Prenatal depression and 5-HTTLPR interact to predict dysregulation from 3 to 36 months – A differential susceptibility model

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Abstract

Background—Childhood dysregulation, which reflects deficits in the capacity to regulate or control one's thoughts, emotions and behaviours, is associated with psychopathology throughout childhood and into adulthood. Exposures to adversity during the prenatal period, including prenatal depression, can influence the development of dysregulation, and a number of candidate genes have been suggested as moderators of prenatal exposure, including polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR). We examined whether prenatal depression and child 5-HTTLPR interact to predict childhood dysregulation.

Method—Sample of $N = 213$ mother-child pairs from the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) project. Mothers reported the IBQ-R at 3 and 6 months, and the ECBQ at 18 and 36 months, from which measures of dysregulation were extracted. Mothers self-reported symptoms of depression on the CES-D at 24–36 weeks gestation, and at 6, 12, 24 and 36 months postnatal. 5-HTTLPR genotype was extracted from buccal swabs. Mixed-model and confirmatory analyses were conducted.

Results—Prenatal maternal depression and 5-HTTLPR interacted to predict dysregulation from 3 to 36 months, within a model of strong differential susceptibility.

Conclusion—Children with the S or L_G alleles, when exposed to prenatal depression, have higher levels of dysregulation, and when exposed to lower or little prenatal depression, have higher capacity for regulation. Our findings support efforts to identify, support and treat prenatal depression.

Keywords

Prenatal; Maternal depression; Gene-environment interaction (GxE); Emotional dysregulation; Child development; Longitudinal studies

Dysregulation, which reflects deficits in the capacity to regulate or control one's thoughts, emotions and behaviours, is highly associated with psychological impairment (Althoff, Verhulst, Rettew, Hudziak, & van der Ende, 2010). Early physiological reactivity or regulation develops as of the first weeks of life, and by three years of age most children can engage in the inhibitory control of reactivity, such as self-soothing (Putnam, Gartstein, & Rothbart, 2006). The inability to inhibit reactivity by age three is associated with life-long patterns of dysregulation and comorbid disorders (Althoff et al., 2010; Holtmann et al., 2011). For example, Meyer et al. (2009) report that dysregulation as early as 18 months of age is associated with mood disorders, suicidal ideation, personality disorders and substance abuse in early adulthood. Conversely, a greater capacity to regulate is associated with better outcomes such as social competence, health-related behaviours, and socioeconomic success (Garner & Waajid, 2012; Nota, Soresi, & Zimmerman, 2004).

We examine dysregulation as a temperamental construct of infancy and early childhood (Gartstein & Rothbart, 2003; Putnam et al., 2006), that is manifested by reactivity patterns easily observable in day-to-day eating and sleep behaviour, play, sensory stimulation, and soothability. This construct of regulatory capacity is especially prominent during infancy, has unique components and a separate trajectory from positive (surgency-extraversion) or negative emotionality, and likely exerts effects on dysregulation in later childhood (i.e., Althoff et al., 2010; Holtmann et al., 2011; Meyer et al., 2009). Given interventions that target dysregulation are complex and often met with uncertain results, a better understanding of the early neuro-developmental pathway is needed to promote new avenues of intervention (Peyre, Speranza, Cortese, Wohl, & Purper-Ouakil, 2012).

Prenatal Programming of Dysregulation

The neural connectivity between the brainstem, limbic and cortical brain regions associated with affect and behaviour regulation undergo rapid development during the third trimester of pregnancy (Geva & Feldman, 2008). Important events during the prenatal period can modify the connectivity between these regions to prepare the foetus for the future environment in a process called *prenatal programming* (Barker, 2004). The influence of prenatal events on dysregulation has been reported as early as the first days of life (Field et al., 2004; O'Connor, Heron, Golding, & Glover, 2003). For example, prenatal affective symptoms and stressors experienced by mothers have been associated with greater negative behavioural reactivity to novelty and slower rate of the behavioural stress-response recovery in infants at 4 months of age (Davis et al., 2011; Davis et al., 2004). Although most prenatal effect studies have been focused on the outcome of prenatal anxiety (e.g., Pluess et al., 2011), there

is evidence that prenatal depressive symptoms can make a separate contribution as they have been linked to elevated neonatal cortisol levels, fussiness and sleep problems (Field et al., 2004), and externalizing symptoms in middle childhood (Luoma et al., 2004).

