Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2009 July 5; 0(5): 670–682. doi:10.1002/ajmg.b.30888.

Effects of Stressful Life Events, Maternal Depression and 5-HTTLPR Genotype on Emotional Symptoms in Pre-Adolescent Children[†]

Ricardo Araya^{1,*}, Xianzhang Hu², Jon Heron³, Mary-Anne Enoch², Jonathan Evans¹, Glyn Lewis¹, David Nutt¹, and David Goldman²

¹Academic Unit of Psychiatry, University of Bristol, Bristol, United Kingdom ²Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH, Rockville, Maryland

³ALSPAC, Social Medicine, University of Bristol, Bristol, United Kingdom

Abstract

There has been a large but inconsistent literature on interactions between the 5-HTTLPR polymorphism of the serotonin transporter gene and adversity on emotional disorders. We investigated these interactions in 4,334 children from a birth longitudinal cohort: the Avon Longitudinal Study of Parents and Children (ALSPAC). We measured emotional symptoms at 7 years with the Strengths and Difficulties Questionnaire. Mothers rated stressful life events between ages 5 and 7 years. Maternal depression was defined as a score ≥ 12 on the Edinburgh Postnatal Depression Scale at 2 or 8 months postnatally. Triallelic genotyping of the 5-HTTLPR polymorphism was performed. We found strong associations between stressful life events (OR 1.19; 1.12–1.26; $P < 0.01$) and maternal postnatal depression (OR 1.91; 1.63–2.24; $P < 0.01$) with emotional symptoms in the children. There were no main 5-HTTLPR genotype effects or significant interactions between genotype and life events or maternal postnatal depression on emotional symptoms. There was marginal evidence ($P = 0.08$) for an interaction between stressful life events and genotype in boys only, with those in the low and high 5-HTTLPR expression groups showing stronger associations. In these 7-year-old children, we did not replicate previously reported G $\times$ E interactions between 5-HTTLPR and life events for emotional symptoms. Gene by environment interactions may be developmentally dependent and show variation depending on the type and levels of exposure and sex. Young cohorts are essential to improve our understanding of the impact of development on gene and environment interactions.

Keywords

genotype; environment; interactions; depression; ALSPAC

[†]This article was published online on 14 November 2008. An error was subsequently identified. Xianzhang Hu's name was incorrectly listed. This notice is included in the online and print versions to indicate that both have been corrected 28 May 2009.

© 2008 Wiley-Liss, Inc.

*Correspondence to: Prof. Ricardo Araya, Academic Unit of Psychiatry, University of Bristol, Cotham House, Cotham Hill, Bristol BS6 6JL, United Kingdom. r.araya@bris.ac.uk.

INTRODUCTION

Symptoms of anxiety and depression often co-occur in childhood [Meltzer et al., 2000; Kessler et al., 2001] and are strong predictors of emotional disorders later in life [Kessler et al., 1997; Fombonne et al., 2001; Bosquet and Egeland, 2006]. Genetic and environmental influences act in complex ways steering children into different emotional developmental trajectories [Rutter and Silberg, 2002; Moffitt et al., 2005].

Twin studies of emotional symptoms show that genetic contributions play an important role [Eaves et al., 1997; Rice et al., 2002] and that the genetic origins of anxiety and depression may overlap [Kendler et al., 1992, 2007; Gorwood, 2007]. Similarly, environmental contributions, such as stressful life events [Heim et al., 1997; Kaufman and Charney, 2001; Sandberg et al., 2001] or parental behavior may also influence the emotional development of children. Animal studies show that maternal behavior can epigenetically reprogram stress responses in the offspring [Weaver et al., 2004] and epidemiological studies demonstrate a clear association between parental depression and emotional symptoms in children [Weissman et al., 1987; Rice et al., 2005]. There is, however, marked individual variation in emotional vulnerability to adversity and some of this variability appears to be genetically determined [Kendler et al., 1995; Rutter and Silberg, 2002].

The interplay between genetic and environmental factors in the aetiology of emotional disorders in children is still poorly understood [Rutter and Silberg, 2002]. There has been interest in a functional polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene. The presence of a short (“s”) allele reduces transcription of the gene, leading to diminished serotonin reuptake [Lesch et al., 1996]. There is some but still disputed evidence suggesting an association between the “s” allele and measures of anxiety and/or fear, neuroticism and harm avoidance [Munafo et al., 2003; Schinka et al., 2004; Sen et al., 2004]. Studies linking the “s” allele and depression have often yielded negative or small effect sizes [Lotrich and Pollock, 2004; Lasky-Su et al., 2005; Willis-Owen et al., 2005; Taylor et al., 2006]. However, larger and more consistent main effects of the “s” allele are found for possible intermediate phenotypes in adults such as emotional reactivity associated with increased amygdala activation in response to emotional stimuli [Hariri et al., 2002; Pezawas et al., 2005; Canli et al., 2006] or changes in frontal–amygdala coupling [Heinz et al., 2007] and in children such as anxiety sensitivity [Stein et al., 2008] or anxiety-related temperament traits [Fox et al., 2005].

Caspi et al. [2003] reported a gene–environment interaction ($G \times E$) between the “s” allele and stressful life events on depressive ideation among young adults in a population-based cohort in Dunedin, New Zealand. The “s” allele carriers reported more depressive symptoms, but only if they had experienced two or more stressful life events [Caspi et al., 2003]. Subsequent studies have reported similar results in adults of both sexes [Kaufman et al., 2004; Kendler et al., 2005; Wilhelm et al., 2006; Zalsman et al., 2006] and in females only [Eley et al., 2004; Grabe et al., 2005; Jacobs et al., 2006; Taylor et al., 2006], but other studies found no $G \times E$ interactions [Gillespie et al., 2004; Surtees et al., 2005]. There are far fewer studies exploring $G \times E$ interactions for anxiety-related disorders.

We found only two studies testing interactions between the 5-HTTLPR locus and adversity for depression in children [Eley et al., 2004; Kaufman et al., 2004]. Eley et al. [2004] found that environmental or genetic risks alone did not predict depression in children aged 12 to 19. However there was a significant interaction of the “s” allele and high psychosocial adversity risk but only in girls. Kaufman et al. [2004, 2006] found 5-HTTLPR and maltreatment/social support interactions in both sexes by comparing a small sample of children who suffered severe forms of adversity with controls [Kaufman et al., 2004, 2006]. Two other small studies found significant interactions between 5-HTTLPR and social support or adversity for anxiety-related endophenotypes. Fox et al. found that 7-year-old children carriers of the “s” allele with low social support had an increased risk for behavioral inhibition, an anxiety-related trait [Fox et al., 2005]. Similarly a study found a significant 5-HTTLPR $\times$ childhood maltreatment interaction for anxiety sensitivity, another intermediate phenotype for anxiety disorders [Stein et al., 2008]. However none of these studies explored the effect of $G \times E$ interactions on anxiety-related symptoms.

Different methodologies and populations may explain some of the variation between-studies but also hint at the overall robustness of this $G \times E$ interaction, since the effect generally survives. In addition, it has recently been appreciated that a single nucleotide substitution (A $\rightarrow$ G) within the “l” allele creates an allele functionally equivalent to the “s” allele with reduced transcription [Nakamura et al., 2000; Hu et al., 2006]. By genotyping the relatively common (frequency 0.09–0.14) L_G allele, variation in serotonin transporter expression can be better predicted, and the L_G allele can be functionally grouped with the “s” allele. Up to now, most studies have used relatively small samples to detect $G \times E$ interactions and the effect of HTTLPR may have been partly masked by the inability to distinguish the reduction-of-function L_G allele from the gain-of function L_A allele.

