beginning to provide evidence for the validity of PO-MDD.

### Summary

The present study suggests that short homozygotes with high exposure to early life stressful life events are vulnerable to the development of preschool-onset depression. These data are consistent with the original report of this association in adults as well as recent meta-analytic data supporting the association between early adversity and adult-onset depression (Caspi et al., 2003; Karg et al., 2011). These data critically extend this previous research by showing that this interaction may be present even for extremely early-onset preschool depression. Further research is needed to: replicate this relationship at this developmental period, examine empirically whether this relationship is diminished in recurrent episodes consistent with the kindling model of depression, and test candidate neurodevelopmental mechanisms.

### Supporting information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Serotonin-transporter-linked polymorphic region (5-HTTLPR) genotype and stressful life events interact to predict preschool-onset depression: A replication and developmental extension (Word document).

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## Key points

- 5-HTTLPR and early life stressful life events interact to predict preschool-onset depression.
- This interaction suggests differential susceptibility; short homozygotes have increased rates of depression in the context of elevated early adversity but reduced rates in the context of low adversity.
- These results converge with recent meta-analytic data and neuroscience research suggesting that the 5-HTTLPR × SLEs interaction may be driven by neurodevelopmental influences.
- We speculate that kindling, may explain some mixed findings within the 5-HTTLPR × SLEs literature.

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