Genetic Moderation

Children may differ in their susceptibility to prenatal events (Field, 2011). A number of candidate genes have been suggested as moderators of the effect of prenatal exposure on the development of dysregulation. Serotonergic cell signalling, for example, is highly active during the third trimester of pregnancy (Geva & Feldman, 2008). Genes in the serotonin (5-HTT) signalling pathway, and specifically functional variations in the promoter region of the serotonin transporter gene (5-HTTLPR), stand out for their association with anxiety, depression and affective regulation (Canli & Lesch, 2007; Hariri, Ahmad & Holmes, 2006; Mann et al., 2000). The SCL6A4 locus of the serotonin gene, which codes for the serotonin transporter, contains a 43 bp variable-number tandem repeat polymorphism in the promoter region (5-HTTLPR) that is believed to be responsible for transporter efficiency. The 'long' (L) and 'short' (S) variants produce the same protein but the S variant results in significantly reduced (about one third) in vitro basal transcription of 5-HTT mRNA (Canli & Lesch, 2007; Little et al., 1998). Although not taken into account in every study of the 5-HTTLPR genotype (Uher, 2008), there is evidence of a further functional variant of the L allele (L_A and L_G) that results from a single nucleotide polymorphism (A→G, rs25531) upstream of 5-HTTLPR (Hu et al., 2006; Nakamura, Ueno, Sano, & Tanabe, 2000). The L_AL_A genotype is associated with a greater 5-HTT binding potential in humans (Praschak-Rieder et al., 2007) and with higher 5-HTT mRNA expression (Hu et al., 2006). However, the L_G genotype has a functionally similar effect on 5-HTT mRNA expression as the S genotype. Carriers of the S allele have been associated with morphometric changes in limbic system regions responsible for negative emotion processing (Pezawas et al., 2005), positive stimuli and general emotional processing (Canli, Omura, Haas, Fallgatter, & Constable, 2005), and emotional regulation (Hariri et al., 2006).

No study to our knowledge has examined how prenatal exposure and genotype predict dysregulation, although there has been some attention to the related construct of Negative Emotionality. Pluess et al. (2011) found that the 5-HTTLPR S allele interacted with prenatal anxiety to predict greater Negative Emotionality in infants at 6 months, while Braithwaite et al. (2013) failed to reproduce this finding at 6 months or later. In separate analyses derived from our sample (Gordon Green et al., 2014; Gordon Green et al., in preparation), prenatal depression was found to interact with 5-HTTLPR to predict Negative Emotionality across infancy to early childhood. The absence of a clear story in the literature examining the genetic moderation of prenatal programming has been the subject of a recent review (Duncan, 2013). Directly relevant methodological improvements, such as the measurement of outcomes across multiple time points, the use of precise functional genotyping (L_G and L_A variants; Wong, Day, Luan, Chan, & Wareham, 2003; Hu et al., 2006) and 'glove-like' statistical analyses (Belsky, Pluess & Widaman, 2013) would address some of the concern about statistical power.

Modeling GxE

The theory of the Biological Sensitivity to Context suggests that genetic variability interacts with pre- and postnatal influences to prepare the infant to match or calibrate their biological and behavioural systems to their postnatal environment (Ellis & Boyce, 2008). Two potential models of prenatal programming could explain how prenatal depression and 5-HTTLPR genotype associate to predict dysregulation. In the *diathesis-stress* model, carriers of genotype variants that associate with increased risk for disorders (S or L_G) when exposed to adverse environmental experiences (e.g., prenatal depression) would have a greater likelihood of developing the negative outcome (e.g., dysregulation). Non-carriers would be insensitive to any environment, and in the absence of adversity individuals with or without the ‘risk’ genotype would show comparable developmental outcomes.

In contrast, the *differential susceptibility* model allows for variability in outcome (Pluess & Belsky, 2009; Boyce & Ellis, 2005). The differential susceptibility model reframed risk as susceptibility after reanalysis of some studies demonstrated that the same genotypes conferring a greater vulnerability under adverse conditions, *promoted* the development of phenotypes associated with resistance to mental illness under more favourable conditions (Pluess & Belsky, 2009; Pluess, Belsky, & Neuman, 2009). This model suggests that ‘risk’ genotypes would be better considered ‘plasticity’ or ‘susceptibility’ genotypes, and that carriers would be susceptible to both adverse and enriched environments, for better and for worse. There is now considerable evidence for the idea that variants of 5-HTTLPR serve as ‘plasticity’ genes (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012).

Purpose of the Study

The overall purpose of this study was to determine whether prenatal depression and child 5-HTTLPR genotype interact to predict the development of infant and early childhood dysregulation over the first three years of life. There were three objectives: (1) To determine whether prenatal depression predicted child dysregulation at 3, 6, 18 and 36 months; (2) To determine whether the association of prenatal depression with dysregulation was moderated by the child’s 5-HTTLPR genotype, in a GxE model; and (3) To determine whether diathesis-stress or differential susceptibility best characterized the GxE model. We tested our hypothesis of differential susceptibility with a novel statistical method, Confirmatory Analysis of Interaction Models (Widaman et al., 2012; Belsky et al., 2013).