Here, in the large ALSPAC birth cohort being longitudinally followed in Avon, England, we have studied the interaction of serotonin transporter genotypes with stressful life events and maternal depression throughout pregnancy and up to 81 months, when emotional symptoms in boys and girls were assessed.

MATERIALS AND METHODS

This study is based on data collected as part of the Avon Longitudinal Study of Parents and Children (ALSPAC) enrolling pregnant women resident in Avon, England, with delivery dates between April 1991 and December 1992. This study enrolled 14,541 women in the early stages of pregnancy and resident in Avon, England and 13,801 mothers remained in the study with 13,985 surviving offspring at 12 months. The ethnic composition of this population is fairly homogeneous with 94.1% of “white” origin. Questionnaires were sent at regular intervals during pregnancy and following childbirth and biological samples were taken from parents and children including blood samples from which DNA was extracted. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

Psychosocial Measures

- A.** Emotional symptoms of the children were measured with the Strengths and Difficulties Questionnaire (SDQ) [Goodman et al., 1998], an instrument that has been translated into more than 40 languages and widely used throughout the world [Smedje et al., 1999; Klasen et al., 2000; Koskelainen et al., 2000; Thabet et al., 2000; Cury and Golfeto, 2003; Ronning et al., 2004; Bourdon et al., 2005; Hayes, 2007; Mazur et al., 2007; Matsuishi et al., 2008]. It has been validated against the several other well-established scales including Rutter scales, Child Behavior Checklist (CBCL), the Child Depression Inventory (CDI), and the Revised Children's Manifest Anxiety Scale (RCMAS) with excellent correlation coefficients for the emotional symptom subscales. Factor analysis identified five behavioral dimensions: conduct problems, emotional symptoms, hyperactivity, peer problems, and pro-social behavior. This factor structure has been confirmed with different samples including the British National Survey of Children [Meltzer et al., 2000; Goodman, 2001], which also validated this questionnaire against DSM -IV diagnoses. The emotional sub-scale reported by parents has shown excellent discriminative ability for emotional disorders [Becker et al., 2004]. The Cronbach alpha or internal consistency of the SDQ in this sample was 0.63. Brief questionnaires, such as the SDQ, are often the only option when interviewing large samples of mothers or their children in different and competing health domains such as in ALSPAC. Furthermore the prevalence of psychiatric disorders is relatively low among pre-pubertal children; thus if we had chosen to use disorders rather than symptoms as outcome measures, tens of thousands of children would need to be interviewed with a long and costly interview to have sufficient statistical power. Given the close relationship between psychiatric symptoms and disorders, the predictive nature of symptoms to become disorders, and the high co-morbidity of depression and anxiety using a questionnaire such as the SDQ that measures common neurotic symptoms seems a reasonable and realistic alternative.

Our study focuses on the emotional symptom subscale administered at 7 years (81 months), the most recent time point for which there was complete outcome data available. This subscale enquires upon the emotions and behaviors of the child over the last 6 months and the mother endorses whether each item is “not true”, “somewhat true,” or “certainly true.” The five items forming this sub-scale with a maximum score of 10 are: (1) Often complains of headaches or stomach aches; (2) Often seems worried; (3) Often unhappy, downhearted or tearful; (4) Often nervous or clingy in new situations; and (5) Many fears, easily scared. Although maternal mood may introduce a bias when mothers report on their children's emotional symptoms, this effect was small in other studies [Youngstrom et al., 1999].

- B.** The self-rated Edinburgh Postnatal Depression Scale (EPDS) [Cox et al., 1987] was used to assess mothers' depressive symptoms. Mothers completed this questionnaire at 18 and 32 weeks of pregnancy and when children were 8 weeks, 8 months, 21 months and 33 months old. This scale can be used either as a continuous or binary score. Validation studies have found that a cut off score of 12/13 identifies major depressive disorder with sensitivity between 100% and 68%

and specificity between 96% and 93% [Eberhard-Gran et al., 2001]. The EPDS has also been validated for use during pregnancy with similar sensitivity and specificity coefficients for identifying major depression using a similar cut-off point [Eberhard-Gran et al., 2001]. Maternal (pregnancy or postnatally) depression can be a potential stressor to the child and may also have an aetiological role in terms of heritable liability. Maternal depression may constitute an external stress factor but it may represent also a shared genetic risk background; something that we do not aim to unravel in this paper if results were positive. It is also important to account for maternal depression as a possible confounder (covariate) in the main analyses. In this study we defined postnatal depression (EPDS/PND) as a score above 12 on the EPDS either at 8 weeks or 8 months postnatally.

- C. Stressful life events were assessed using a questionnaire completed by mothers at 7 years who recorded whether the child had experienced any of 16 upsetting events since the child was 5 years old. The total number of events was used as the main environmentally predictive variable. Ideally one would like to ask young children directly for their events and impact on their lives but this is rarely done for practical as well as ethical reasons. Also the measure we had available did not allow extracting the exact timing of these life events but rather provided a cumulative measure since the age of 5. The items included in this questionnaire were taken from previous studies [Brown and Harris, 1978; Barnett et al., 1983] and a list is available upon request to the authors.

The following additional data obtained at recruitment were used to compare the composition of the original sample with the sample analyzed: ethnicity, housing tenure, highest educational level achieved by mother, crowding, and parity.

Biological Measures

Genotyping—DNA was extracted and processed from cord or peripheral blood as described previously [Jones et al., 2000]. We have shown that 5-HTTLPR is functionally triallelic. $L_{A>G}$ (rs 25531), a common substitution within the L allele, creates a functional AP2 transcription factor-binding site that suppresses transcription [Nakamura et al., 2000; Hu et al., 2006]. No effect of the rs25531 G allele on the activity of the S-promoter has yet been determined, in part due to the low frequency of the S_G allele. The six genotypes were grouped by expression level: Low: SS, SL_G , L_GL_G ; Intermediate: SL_A , L_GL_A ; High: L_AL_A .

Two-stage 5' nuclease genotyping of HTTLPR, including S, L_A , and L_G alleles

—Oligonucleotide primers and dye-labeled probes were designed to optimize allele discrimination using Primer Express™ software (ABI, Applied Biosystems, Inc., Foster City, CA). The L amplicon was 182 bp and the S amplicon was 138 bp. Genotyping was accomplished in two stages. Stage 1 discriminated S versus L alleles. Stage 2 discriminated L_A versus L_G alleles. We used two fluorogenic probes for each stage. For Stage 1, the allele discriminating probe (ADP) was capable of hybridizing once, and once only, to the 43 bp L insertion and an internal control probe (ICP) hybridized to a sequence located within the same amplicon but specific to a divergent repeat found only once in the amplicon and not involved in the insertion/deletion. For Stage 2, probes were designed that were specific for

the L_A and L_G alleles. Although this assay does not reliably distinguish the S_G allele from S_L , the S_G allele is uncommon and apparently does not alter promoter function. The fluorogenic probes were labeled at the 5' end with either FAM or VIC. PCR was carried out in a 25 μ l volume: 25–50 ng DNA, 120 nmol ADP, 60 nmol ICP, PCR primers (200 nmol of each), DMSO (4% by volume), and 1 $\times$ Mastermix (ABI). Amplification conditions were: 2 min at 50°C, 10 min at 95°C, then 40 cycles at 96°C for 15 s and 62.5°C for 90 s. Genotypes were generated using ABIPRISM 7700 Sequence Detection system software. Stage 1 and Stage 2 genotypes were combined to assign samples as one of six genotypes: SS, SL_A , SL_G , L_AL_A , L_AL_G , or L_GL_G . On each plate, previously sequenced standards were introduced: Stage 1 standards were SS, LS, and LL. Stage 2 standards were L_AL_A , L_AL_G , and L_GL_G . This genotyping can also be performed by resolving the S and L alleles on the basis of size in Stage 1, for example in a fluorescent sequencer. However for this large-scale genotyping the assay was performed as described above. For primer and probe sequences see Hu et al. [2005]. Genotyping failure rates were 3.28% (230 samples) and 12.84% (900 samples) for SNP and VNTR respectively. This left us with 162 and 147 successful duplicate pairs respectively with which to assess genotyping error rates. Estimated error rates were: SNP: 0.62% (95% CI 0.17–2.23%) and VNTR: 3.4% (1.86–6.15%).

The distribution of 5-HTTLPR tri-allelic genotype was: SS (16.6%), SL_A (44.2%), SL_G (5.9%), L_AL_A (25.5%), L_AL_G (7.0%), L_GL_G (0.8%). The distribution of genotype and allele frequencies (S: 0.416, L_A : 0.511, L_G : 0.073) is similar to that of other Caucasian populations [Hu et al., 2006]. The genotype frequencies for the three expression groups used in the analysis were: low expression group 23.3%, intermediate expression 51.1%, and high expression group 25.5%. There were no significant differences in genotype distribution between boys and girls. The three groupings ($\chi^2 = 2.32$, $P = 0.128$) and the six groupings ($\chi^2 = 5.92$, $P = 0.116$) were in Hardy–Weinberg equilibrium.

Data Analyses

Firstly we investigated whether missing data could have introduced bias by comparing characteristics of missing children with our sample using chi square tests. At age 7 years, there was complete data for SDQ, life events and 5-HTTLPR genotype for 4,334 children and this was the dataset used in this study. DNA from 9198 children was available for genotyping but 2,818 of these samples—almost all cord-blood samples treated with heparin—were failures and removed from the analysis. There was incomplete data for another 2,046 individuals most of which originated from non-attendance to the clinics. Differences between children in our sample and those left out are presented in Table I. Children excluded reported more stressful life events and negative emotional outcomes. The children analyzed were marginally more likely to have experienced three or more stressful life events and less likely to have high SDQ scores. There were also socio-demographic differences, suggesting that the studied sample enjoyed a better socio-economic position. The ALSPAC cohort is moderately homogeneous on an ethnic basis, with almost all sample classified as of “white origin.”

In the main analyses we used ordinal logistic regression models to investigate the relationship of children’s emotional symptoms assessed by the SDQ at 7 years with (1) 5-

HTTPLR genotype, (2) stressful life events, and (3) postnatal depression. For the SDQ emotional sub-scale scores children were grouped into four categories based on scores of 0, 1, 2, and 3+. In ordinal logistic modeling, the transition between each adjacent pair of levels on the outcome variable is modeled as a logistic regression equation. In our example, SDQ has four levels resulting in three such equations. The proportional odds assumption restricts the effects of each covariate to be constant across these three equations, that is, that the odds ratio for being above or below a cut-off on the outcome is the same irrespective of your location on the outcome scale. The result is a single regression estimate for each covariate which has the interpretation of being the ratio of odds of being above versus below a randomly chosen cut-point.

For stressful life events we generated a total score by adding all events. We opted for this approach rather than weighting specific events to facilitate comparisons with previous papers that have used the number of un-weighted stressful life events [Caspi et al., 2003; Eley et al., 2004; Gillespie et al., 2004]. However we briefly present the most relevant results with a measure of weighted stressful life events according to how mother rated the impact of the event on the child.

We examined the association between postnatal depression, defined as a score above 12 on the EPDS either at 8 weeks or 8 months postnatal, and emotional symptoms in children. We also investigated the effect of adjusting results for depression during pregnancy (mean EPDS scores at 18 and 33 months during pregnancy) and maternal depression (mean EPDS scores at 21 and 33 months postnatal) because maternal depression can affect children possibly through different mechanisms depending on the stage of development.

Three genotypes representing low (SS, SL_G, L_GL_G), intermediate (SL_A, L_GL_A), and high (L_AL_A) expression were used. In order to test for the association between life events and genotype we used a three-level multinomial regression model with genotype as the outcome. In view of the highly skewed distribution of emotional symptoms it was not possible to use linear regression models. We tested for interactions using ordinal regression models between life events and emotional symptoms stratified by genotype using likelihood ratio tests. This analysis was undertaken for the total sample and for the sample stratified according to sex. A similar analysis was performed to investigate interactions between post-natal depression and genotype. We tested models unadjusted and adjusted for maternal psychopathology. All analysis was performed using STATA 8.0 [STATA 2003].

RESULTS

As shown in Table I, approximately one-third of the children had two or more emotional symptoms. The sex distribution of SDQ scores was virtually identical (two symptoms = 16% of both sexes, 3 symptoms = 9% of boys and 11% of girls, 4 or more symptoms = 12% of both). The number of stressful life events had a skewed distribution with approximately one third of children experiencing two or more events in the 21 months since their fifth birthday, with equal frequency in boys and girls.

Results of adjusted and unadjusted associations between emotional symptoms and the exposure variables of interest (i.e., life events, and maternal postnatal depressive symptoms) are presented in the tables stratified according to genotype groupings. The significance of these associations was examined using odds ratios based on the proportional odds models. In these models multiple levels of an ordinal outcome variable (emotional symptoms in four groups) are analyzed together in order to produce a single odds ratio which best represents the association of the exposure (life events) with the outcome of interest.

There was no evidence of an association (main effects) between the 5-HTTLPR genotype and emotional symptoms (Table II). Girls with the intermediate expression and boys with the high expression genotype showed some tendency to present more emotional symptoms than other groups but the strength of these associations was weak. There were no clear genotype and sex interactions contributing to variability in emotional symptoms ($\chi^2 = 4.95$, $P = 0.08$). The 5-HTTLPR genotype was not associated with the number of stressful life events. For instance, the odds ratios for an increase of one stressful life event in the high expression compared to the low expression group were 1.02 (0.93–1.09) for the total sample, 1.01 (0.81–1.27) and 1.01 (0.82–1.25) in girls and boys respectively.

There was a clear and consistent association between stressful life events and emotional symptoms in the total sample (OR 1.19, 1.12–1.26, $P < 0.01$) and across all genotype groupings (Table III). In other words, the likelihood of presenting emotional symptoms increased significantly with a higher number of stressful life events and these results were consistent before and after adjusting for EPDS postnatal scores of the mothers.

There were no interactions ($P < 0.05$) between stressful life events and the 5-HTTLPR genotype influencing the presence of emotional symptoms in the total sample or in girls. However there was some marginal evidence [unadjusted ($\chi^2 = 6.23$, $P = 0.04$) and after adjustment for EPDS postnatal scores ($\chi^2 = 5.00$, $P = 0.08$)] of a possible interaction between stressful life events and genotype in boys only, with those in the extreme expression groups (low and high) showing stronger associations (Table III). In the analysis using life events weighted according to severity similar results were obtained. Similarly we found no main effects or interactions between the genotype and life events for the full sample ($\chi^2 = 0.04$, $P = 0.851$), girls ($\chi^2 = 2.56$, $P = 0.110$), or boys ($\chi^2 = 1.67$, $P = 0.197$) when using the traditional 5-HTTLPR S/L classification.

Plotting crude data (Figs. 1–3) revealed a complex crossover pattern of 5-HTTLPR genotype effects on emotional symptoms depending on number of stressful events reported. At higher levels of stress exposure (3+ events), higher 5-HTTLPR expression children reported more emotional symptoms with an allele dosage effect but none of these associations was statistically significant at a P -value < 0.05 .