METHOD

Participants

Participants were mother-child pairs from the ongoing longitudinal Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) project (see Table 1). The MAVAN is a Canadian community-based birth cohort that recruited 578 women from Montreal (Qc.) and Hamilton (On.). Women were recruited between 2003 and 2009 during routine ultrasound examinations in maternity hospitals. Eligibility criteria for women were age 18 years of age or over at the expected date of delivery and singleton and term pregnancy (≥ 37 weeks). Exclusion criteria were the presence of severe chronic maternal illness, past obstetrical

complications or major foetal/infant anomaly. Women were on average 30.2 years of age at recruitment, and approximately half were in the “University graduate” or higher category. The demographic and socioeconomic distribution of women in this study was similar to that of women from the Generation R Study and the Avon Longitudinal Study of Parents and their Children, two comparable prenatal cohort studies (van Batenburg-Eddes et al., 2013). Children exhibiting significant developmental delays were to be removed from the study. A detailed description of the recruitment, procedure and measures has been published (see O’Donnell et al., 2014).

Retention rates for the MAVAN subjects were 97.4% at 6 months, 84.0% at 18 months, and 80.6% ($N = 466$) at 36 months. The present study included 213 mother-child dyads with complete measures at 36 months. The reduction of sample size from 578 participants to 213 participants is explained as follows: 112 drop-outs; 60 children were missing prenatal data; 168 were missing genomic data (due to partial funding); 21 had not reached the age of 36 months; and 3 were outliers. Compared to mothers who remained in the study, mothers who left the study did not differ significantly on measures of age at delivery, depression, or education. Compared to children who remained in the study, children lost to follow-up did not differ significantly on measures of dysregulation assessed at available time points; however, they had significantly lower birth weight. There was an almost equal distribution of male to female participants (Table 1).

Measures

Women consenting to participate were interviewed at 24–36 weeks of pregnancy to obtain data on demographic, medical and obstetric history, stressors, social support, and pregnancy. At each time point, mothers were assessed with extensive socio-demographic and psychological measures and children with neurodevelopmental, behavioural and socio-emotional measures.

Dysregulation—The Infant Behavior Questionnaire-Revised (IBQ-R; Gartstein & Rothbart, 2003) is a measure of child temperament that was completed by mothers when their infant was 3 and 6 months old. In the original development of the scale, the IBQ-R led to three factors: Negative Emotionality, Surgency-Extraversion, and Regulation (Dysregulation). Our measure of dysregulation is constructed with the regulation factor to reflect that regulation and dysregulation exist on a continuum. In our sample, a factor analysis only led to two factors, Surgency-Extraversion and Negative Emotionality, at both the 3 and 6 month time points. Our factor of dysregulation was thus constructed using the published subscales, as per the direction of the authors (Gartstein, personal communication, May 15, 2012). The five subscales (Smiling/Laughter, Low-Intensity Pleasure, Cuddliness/Affiliation, Duration of Orienting, and Soothability) were standardized and aggregated to create a dysregulation factor at 3 months ($\alpha = .73$) and 6 months of age ($\alpha = .67$; further details available).

The Early Childhood Behavior Questionnaire (ECBQ; Putnam et al., 2006), an age-appropriate version of the IBQ-R, was completed by mothers when their child was 18 and 36 months old. In the original development of the scale as well as in our own sample, the

ECBQ led to three factors: Negative Emotionality, Surgency-Extraversion, and Dysregulation. Dysregulation explained 29.6% of the variance at 18 months (eigenvalue = 2.03), and 25.6% of the variance at 36 months (eigenvalue = 1.74). The eight subscales (i.e., Attention Shifting, Low-Intensity Pleasure, Cuddliness, Attention Focusing, Inhibitory Control, Perceptual Sensitivity, Sociability, and Soothability) were standardized and aggregated to create a dysregulation factor at 18 months ($\alpha = .69$) and 36 months of age ($\alpha = .72$) (Appendix 1).

Dysregulation scores were normally distributed at all four time points and did not differ by gender. Only findings for dysregulation are reported here. Findings pertaining to the prediction of Negative Emotionality are mentioned in the introduction and are reported elsewhere (Gordon Green et al., 2013; Gordon Green et al., in preparation), while those pertaining to Surgency-Extraversion were not significant.

Prenatal depression—The Center for Epidemiologic Studies Depression Scale (CES-D), a 20-item self-report measure of depressive symptomatology (Radloff, 1977) validated for pregnancy (e.g., Davis et al., 2011), was completed by the mothers at 24–36 weeks gestation. The highest score is 60 and a score of 16 is suggestive of a depressive disorder. Scores were centered.