There was a strong and consistent association between EPDS postnatal depression and children's emotional symptoms in the unadjusted models (OR 1.91, 1.63–2.24, $P < 0.01$) (Table IV). The strength of this association after adjustment for other maternal EPDS scores remained consistent in boys ($P = 0.04$ and < 0.01) but declined in girls ($P = 0.12$ and 0.10). Likewise there were reasonably consistent associations between EPDS postnatal depression

and emotional symptoms for all genotype groupings in both sexes in the unadjusted models (Table V). However the statistical significance of most of these associations dropped markedly after adjusting for other EPDS scores. Among girls the strongest association between emotional symptoms and EPDS postnatal depression was found in the low expression group before and after adjusting for other maternal EPDS scores. On the contrary, among boys, stronger associations were found for emotional symptoms and the high expression group that were attenuated by maternal depression. The 5-HTTLPR genotype and EPDS postnatal depression did not clearly interact to modify emotional symptoms.

DISCUSSION

This study investigated whether interactions between the 5-HTTLPR genotype and stressful life events or postnatal depression might modify the presence of emotional symptoms in pre-pubertal children. Although several studies have examined similar interactions, we found no other large population-based, birth cohort of pre-pubertal children in which these interactions had been tested.

Two previous studies investigated 5-HTTLPR interactions with adversity in children and adolescents [Eley et al., 2004; Kaufman et al., 2004, 2006] but both used small samples and mixed children and adolescents limiting their ability to detect effects that may be specific to pre-adolescent children such as we studied. Also these studies used exposure and outcome measures different from those used in our study and this may partly explain the discrepancies with our findings. For instance the definitions of adversity (exposure) used in these studies focused in more severe life events than in our study. Thus it is possible that the threshold at which a moderating effect of adversity may work was not reached in our study. It is important to point out, however, that the severity distribution of life events in our study is representative of the population under study allowing a better understanding of the public health impact of any findings. A general limitation of the literature in this field is that studies use different definitions of adversity and there is a tendency to treat the environment as a homogeneous concept. Similarly both these previous studies [Eley et al., 2004; Kaufman et al., 2004, 2006] used depression questionnaires—the Mood and Feelings Questionnaire (MFQ)—as outcome measures. Our study used an unspecific emotional symptom questionnaire that indexes depressive symptoms but also other common emotional symptoms, especially anxiety-related. The developmental trajectories of anxiety and depressive disorders are still contested and likely to be dissimilar [Roza et al., 2003]. However anxiety disorders tend to predominate in the pre-adolescence period and depressive disorders later in life often are preceded by anxiety disorders in childhood. Thus it is likely that in some of our cases anxiety may have predominated but it is equally likely that many of these children will turn out to be cases of depression later in life. The most consistent previous evidence of a $G \times E$ interaction between life events and the 5-HTTLPR gene is related to depressive outcomes in children and thus our outcome measure may have failed to capture those cases. The specificity of outcomes has been highlighted as a highly desirable factor when evaluating the robustness of $G \times E$ interaction [Moffitt et al., 2005]. However it must be borne in mind that no scale would be able to capture fully specific disorders that remain stable over time given the fluctuating trajectories of emotional disorders throughout

early development. Thus for all these reasons, strictly speaking, this study cannot be regarded as a replication study but as a different and novel study testing similar interactions but in different populations and with different measures.

We found strong associations of stressful life events and postnatal depression with emotional symptoms but no clear interactions between the 5-HTTLPR genotype and stressful life events or postnatal depression. We did find a modest interaction ($P = 0.04$) between 5-HTTLPR genotype and life events among boys only, in which those in the extreme genotype expression groups (low and high) showed stronger associations with emotional symptoms. However since there is no biologically plausible explanation for an increased risk among children with both high and low expression levels, this is likely to be a spurious association arising from multiple statistical testing. Our study sample size was between 5 and 10 times larger than in previous studies that reported similar and significant 5-HTT and adversity interactions [Caspi et al., 2003; Eley et al., 2004]; thus there should have been sufficient statistical power to test for interactions of a similar size.

Although we found no clear interactions, there was a complex crossover in these variables suggesting that interactions between genes and adversity may appear only above a certain level of stress exposure (Figs. 1–3). This may also help to explain the interactions found in studies involving levels of adversity way above those included in our study. There was an increase in the risk of emotional symptoms among those in the 5-HTTLPR low expression group. However this effect was reversed at higher levels of stress in which those pre-adolescent children carrying the L_A allele show increased emotionality in the presence of life events, although it is worth bearing in mind that these findings are statistically weak. Similar findings had been previously reported. For instance, Kendler et al. [2005] found $G \times E$ interactions between the “s” allele and life events but only in relation to mild/moderate rather than the uncommon high threat events [Kendler et al., 2005] and crossover effects were reported in at least two other depression/suicidality studies [Caspi et al., 2003; Roy et al., 2007].

Recent neurobiological studies of anxiety disorders have revealed differences between children and adults consistent with developmental influences in the associations between the 5-HTTLPR genotype and emotionality. In adults, the reduction-of-function S and L_G alleles consistently predicted anxiety and a stronger amygdala activation during passive viewing of emotion evocative faces [Hariri et al., 2002]. Contrary to these findings, behaviorally inhibited (anxious) adolescents showed amygdala deactivation during passive viewing of emotion evocative faces. In these youngsters, the amygdala became activated only when these youngsters were asked to provide subjective fear ratings, in other words when forced to connect with emotional circuitry [McClure et al., 2007; Perez-Edgar et al., 2007]. These findings illustrate the complexity of emotion developmental processes and suggest that brain circuits underlying emotional responses are in a process of continuous development possibly until well-advanced adolescence. A moderating effect of genotype on these complex associations between brain activity and emotional responses to stimuli that varies across development is yet to be found. From an evolutionary perspective this possibility would add an important explanation for the very high abundance and worldwide distribution of

functionally divergent serotonin transporter alleles, and the existence of orthologous serotonin transporter polymorphisms in non-human primates.

Interestingly the prevalence of stressful life events and emotional symptoms were similar in boys and girls, which could reflect their pre-adolescent age. As adolescence ensues, the number of reported adverse life events is likely to increase [Cyranowski et al., 2000] and this may also help to explain the possible emergence of different patterns of gene $\times$ adversity interactions according to sex. However, it is worth bearing in mind that there is still disagreement as to whether most new incident cases of adolescent depression are preceded by a clearly identified adverse life events [Goodyer, 2003].

Although we found a robust main effect association between postnatal depression and emotional symptoms, in keeping with previous studies [Weissman et al., 1987; Warner et al., 1995; Rice et al., 2005], we found no interactions between postnatal depression and the 5-HTTLPR genotype on the risk of emotional symptoms. We adjusted for the effects of maternal depression during pregnancy and after delivery because there have been suggestions that the effect of maternal depression during pregnancy or later in life may differ. Maternal depression may influence childhood emotional symptoms because of exposure to high levels of cortisol in utero [O'Connor et al., 2005] or through postnatal mother-child interactions [Plotsky and Meaney, 1993; Brummelte et al., 2006] or indeed through other pathways [Ramchandani et al., 2005]. Overall adjusting for pregnancy or later maternal depression levels decreased substantially the strength of most associations.

Among the major strengths of this study are the large sample size and the focus on children which contributes to the elucidation of the early phase of the interplay between genetic and environmental risks, and finally the long follow-up of more than 5 years. Other strengths are the inclusion of mothers' depressive state which is an important risk factor for distress in the offspring as well as using a tri-allelic 5-HTTLPR, instead of the simple short/long variant.