Genotype—Child and mother genotype for 5-HTTLPR was obtained from buccal swabs, using the standard TaqMan method on the ABI-7000 for Single Nucleotide Polymorphism markers and on the ABI-3100 for repeat polymorphisms. Any ambiguous genotypes were discarded and the subjects were re-genotyped until the results became unambiguous. Each 20th marker was re-genotyped to check for error rates (0.5%). The 5-HTTLPR and rs25531 polymorphism were genotyped to optimize SLC6A4 genotyping. The genotype was coded dichotomously: (i) S/L_G - carriers of any functionally similar S or L_G allele (Hu et al., 2006); and (ii) L_AL_A - carriers homozygous for the long allele. The distribution conformed to the Hardy Weinberg equilibrium for both sites. There were no gender differences by 5-HTTLPR genotype ($\chi^2 = .02$, $df = 1$, $p > .05$), and the genotype distribution represented that of a predominantly Caucasian population sample.

Covariates—Covariates were obtained from the Health and Well Being of Mothers and their Newborns questionnaire (Kramer et al., 2009) administered prenatally and at 6, 12, 24 and 36 months postnatal. Maternal postnatal depression was assessed with the CES-D at 6, 12, 24 and 36 months postnatal. Maternal education, assessed prenatally, was dichotomized as 'University graduate or higher' or 'other'. The original categories (Table 1) were collapsed into two groups due to small sized categories.

Analyses

Mixed-model—A mixed-model for repeated measures included prenatal depression (continuous) and infant/child genotype as predictors, and infant/child dysregulation (continuous) as the outcome. The proportion of variance in dysregulation accounted for by site of recruitment, measured using Intraclass Correlation, was .03 at 3 months ($p > .05$), and 0 at 6, 18 and 36 months (p 's $> .05$). A random effect for site was not necessary.

Heteroscedasticity was addressed. Outliers with studentized residual values greater than 2.80 or greater than 2.00 with a combined leverage larger than $2p/n$ (Hoaglin & Welsch, 1978) were removed: 1 at 3 months, 1 at 6 months, 4 at 18 months, and 3 at 36 months.

Confirmatory—To test whether S/L_G carriers were *at risk* (diathesis-stress) or *susceptible* (differential susceptibility) when exposed to prenatal depression, confirmatory regression models were used with a re-parameterized equation (Widaman et al., 2012):

$$Y = \beta_0 + \beta_1 (\text{CES- D} - C) + \varepsilon, \text{ for } L_A L_A \text{ carriers}$$

$$Y = \beta_0 + \beta_2 (\text{CES- D} - C) + \varepsilon, \text{ for } S/L_G \text{ carriers}$$

The parameters in this equation are the intercept (β_0), the slope for L_AL_A carriers (β_1), the slope for S/L_G carriers (β_2), and the cross-over point between the two slopes (C). The magnitude of the crossover point (C) distinguishes a diathesis-stress from a differential susceptibility model. If the magnitude is zero (diathesis-stress), then the two lines meet at the left of the graph (no cross-over) and S/L_G carriers cannot have a better outcome than L_AL_A carriers. If the magnitude of C is not zero (differential susceptibility), then the two lines cross-over in the middle of the graph and the S/L_G carriers can have a better outcome than L_AL_A carriers.

Both the diathesis-stress and differential susceptibility models assume that carriers with no susceptibility alleles would not be influenced by the environment (prenatal depression), i.e. that $\beta_1 = 0$. However, since there remains the possibility that the environment exerts a slight effect even on non-carriers, the diathesis-stress and differential susceptibility models are further separated into two groups: a weak model ($\beta_1 = 0$ and $\beta_1 < \beta_2$) and a strong model ($\beta_1 = 0$). Accordingly, there is a possibility of four different models, namely weak or strong diathesis-stress, and weak or strong differential susceptibility. The Akaike information criteria (AIC) with significance testing at a 95% confidence interval is used to determine which of the four models best fit the data at each time-point. Only the strong diathesis-stress and strong differential susceptibility model testing are reported; however, all four models were tested (details available in Appendix 2).

RESULTS

Covariates were identified in preliminary analyses driven by theoretical conception, and were included in all subsequent analyses. Covariates associated with both a predictor and the outcome included maternal postnatal depression (all time points) and maternal age at birth (36 months only). Child gender was also included. Variables considered as covariates but not retained were maternal 5-HTTLPR genotype, maternal education, family income, child birth-weight, and child BSID-II scores. Mother 5-HTTLPR genotype was not significantly associated to prenatal depression or child dysregulation, and child 5-HTTLPR genotype was not significantly associated to prenatal depression.

Prediction from Mixed-Model Analysis

There was a significant interaction effect between prenatal depression and infant/child 5-HTTLPR on the outcome of dysregulation at 3, 6, 18 and 36 months ($\beta = -.11$, $SE = .04$, $p < .01$). The effect size was moderate (McFadden $R^2 = .40$; likelihood ratio test $\chi^2 = 231.1$, $df = 9$, $p < .0001$). The results remained consistent after adjusting for covariates ($\beta = -.11$, $SE = .04$, $p < .01$; McFadden $R^2 = .40$; likelihood ratio test $\chi^2 = 231.67$, $df = 9$, $p < .0001$), as none of the covariates were significant.

Prediction from Confirmatory Analyses

The strong differential susceptibility model had the smallest AIC at all time-points (Table 2). At 3 months, the interaction was significant, and the cross-over point (C) was not significant. We remind the reader that the magnitude of the cross-over point indicates whether S/L_G carriers can have better regulation than L_AL_A carriers, when exposed to lower levels of prenatal depression. Since the crossover point was not significant, it is unclear whether the 3 month time point represented diathesis-stress or differential susceptibility. At all other time-points, the interaction and cross-over points were significant. Confirmatory models at all time-points remained significant after adjusting for covariates (details available in Appendix 3). The only covariate with a significant effect on dysregulation was maternal postnatal depression at 18 months ($\beta = -.08$, $SE = .04$, $p < .05$) and 36 months ($\beta = -.08$, $SE = .03$, $p < .01$).

Figure 1 represents the differential susceptibility model for the prediction of dysregulation (standardized) at all time-points. Carriers of the L_AL_A genotype were insensitive to prenatal depression exposure, with stable scores of dysregulation throughout. Carriers of the S/L_G genotypes, however, had higher levels of dysregulation as a function of exposure to greater levels of prenatal depression. With lower prenatal depression, S/L_G carriers had *lower* levels of dysregulation than L_A carriers.

DISCUSSION

Our findings suggest that prenatal depression and the 5-HTTLPR genotype interact in a differential susceptibility model to predict infant and early childhood dysregulation from 3 to 36 months of age. These unique findings are strengthened by a prenatal longitudinal design with repeated measures, refined functional genotyping of the L allele, complementary analyses and novel analyses.

Three immediate conclusions are suggested. The principal finding is that our prediction is stable and clinically significant. As of 3 months of age, dysregulation emerges from a two-way interaction between prenatal depression and the 5-HTTLPR genotype, with interaction estimates that are stable across the first three years of life (i.e., between $-.09$ and $-.10$). The interaction effect is modest, however, the magnitude of the difference between the dysregulation scores when examining extremes of exposure to prenatal depression, for children with susceptible genotypes, is between two to three standard deviations (i.e., clinically significant). These findings support the *prenatal programming* of dysregulation, and are consistent with previous findings that prenatal affective symptoms experienced by

mothers are associated with greater negative behavioural reactivity to novelty and slower rate of the behavioural stress-response recovery in infants at 4 months of age (Davis et al., 2011; Davis et al., 2004). Our findings refine the existing literature by identifying genetic moderation by the 5-HTTLPR genotype.

Second, the association between prenatal depression and dysregulation was not better explained by the effect of maternal *postnatal* depression. Consistent with the literature (Field, 2011), maternal postnatal depression predicted dysregulation, however, independently from prenatal depression. A separate mechanism for the effect of prenatal depression has also been suggested by the finding that unlike with prenatal depression, maternal postnatal depression *did not* interact with child 5-HTTLPR genotype to predict dysregulation (Babineau et al., 2014). Similarly, Pearson et al. (2013) reported that only maternal postnatal depression (but not prenatal depression) interacted with maternal education to predict offspring depression. The overall prediction of dysregulation appears to be strengthened by maternal depression spanning the pre- to the postnatal period; however, the influences of prenatal and postnatal depression seem to be differentiated by separate mechanisms and pathways.

Third, our findings are best characterized by a model of differential susceptibility, whereby exposure to prenatal depression is moderated in a bi-directional manner for better and for worse. More specifically, children exposed to higher levels of prenatal depression had higher levels of dysregulation if they were S/L_G carriers than if they were L_AL_A carriers. Conversely, children exposed to lower levels of prenatal depression had *lower* levels of dysregulation if they were S/L_G carriers than if they were L_AL_A carriers. L_AL_A carriers, however, seemed to be impervious to exposure level of prenatal depression. This is consistent with previous evidence of the S/L_G genotype of 5-HTTLPR as a susceptibility or plasticity factor (Pluess et al., 2011; van IJzendoorn et al., 2012).