This study has some limitations too. First, our original sample experienced considerable attrition. In the end those individuals included in our sample were of somewhat higher socio-economic status than the original cohort. It is difficult to know precisely to what extent this affected our results but if it did it was most likely in the direction of attenuating our estimates given the well-known association of low socio-economic status and emotional symptoms. Second, the instrument used to measure emotional disorders (SDQ) may not be ideal to classify the phenotype. It is a list of symptoms and behaviors whose presence is ascertained by mothers rather than a diagnostic interview administered by an independent assessor. Nonetheless the SDQ has shown excellent psychometric properties and good discriminative ability for diagnosed psychiatric cases. Other studies have shown that results obtained with questionnaires are similar to those obtained when using psychiatric interviews [Caspi et al., 2003; Kaufman et al., 2004; Jacobs et al., 2006] and this study did not aim to establish associations with specific psychiatric diagnoses. The two previous studies in children [Eley et al., 2004; Kaufman et al., 2004] both used questionnaires. Third, we opted for prevalent rather than incident emotional symptoms; at this early age chronic cases are unlikely and restricting our analysis to incident cases would have limited further the statistical power of the analyses. Fourth, there is always the possibility that the mental state

of the mother may influence the ratings given to the children. However maternal depression may also influence the ratings of children's emotional symptoms in other ways. For instance Weissman et al. [1987] found that agreement between maternal and child reports of their own symptoms increased when the mother was depressed; depressed mothers were more sensitive to their children's symptoms [Weissman et al., 1987]. In any case we adjusted our results for the mental state of the mothers in order to decrease any possible confounding effect of maternal depression. Maternal genetic predisposition for depression may contribute to the genesis of emotional symptoms in children either by increasing heritability or influencing the environment in which the child lives. Fifth, we used a composite measure of acute stressful, independent life events impacting preferentially on the child and we did not include indicators of chronic social adversity, in keeping with previous studies [Caspi et al., 2002, 2003]. Mothers reported stressful life events and their mental status could have biased their reporting. Nonetheless asking young children (aged 5–7) about life events is rarely done in large surveys such as this. Lastly, although we adjusted our results for a wide range of potential confounders there is always the possibility to add more variables to the models. Nonetheless there is also the risk of over-adjusting and we opted for limiting these adjustments to a reasonable minimum.

Overall our results were negative for either main effects or interactions between the 5-HTTLPR genotype and stressful life events or postnatal depression on the risk of emotional symptoms in these pre-adolescent children. There remains the possibility of the existence of complex crossover effects of the alleles with different degree of risk exposure, as seen in other studies in adults. Equally interactions between the 5-HTTLPR genotype and stress may also be dependent [Rice et al., 2002] on developmental stages. These issues may be further clarified within this age cohort as it matures including the passage of these children through the period of adolescence and early adulthood when many psychiatric diseases have their onset.

Acknowledgments

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council, the Wellcome Trust and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and R Araya will serve as guarantor for the contents of this paper. The Intramural Research Program of the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, USA specifically funded this research.

References

- Barnett B, Hanna B, Parker G. Life event scales for obstetric groups. *J Psychosom Res.* 1983; 27(4): 313–320. [PubMed: 6620208]
- Becker A, Woerner W, Hasselhorn M, Banaschewski T, Rothenberger A. Validation of the parent and teacher SDQ in a clinical sample. *Eur Child Adolesc Psychiatry.* 2004; 13(Suppl 2):II11–II16. [PubMed: 15243781]
- Bosquet M, Egeland B. The development and maintenance of anxiety symptoms from infancy through adolescence in a longitudinal sample. *Dev Psychopathol.* 2006; 18:517–550. [PubMed: 16600066]
- Bourdon KH, Goodman R, Rae DS, Simpson G, Koretz DS. The strengths and difficulties questionnaire: U.S. normative data and psychometric properties. *J Am Acad Child Adolesc Psychiatry.* 2005; 44(6):557–564. [PubMed: 15908838]

- Brown, AC.; Harris, TO. Social origins of depression: A study of psychiatric disorder in women. New York: Free Press; 1978.
- Brummelte S, Pawluski JL, Galea L. High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behavior of offspring: A model of post-partum stress and possible depression. *Horm Behav.* 2006; 50:370–382. [PubMed: 16780843]
- Canli T, Qiu M, Omura K, Congdon E, Haas BW, Amin Z, Herrmann MJ, Constable RT, Lesch KP. Neural correlates of epigenesis. *Proc Natl Acad Sci.* 2006; 103(43):16033–16038. [PubMed: 17032778]
- Caspi A, McClay J, Moffitt T, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. *Science.* 2002; 297:851–854. [PubMed: 12161658]
- Caspi A, Sugden K, Moffitt T, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science.* 2003; 301:386–389. [PubMed: 12869766]
- Cox J, Holden J, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry.* 1987; 150:782–786. [PubMed: 3651732]
- Cury CR, Golfeto JH. Strengths and difficulties questionnaire (SDQ): A study of school children in Ribeirao Preto. *Rev Bras Psiquiatr.* 2003; 25(3):139–145. [PubMed: 12975687]
- Cyranowski JM, Frank E, Young E, Shear K. Adolescent onset of the gender difference in lifetime rates of major depression. *Archives General Psychiatry.* 2000; 57:21–27.
- Eaves L, Silberg J, Meyer JM, Maes H, Simonoff E, Pickles A, Rutter M, Neale MC, Reynolds CA, Erikson MT, Heath AC, Loeber R, Tuett KR, Hewitt JK. Genetics and developmental psychopathology: 2. The main effects of genetics and environment of behavioral problems in the Virginia twin study of adolescent behavioral development. *J Child Psychol Psychiatry.* 1997; 38:965–980. [PubMed: 9413795]
- Eberhard-Gran M, Eskild A, Tambs K, Opjordsmoen S, Samuelsen SO. Review of validation studies of the Edinburgh Postnatal Depression Scale. *Acta Psychiatr Scand.* 2001; 104(4):243–249. [PubMed: 11722298]
- Eley TC, Sugden K, Corsico A, Gregory AM, Sham P, McGuffin P, Plomin R, Craig IW. Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol Psychiatry.* 2004; 9:908–915. [PubMed: 15241435]
- Fombonne E, Wostear G, Cooper V, Harrington R, Rutter M. The Maudsley long-term follow-up of child and adolescent depression. 2. Suicidality, criminality and social dysfunction in adulthood. *Br J Psychiatry.* 2001; 179:218–223. [PubMed: 11532798]
- Fox NA, Nichols KE, Henderson HA, Rubin K, Schmidt L, Hamer D, Ernst M, Pine DS. Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychol Sci.* 2005; 16(12):921–926. [PubMed: 16313653]
- Gillespie NA, Whitfield JB, Williams B, Heath AC, Martin NG. The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression. *Psychol Med.* 2004; 34:1–11.
- Goodman R. Psychometric properties of the strengths and difficulties questionnaire (SDQ). *J Am Acad Child Adolesc Psychiatry.* 2001; 40:1337–1345. [PubMed: 11699809]
- Goodman R, Meltzer H, Bailey V. The strengths and difficulties questionnaire: A pilot study on the validity of the self-report version. *Eur J Child Adolesc Psychiatry.* 1998; 7:125–130.
- Goodyer, I. Unipolar depression: A lifespan perspective. Oxford: Oxford University Press; 2003.
- Gorwood P. Generalized anxiety disorder and major depressive disorder comorbidity: An example of genetic pleiotropy? *Eur Psychiatry.* 2007; 19:27–33. [PubMed: 14969778]
- Grabe HJ, Lange M, Wolff B, Volzke H, Lucht M, Freyberger HJ, John U, Cascorbi I. Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. *Mol Psychiatry.* 2005; 10:220–224. [PubMed: 15263905]
- Hariri AR, Mattay VS, Tessitore A, Kolachana BS, Fera F, Goldman D, Egan MF, Weinberger DR. Serotonin transporter genetic variation and the response of the human amygdala. *Science.* 2002; 297:400–403. [PubMed: 12130784]

- Hayes L. Problem behaviours in early primary school children: Australian normative data using the Strengths and Difficulties Questionnaire. *Aust N Z J Psychiatry*. 2007; 41(3):231–238. [PubMed: 17464704]
- Heim C, Owens M, Plotsky P, Nemeroff C. The role of early adverse life events in the etiology of depression and posttraumatic stress disorder: Focus on corticotropin-releasing factor. *Ann NY Acad Sci*. 1997; 821:194–207. [PubMed: 9238204]
- Heinz A, Smolka MN, Braus DF, Wrase J, Beck A, Flor H, Mann K, Schumann G, Buchel C, Hariri AR, Weinberger DR. Serotonin Transporter Genotype (5-HTTLPR): Effects of neutral and undefined conditions on amygdala activation. *Biol Psychiatry*. 2007; 61(8):1011–1014. [PubMed: 17157270]
- Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcohol Clin Exp Res*. 2005; 29(1):8–16. [PubMed: 15654286]
- Hu X, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Human Genetics*. 2006; 78:815–826. [PubMed: 16642437]
- Jacobs N, Kenis G, Peeters F, Derom C, Vlietink R, Van Os J. Stress-related negative affectivity and genetically altered serotonin transporter function: Evidence of synergism in shaping risk of depression. *Arch Gen Psychiat*. 2006; 63:989–996. [PubMed: 16953001]
- Jones R, Ring S, Tyfield L, Hamvas R, Simmons H, Pembrey M, Golding J. Study Team ALSPAC. A new DNA resource: A DNA bank established as part of the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). *Eur J Hum Genet*. 2000; 8:653–660. [PubMed: 10980570]
- Kaufman J, Charney D. Effects of early stress on brain structure and function: Implications for understanding the relationship between child maltreatment and depression. *Dev Psychopathol*. 2001; 13:451–471. [PubMed: 11523843]
- Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH, Gelertner J. Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci USA*. 2004; 101:17316–17321. [PubMed: 15563601]
- Kaufman J, Yang B-Z, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, Krystal JH, Gelertner J. Brain-derived neurotrophic factor—5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biol Psychiatry*. 2006; 59:673–680. [PubMed: 16458264]
- Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves L. Major depression and generalized anxiety disorder: Same genes, (partly) different environments? *Arch Gen Psychiatry*. 1992; 49:716–722. [PubMed: 1514877]
- Kendler KS, Kessler RC, Walter EE, Mac Lean C, Neal MC, Heath AC, Eaves LJ. Stressful life events, genetic liability, and onset of an episode major depression in women. *Am J Psychiatry*. 1995; 152:833–842. [PubMed: 7755111]
- Kendler KS, Kuhn JW, Vittum J, Prescott CA, Riley B. The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes in major depression. *Arch Gen Psychiatry*. 2005; 62:529–535. [PubMed: 15867106]
- Kendler KS, Gardner CO, Gatz M, Pedersen NL. The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. *Psychol Med*. 2007; 37(3):453–462. [PubMed: 17121688]
- Kessler R, Davis CG, Kendler KS. Childhood adversity and adult psychiatric disorder in the U.S. National Comorbidity Survey. *Psychol Med*. 1997; 27:1101–1119. [PubMed: 9300515]
- Kessler R, Avenevoli S, Merikangas K. Mood disorders in children and adolescents: An epidemiologic perspective. *Biol Psychiatry*. 2001; 49:1002–1014. [PubMed: 11430842]
- Klasen H, Woerner W, Wolke D, Meyer R, Overmeyer S, Kaschnitz W, Rothenberger A, Goodman R. Comparing the German versions of the strengths and difficulties questionnaire (SDQ-Deu) and the child behavior checklist. *Eur Child Adolesc Psychiatry*. 2000; 9(4):271–276. [PubMed: 11202102]
- Koskelainen M, Sourander A, Kaljonen A. The strengths and difficulties questionnaire among Finnish school-aged children and adolescents. *Eur Child Adolesc Psychiatry*. 2000; 9(4):277–284. [PubMed: 11202103]

- Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter and affective disorders. *Am J Med Genet Part B*. 2005; 133B:110–115. [PubMed: 15578606]
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. A gene regulatory region polymorphism alters serotonin transporter expression and is associated with anxiety-related personality traits. *Science*. 1996; 274:1527–1531. [PubMed: 8929413]
- Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatr Genet*. 2004; 14:121–129. [PubMed: 15318024]
- Matsuishi T, Nagano M, Araki Y, Tanaka Y, Iwasaki M, Yamashita Y, Nagamitsu S, Iizuka C, Ohya T, Shibuya K, Hara M, Matsuda K, Tsuda A, Kakuma T. Scale properties of the Japanese version of the Strengths and Difficulties Questionnaire (SDQ): A study of infant and school children in community samples. *Brain Dev*. 2008; 30(6):410–415. [PubMed: 18226867]
- Mazur J, Tabak I, Kololo H. Towards a better assessment of child and adolescent mental health status. Polish version of strengths and difficulties questionnaire. Experiences from two population studies. *Med WiekuRozwoj*. 2007; 11(1):13–24.
- McClure EB, Monk CS, Nelson EE, Parrish JM, Adler A, Blair JR, Fromm S, Charney DS, Leibenluft E, Ernst M, Pine DS. Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. *Arch Gen Psychiatry*. 2007; 64:97–106. [PubMed: 17199059]
- Meltzer, H.; Gatward, R.; Goodman, R.; Ford, F. Mental health of children and adolescents in Great Britain. London: The Stationery Office; 2000.
- Moffitt T, Caspi A, Rutter M. Strategy for investigating interactions between measured genes and measured environments. *Arch Gen Psychiatry*. 2005; 62:473–481. [PubMed: 15867100]
- Munafo MR, Clark TG, Moore LR, Payne E, Walton R, Flint J. Genetic polymorphisms and personality in healthy adults: A systematic review and meta-analysis. *Mol Psychiatry*. 2003; 8:471–484. [PubMed: 12808427]
- Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry*. 2000; 5(32):38.
- O'Connor TG, Ben-Shlomo Y, Heron J, Golding J, Adams D, Glover V. Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. *Biol Psychiatry*. 2005; 58(3):211–217. [PubMed: 16084841]
- Perez-Edgar K, Roberson-Nay R, Hardin MG, Poeth K, Guyer AE, Nelson EE, McClure EB, Henderson HA, Fox NA, Pine DS, Ernst M. Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. *Neuroimage*. 2007; 35(4):1538–1546. [PubMed: 17376704]
- Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BA, Munoz KE, Kolachana BS, Egan MF, Mattay VS, Hariri AR, Weinberger DR. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: A genetic susceptibility mechanism for depression. *Nat Neurosci*. 2005; 8:828–834. [PubMed: 15880108]
- Plotsky P, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress induced release in adult rats. *Mol Brain Res*. 1993; 18:195–200. [PubMed: 8497182]
- Ramchandani P, Stein A, Evans J, O'Connor TG. Paternal depression in the postnatal period and child development: A prospective population study. *Lancet*. 2005; 365(9478):2201–2205. [PubMed: 15978928]
- Rice F, Harold G, Thapar A. The genetic aetiology of childhood depression: A review. *J Child Psychol Psychiatry*. 2002; 43(1):65–79. [PubMed: 11848337]
- Rice F, Harold G, Thapar A. The link between depression in mothers and offspring: An extended twin analysis. *Behav Genet*. 2005; 35(5):565–577. [PubMed: 16184485]
- Ronning JA, Handegaard BH, Sourander A, Mørch WT. The strengths and difficulties self-report questionnaire as a screening instrument in Norwegian community samples. *Eur Child Adolesc Psychiatry*. 2004; 13(2):73–82. [PubMed: 15103532]
- Roy A, Hu XZ, Janal MN, Goldman D. Interaction between childhood trauma and serotonin transporter gene variation in suicide. *Neuro-psychopharmacology*. 2007; 32(9):2046–2052.