Limitations

The interpretation of our findings should be made with caution in light of certain limitations. For example, results might indicate a gene by environment correlation (rGE). Our finding that prenatal depression was not associated with infant/child genotype, and that maternal genotype did not confound our association, make it unlikely that passive rGE were at play. We cannot eliminate other mechanisms such as evocative rGE, although these are less likely with findings emerging as early as 3 months of age.

Maternal reports of child regulation were used. Although parent-reported measures reflect a longer observation period and reduce bias by inquiring only about recently occurring events and concrete infant behaviors (Gartstein & Rothbart, 2003), they might be influenced by the parents' mood states (Atella, DiPietro, Smith, & St James-Roberts, 2003). Accordingly, we adjusted all analyses for mother's depression scores at the time of reporting.

When compared to other genetic studies, the MAVAN has a relatively smaller number of participants. Our power, however, is strengthened by the accuracy of our genotyping method (Wong et al., 2003), precise functional sub-categorization of the L allele (L_A or L_G), and confirmatory analyses.

We do not include data on prenatal antidepressant medication exposure. Community estimates of antidepressant use suggest that about 6% of our sample might have been exposed during pregnancy (Cooper, Willy, Pont, & Ray, 2007). There is a slight possibility that the association between prenatal depression and dysregulation might be in part explained by the associated antidepressant exposure in a few cases. Even then, questions remain as to whether antidepressant exposure predicts developmental outcomes via direct causal processes, or represents a marker of the severity for the associated prenatal depression (Weikum et al., 2013).

Finally, with analyses spanning the first three years of life, we are not in a position yet to compare our measure of dysegregation with those from previous longitudinal studies (e.g., Althoff et al., 2010; Holtmann et al., 2011). As we examine time points in middle childhood and anchor our measures of dysregulation across each time point, including laboratory observations and psychiatric interviews, we will be in a better position to discuss the stability and continuity of the prediction of dysregulation from infancy to early childhood and beyond.

Implications

The generalizability of our findings is supported by the characteristics of our sample, namely pregnant women recruited in the community with close to average rates of maternal depression, socioeconomic status, and maternal age at delivery. Although more women are affected by symptoms of prenatal depression (20 to 38%; Vesga-López et al., 2008) than by symptoms of postpartum depression, only 5 to 14% of affected women are seeking treatment (Field, 2011). Our findings support existing efforts to research treatment options for prenatal distress. Early identification and treatment for women with prenatal distress is associated with reduced risk of postpartum depression and beneficial carryover effects for the developing foetus and child (see O'Connor, Monk, & Fitelson, 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Althoff RR, Verhulst FC, Rettew DC, Hudziak JJ, van der Ende J. Adult outcomes of childhood dysregulation: A 14-year follow-up study. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2010; 49:1105–1116. [PubMed: 20970698]
- Atella LD, DiPietro JA, Smith BA, James-Roberts IS. Contributions to maternal and paternal reports of early infant difficulties. *Parenting: Science and Practice*. 2003; 3:265–284.
- Babineau, V., Gordon Green, C., Jolicoeur-Martineau, A., Minde, K., Sassi, R., St-Andr, M., ... Wazana, A. The continuity of dysregulation from infancy to early childhood as predicted by prenatal