- Roza SJ, Hofstra MB, van der Ende J, Verhulst FC. Stable prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood: A 14-year follow-up during childhood, adolescence, and young adulthood. *Am J Psychiatry*. 2003; 160(12):2116–2121. [PubMed: 14638580]
- Rutter M, Silberg J. Gene-environment interplay in relation to emotional and behavioural disturbance. *Annu Rev Psychol*. 2002; 53:463–490. [PubMed: 11752493]
- Sandberg S, Rutter M, Pickles A, McGuinness D, Angold A. Do high-threat life events really provoke the onset of psychiatric disorder in children? *J Child Psychol Psychiatry*. 2001; 42:523–532. [PubMed: 11383968]
- Schinka JA, Busch RM, Robichaux-Keene N. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol Psychiatry*. 2004; 9:197–202. [PubMed: 14966478]
- Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *Am J Med Genet Part B*. 2004; 127B(1):85–89. [PubMed: 15108187]
- Smedje H, Broman JE, Hetta J, von Knorring AL. Psychometric properties of a Swedish version of the “strengths and difficulties questionnaire”. *Eur Child Adolesc Psychiatry*. 1999; 8(2):63–70. [PubMed: 10435454]
- STATA. STATA Version 8.0. Texas: Stata Corp; 2003.
- Stein MB, Schork NJ, Gelernter J. Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology*. 2008; 33:312–319.
- Surtees PG, Wainwright NWJ, Willis-Owen SAG, Luben R, Day NE, Flint J. Social adversity, the serotonin transporter (5-HTTLPR) polymorphism and major depressive disorder. *Biol Psychiatry*. 2005; 59(3):224–229. [PubMed: 16154545]
- Taylor SE, Way BM, Welch WT, Hilmert CJ, Lehman BJ, Eisenberger NI. Early family environment, current adversity, the serotonin transporter promoter polymorphism, and depressive symptomatology. *Biol Psychiatry*. 2006; 60(7):671–676. [PubMed: 16934775]
- Thabet AA, Stretch D, Vostanis P. Child mental health problems in Arab children: Application of the strengths and difficulties questionnaire. *Int J Soc Psychiatry*. 2000; 46(4):266–280. [PubMed: 11201348]
- Warner V, Mufson L, Weissman MM. Offspring at high and low risk for depression and anxiety: Mechanisms of psychiatric disorder. *J Am Acad Child Adolesc Psychiatry*. 1995; 34:786–797. [PubMed: 7608053]
- Weaver IC, Cervoni N, Champagne FA, D’Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004; 7(8):847–854. [PubMed: 15220929]
- Weissman MM, Wickramaratne P, Warner V, John K, Prusoff A, Merikangas K, Gammon DG. Assessing psychiatric disorders in children—Discrepancies between mothers and children’s reports. *Arch Gen Psychiatry*. 1987; 44(8):744–753.
- Wilhelm K, Mitchell PB, Niven H, Finch A, Wedgwood L, Scimone A, Blair IP, Parker G, Schofield PR. Life events, first depression onset and the serotonin transporter gene. *Br J Psychiatry*. 2006; 188:210–215. [PubMed: 16507960]
- Willis-Owen SAG, Turri MG, Munafo MR, Surtess PG, Wainwright NWJ, Brixey RD, Flint J. The serotonin transporter length polymorphism, neuroticism, and depression: A comprehensive assessment of association. *Biol Psychiatry*. 2005; 58(6):451–456. [PubMed: 16023085]
- Youngstrom E, Izard C, Ackerman B. Dysphoria-related bias in maternal ratings of children. *J Consult Clin Psychol*. 1999; 67(6):905–916. [PubMed: 10596512]
- Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, Ellis SP, Goldman D, Mann JJ. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *Am J Psychiatry*. 2006; 163(9):1588–1593. [PubMed: 16946185]

FIG. 1.

Association between stressful life events, 5-HTTLPR genotype and emotional symptoms in children (total sample, unadjusted data).

FIG. 2. Association between stressful life events, 5-HTTLPR genotype and emotional symptoms in children (girls only, unadjusted data).

FIG. 3. Association between stressful life events, 5-HTTLPR genotype and emotional symptoms in children (boys only, unadjusted data).

TABLE I

Characteristics and Comparison of the Studied and Missing Subjects From the ALSPAC Cohort: Avon, England N (%)

	N	Missing subjects	Study sample	Statistical significance, χ^2 , P
Car ownership				
Yes	11,637	7,585 (86.2)	4,052 (95.5)	258.14, <0.01
No	1,407	1,216 (13.8)	191 (4.5)	
Housing tenure				
Mortgage/owned	9,567	6,035 (68.6)	3,532 (83.3)	337.06, <0.01
Council	1,862	1,544 (17.6)	318 (7.5)	
Other rented	1,606	1,217 (13.8)	389 (9.2)	
Crowding index				
<1 person/room	10,280	6,624 (76.7)	3,656 (87.0)	195.15, <0.01
1 person/room	1,676	1,289 (14.9)	387 (9.2)	
>1 person/room	879	721 (8.4)	158 (3.8)	
Mother's education				
<O level or less than 10 years	3,730	2,879 (35.2)	851 (20.0)	377.55, <0.01
O level or 10 years	4,302	2,800 (34.3)	1,502 (35.3)	
>O level or more than 10 years	4,394	2,491 (30.5)	1,903 (44.7)	
Parity				
0 children	5,729	3,851 (44.2)	1,878 (44.4)	18.57, <0.01
1 child	4,505	2,954 (33.9)	1,551 (36.7)	
2+ children	2,713	1,912 (21.9)	801 (18.9)	
Ethnicity				
White	13,421	9,251 (93.5)	4,170 (96.5)	50.94, <0.01
Non-white	792	641 (6.5)	151 (3.5)	
Emotional symptoms (SDQ scores)				
0	3,007	1,421 (34.7)	1,586 (36.6)	12.34, <0.01
1	2,132	1,009 (24.6)	1,123 (25.9)	
2	1,355	661 (16.1)	694 (16.0)	
+3	1,938	1,007 (24.6)	931 (21.5)	
Adverse life events				
0	2,557	1,180 (30.3)	1,377 (31.8)	9.21, 0.03
1	2,524	1,168 (30.0)	1,356 (31.3)	
2	1,636	783 (20.1)	853 (19.7)	
+3	1,514	766 (19.7)	748 (17.3)	

TABLE II
Association Between Emotional Symptoms* and 5-HTTLPR Genotype According to Sex

5-HTTLPR Emotional symptoms	Full sample			Girls			Boys		
	Odds ratios ^d	95% CI	P	Odds ratios ^d	95% CI	P	Odds ratios ^d	95% CI	P
3 groups									
Low expression ^a	1.00			1.00			1.00		
Medium expression ^b	1.09	[0.95, 1.25]	0.44	1.19	[0.98, 1.45]	0.08	1.02	[0.85, 1.23]	0.40
High expression ^c	1.07	[0.91, 1.24]		0.99	[0.79, 1.25]		1.14	[0.92, 1.41]	
Dose effect	1.03	[0.95, 1.11]	0.44	0.99	[0.89, 1.11]	0.88	1.07	[0.96, 1.19]	0.22
6 groups									
SS	1.00			1.00			1.00		
SL _A	1.10	[0.94, 1.28]	0.65	1.19	[0.95, 1.49]	0.15	1.03	[0.83, 1.28]	0.50
SL _G	1.03	[0.79, 1.34]		1.17	[0.80, 1.72]		0.92	[0.64, 1.32]	
L _A L _A	1.08	[0.92, 1.29]		1.03	[0.81, 1.32]		1.14	[0.90, 1.44]	
L _G L _A	1.21	[0.94, 1.54]		1.54	[1.08, 2.19]		0.96	[0.68, 1.36]	
L _G L _G	1.37	[0.74, 2.54]		1.01	[0.44, 2.32]		1.98	[0.78, 5.03]	
	4,334			2,028			2,306		

* SDQ symptom scores into 4-group modeled as an ordinal measure with the proportional odds assumption.