- depression and genotype. Poster session presented at the annual meeting of the World Congress on Brain, Behavior and Emotions (WCBBE); Montreal, Qc. 2014.
- Barker DJP. The developmental origins of adult disease. *Journal of the American College of Nutrition*. 2004; 23:588S–595S. [PubMed: 15640511]
- Bayley, N. Bayley scales of infant development. 2. San Antonio, Texas: Psychological Corp; 1993.
- Belsky J, Pluess M, Widaman KF. Confirmatory and competitive evaluation of alternative gene-environment interaction hypotheses. *Journal of Child Psychology and Psychiatry*. 2013; 54:1135–1143. [PubMed: 23617948]
- Boyce WT, Ellis B. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology*. 2005; 17:271–301. [PubMed: 16761546]
- Braithwaite, EC., Ramchandani, PG., O'Connor, TG., van Ijzendoorn, MH., Bakermans-Kranenburg, MJ., ... Murphy, SE. No moderating influence of the serotonin transporter polymorphism (5-HTTLPR) on the association between antenatal maternal mood and infant temperament. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2013. <http://www.jaacap.com/article/S0890-8567%2813%2900114-7/abstract>
- Canli T, Omura K, Haas BW, Fallgatter A, Constable RT. Beyond affect : A role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. *Proceedings of the National Academic of Sciences of the United States of America*. 2005; 102:12224–12229.
- Canli T, Lesch KP. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience*. 2007; 10:1103–1109. [PubMed: 17726476]
- Cooper WO, Willy ME, Pont SJ, Ray WA. Increasing use of antidepressants in pregnancy. *American Journal of Obstetrics and Gynecology*. 2007; 196:544.e1–544.e5. [PubMed: 17547888]
- Davis EP, Glynn LM, Waffarn F, Sandman CA. Prenatal maternal stress programs infant stress regulation. *Journal of Child Psychology and Psychiatry*. 2011; 52:119–129. [PubMed: 20854366]
- Davis EP, Snidman N, Wadhwa PD, Glynn LM, Schetter CD, Sandman CA. Prenatal maternal anxiety and depression predict negative behavioral reactivity in infancy. *Infancy*. 2004; 6:319–331.
- Duncan LE. Paying attention to all results, positive and negative. *Journal of the American Academy of Child and Adolescent Psychiatry*. 2013; 52:462–465. [PubMed: 23622847]
- Ellis BJ, Boyce WT. Biological sensitivity to context. *Current Directions in Psychological Science*. 2008; 17:183–187.
- Field T. Prenatal depression effects on early development: A review. *Infant Behaviour and Development*. 2011; 34:1–14.
- Field T, Diego M, Hernandez-Reif M, Vera Y, Gil K, Schanberg S, ... Gonzalez-Garcia A. Prenatal maternal biochemistry predicts neonatal biochemistry. *International Journal of Neuroscience*. 2004; 114:981–993.
- Garner PW, Waajid B. Emotion knowledge and self-regulation as predictors of preschoolers' cognitive ability, classroom behaviour, and social competence. *Journal of Psychoeducational Assessment*. 2012; 30:330–343.
- Gartstein MA, Rothbart MK. Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development*. 2003; 26:64–86.
- Geva R, Feldman R. A neurobiological model for the effects of early brainstem functioning on the development of behavior and emotion regulation in infants: Implications for prenatal and perinatal risk. *Journal of Child Psychology and Psychiatry*. 2008; 49:1031–1041. [PubMed: 18771507]
- Gordon Green, C., Babineau, V., Bouvette-Turcot, A., Jolicoeur-Martineau, A., Minde, K., St-Andre, ... Wazana, A. Investigating the moderating effect of prenatal stress on serotonin transporter polymorphism 5-HTTLPR in predicting negative emotionality. Symposium session presented at the biennial meeting of the Society for Research in Child Development (SRCD); Seattle, WA. 2013.
- Gordon Green C, Babineau V, Bouvette-Turcot A, Jolicoeur-Martineau A, Minde K, St-Andre, ... Wazana A. Investigating the moderating effect of prenatal stress on 5-HTTLPR, DRD4, and cumulative genetic risk in predicting negative emotionality across the first two years of childhood. in preparation.