^aLow: SS, SL_G, L_GL_G.

^bMedium: SL_A, L_GL_A.

^cHigh: L_AL_A.

^dFigures are odds ratio for an increase of one point in emotional symptom scale (SDQ).

Unadjusted and Adjusted Associations Between Emotional Symptoms*, Stressful Life Events, and 5-HTTLPR Genotype According to Sex

TABLE III

5-HTTLPR genotype																	
All genotypes				Low expression ^d				Medium expression ^d				High expression ^d		Gene 3 life event interaction χ^2 , <i>P</i>			
N	OR ^b	95% CI	<i>P</i>	N	OR ^b	95% CI	<i>P</i>	N	OR ^b	95% CI	<i>P</i>	N	OR ^b		95% CI	<i>P</i>	
Unadjusted association between children emotional symptoms and stressful life events																	
Full sample	4,334	1.19	1.12–1.26	<0.01	1,011	1.29	1.14–1.45	<0.01	2,216	1.14	1.05–1.23	<0.01	1,107	1.20	1.07–1.34	<0.01	2.93, 0.23
Girls	2,028	1.21	1.12–1.32	<0.01	489	1.28	1.07–1.53	0.01	1,015	1.24	1.10–1.40	<0.01	524	1.10	0.93–1.29	0.27	2.11, 0.35
Boys	2,306	1.17	1.08–1.26	<0.01	522	1.30	1.10–1.53	<0.01	1,201	1.06	0.95–1.18	0.27	583	1.30	1.12–1.52	<0.01	6.23, 0.04
χ^2 , <i>P</i> interaction	0.29, 0.59			0.02, 0.88			3.29, 0.07			2.26, 0.133							
Association between children emotional symptoms and stressful life events adjusted by EPDS postnatal depression (EPDS/PND) ^c																	
Full sample	4,257	1.16	1.10–1.23	<0.01	994	1.27	1.12–1.43	<0.01	2,175	1.13	1.04–1.22	<0.01	1,088	1.15	1.02–1.28	0.02	2.85, 0.24
Girls	2,003	1.21	1.11–1.31	<0.01	482	1.28	1.06–1.53	<0.01	1,000	1.24	1.10–1.40	<0.01	521	1.08	0.92–1.27	0.35	2.63, 0.27
Boys	2,254	1.13	1.04–1.22	<0.01	512	1.27	1.08–1.49	<0.01	1,175	1.03	0.93–1.15	0.55	567	1.21	1.03–1.42	0.02	5.00, 0.08
χ^2 , <i>P</i>	1.01, 0.31			<0.01, 0.99			4.25, 0.04			1.14, 0.29							

* SDQ symptom scores into 4-group modeled as an ordinal measure with the proportional odds assumption.

^d Low: SL, SLG; Medium: SLA, LGLA; High: LALA.

^b Figures are odds ratio per SD of life events.

^c Adjusted by EPDS score >12 at 8 weeks or 8 months postnatal.

TABLE IV
The Association Between Emotional Symptoms* and EPDS Postnatal Depression (EPDS/PND) According to Sex

Full sample			Girls			Boys			EPDS/PND 3 sex interaction χ^2 , <i>P</i>
OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	
Unadjusted association between children emotional symptoms and EPDS/PND									
EPDS/PND ^a									
No	1.00	—	1.00	—	—	1.00	—	—	
Yes	1.91	1.63–2.24	<0.01	1.80	1.43–2.28	<0.01	2.02	1.62–2.51	<0.01
N	4,381		2,055			2,326			0.73, 0.39
Association between children emotional symptoms and EPDS/PND adjusted by other mean maternal EPDS scores ^b									
EPDS/PND ^a									
No	1.00	—	1.00	—	—	1.00	—	—	
Yes	1.27	1.06–1.53	0.01	1.24	0.95–1.62	0.12	1.30	1.01–1.67	0.04
N	4,296		2,012			2,284			0.42, 0.52
Association between children emotional symptoms and EPDS/PND adjusted by mean pregnancy EPDS scores ^c									
EPDS/PND ^a									
No	1.00	—	1.00	—	—	1.00	—	—	
Yes	1.39	1.14–1.70	<0.01	1.27	0.96–1.69	0.10	1.51	1.15–1.99	<0.01
N	3,672		1,732			1,940			1.12, 0.29

* SDQ symptom scores into 4-group modeled as an ordinal measure with the proportional odds assumption.
^a EPDS/PND defined as EPDS score >12 at 8 weeks or 8 months postnatal.
^b Adjusted by mean EPDS score at 21 and 33 months postnatal.
^c Adjusted by mean EPDS score at 18 and 32 weeks during pregnancy.

TABLE V

The Unadjusted and Adjusted Associations Between Emotional Symptoms^{*}, EPDS Postnatal Depression (EPDS/PND), and 5-HTTLPR Genotype According to Sex

5-HTTLPR genotype										
Comparison	Low expression ^d			Medium expression ^d			High expression ^d			EPDS/PND 3 gene interaction χ^2 , <i>P</i>
	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>	
Unadjusted association between children emotional symptoms and EPDS/PND depression ^b										
Full sample	1.92	1.33–2.75	<0.01	1.89	1.53–2.35	<0.01	1.93	1.39–2.68	<0.01	0.01, 0.99
Girls	2.60	1.48–4.57	<0.01	1.66	1.22–2.27	<0.01	1.61	1.00–2.58	0.05	2.30, 0.32
Boys	1.58	0.98–2.53	0.06	2.12	1.58–2.85	<0.01	2.28	1.44–3.59	<0.01	1.28, 0.53
χ^2 , <i>P</i>	1.61, 0.21			1.71, 0.19			1.11, 0.29			
Association between children emotional symptoms and EPDS/PND adjusted by mean maternal EPDS scores ^c										
Full sample	1.17	0.77–1.77	0.47	1.32	1.03–1.69	0.03	1.24	0.86–1.79	0.26	0.20, 0.91
Girls	1.98	1.05–3.75	0.04	1.15	0.81–1.65	0.44	0.98	0.58–1.68	0.95	1.57, 0.46
Boys	0.78	0.45–1.36	0.39	1.47	1.05–2.07	0.03	1.52	0.91–2.54	0.11	1.74, 0.42
χ^2 , <i>P</i>	1.53, 0.22			1.26, 0.26			0.83, 0.36			
Association between children emotional symptoms and EPDS/PND adjusted by mean pregnancy EPDS scores ^d										
Full sample	1.32	0.85–2.07	0.22	1.35	1.04–1.76	0.03	1.58	1.06–2.34	0.02	0.69, 0.71
Girls	1.54	0.80–2.97	0.20	1.18	0.80–1.73	0.40	1.25	0.71–2.20	0.45	1.48, 0.45
Boys	1.16	0.63–2.14	0.63	1.49	1.03–2.16	0.04	1.96	1.13–3.41	0.02	0.11, 0.92
χ^2 , <i>P</i>	0.16, 0.69			1.17, 0.28			0.93, 0.33			

^{*} SDQ symptom scores into 4-group modeled as an ordinal measure with the proportional odds assumption.

^a Low: SS, SLG; Medium: SLA, LGLA; High: LALA.

^b EPDS/PND defined as EPDS score >12 at 8 weeks or 8 months postnatal.

^c Adjusted by mean EPDS score at 21 and 33 months postnatal.

^d Adjusted by mean EPDS score at 18 and 32 weeks during pregnancy.