- Hariri AR, Ahmad R, Holmes A. Genetics of emotional regulation: The role of the serotonin transporter in neural function. *Trends in Cognitive Sciences*. 2006; 10:182–191. [PubMed: 16530463]
- Hoaglin DC, Welsch RE. The hat matrix in regression and anova. *The American Statistician*. 1978; 32:17–22.
- Holtmann M, Buchmann AF, Esser G, Schmidt MH, Banaschewski T, Laucht M. The Child Behavior Checklist-Dysregulation profile predicts substance use, suicidality, and functional impairment: A longitudinal analysis. *Journal of Child Psychology and Psychiatry*. 2011; 52:139–147. [PubMed: 20854363]
- Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, ... Goldman D. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive-disorder. *The American Journal of Human Genetics*. 2006; 78:815–826. [PubMed: 16642437]
- Kramer MS, Wilkins R, Gouler L, Seguin L, Lydon J, Kahn SR, ... Platt. Investigating socio-economic disparities in preterm birth: Evidence for selective study participants and selection bias. *Paediatric and Perinatal Epidemiology*. 2009; 23:301–309. [PubMed: 19523077]
- Little KY, McLaughlin DP, Zhang L, Livermore CS, Dalack GW, McFinton PR. Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *American Journal of Psychiatry*. 1998; 155:207–213. [PubMed: 9464199]
- Luoma I, Tamminen T, Kaukonen P, Laippala P, Puura K, Salmelin R. A longitudinal study of maternal depressive symptoms, negative expectations and perceptions of child problems. *Child Psychiatry and Human Development*. 2004; 35:37–53. [PubMed: 15626324]
- Mann JJ, Huang Y, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, Dwork AJ, Arango V. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *American Medical Association*. 2000; 57:729–738.
- Meyer SE, Carlson GA, Youngstrom E, Ronsaville D, Martinez PE, Gold PW, Hakak R, Radke-Yarrow M. Long-term outcomes of youth who manifested the CBCL-Pediatric Bipolar Disorder phenotype during childhood and/or adolescence. *Journal of Affective Disorders*. 2009; 113:227–235. [PubMed: 18632161]
- Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Molecular Psychiatry*. 2000; 5:32–38. [PubMed: 10673766]
- Nota L, Soresi S, Zimmerman BJ. Self-regulation and academic achievement and resilience: A longitudinal study. *International Journal of Educational Research*. 2004; 41:198–215.
- O'Connor TG, Monk C, Fitelson EM. Practitioner review: Maternal mood in pregnancy and child development – Implications for child psychology and psychiatry. *Journal of Child Psychology and Psychiatry*. 2014; 55:99–111. [PubMed: 24127722]
- O'Connor TG, Heron J, Golding J, Beveridge M, Glover V. Maternal antenatal anxiety and behavioral/emotional problems in children: A test of programming hypothesis. *Journal of Child Psychology and Psychiatry*. 2003; 44:1025–1036. [PubMed: 14531585]
- O'Donnell K, Gaudreau H, Colalillo S, Steiner M, Atkinson L, Moss E, ... Meaney M. The Maternal Adversity Vulnerability and Neurodevelopment (MAVAN) Project: Theory and methodology. *The Canadian Journal of Psychiatry*. Accepted.
- Pearson RM, Evans J, Kounali D, Lewis G, Heron J, Ramchandani P, O'Connor TG, Stein A. Maternal depression during pregnancy and the postnatal period: Risks and possible mechanisms for offspring depression at age 18 years. *JAMA Psychiatry*. 2013; 70:1312–1319. [PubMed: 24108418]
- Peyre H, Speranza M, Cortese S, Wohl M, Purper-Ouakil D. Do ADHD children with and without Child Behaviour Checklist-Dysregulation Profile have different clinical characteristics, cognitive features, and treatment outcomes? *Journal of Attention Disorders*. 2012:1–9.
- Pezawas L, Meyer-Lindenberg A, Drabant EM, Verchinski BE, Munoz KE, Kolachana BS, ... Weinberger DR. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience*. 2005; 8:828–834. [PubMed: 15880108]
- Pluess M, Belsky J, Neuman RJ. Prenatal smoking and attention-deficit/hyperactivity disorder: DRD4-7R as a plasticity gene. *Biological Psychiatry*. 2009; 66:e5–e6. [PubMed: 19500778]

- Pluess M, Velders FP, Belsky J, van IJzendoorn MH, Bakermans-Kranenburg MJ, Jaddoe VWV, ... Tiemeier H. Serotonin transporter polymorphism moderates effects of prenatal maternal anxiety on infant negative emotionality. *Biological Psychiatry*. 2011; 69:520–525. [PubMed: 21126730]
- Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M, ... Meyer JH. Novel 5-HTTLPR allele associates with higher serotonin transporter binding in Putamen: A [¹¹C] DASB positron emission tomography study. *Biological Psychiatry*. 2007; 62:327–331. [PubMed: 17210141]
- Putnam SP, Gartstein MA, Rothbart MK. The Early Childhood Behavior Questionnaire. *Infant Behavior & Development*. 2006; 29:386–401. [PubMed: 17138293]
- Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement*. 1977; 1:385–401.
- Uher R. The implications of gene-environment interactions in depression: Will cause inform cure? *Molecular Psychiatry*. 2008; 13:1070–1078. [PubMed: 18679406]
- van Batenburg-Eddes T, Brion MJ, Henrichs J, Jaddoe VWV, Hofman A, Verhulst FC, ... Tiemeier H. Parental depressive and anxiety symptoms during pregnancy and attention problems in children: A cross-cohort consistency study. *Journal of Child Psychology and Psychiatry*. 2013; 54:591–600. [PubMed: 23215861]
- van IJzendoorn MH, Belsky J, Bakermans-Kranenburg M. Serotonin transporter genotype 5-HTTLPR as a marker of differential susceptibility? A meta-analysis of child and adolescent gene-by-environment studies. *Translational Psychiatry*. 2012; 2:e147. [PubMed: 22872162]
- Vesga-López O, Blanco C, Keyes K, Olfson M, Grant BF, Hasin DS. Psychiatric disorders in pregnant and postpartum women in the United States. *Archives of General Psychiatry*. 2008; 65:805–815. [PubMed: 18606953]
- Weikum WM, Brain U, Chau CM, Grunau RE, Boyce WT, Diamond A, Oberlander TF. Prenatal serotonin reuptake inhibitor (SRI) antidepressant exposure and serotonin transport promoter genotype (SLC6A4) influence executive functions at 6 years of age. *Frontiers in Cellular Neuroscience*. 2013; 7:1–12. [PubMed: 23355802]
- Widaman KF, Helm JL, Castro-Schilo L, Pluess M, Stallings MC, Belsky J. Distinguishing ordinal and disordinal interactions. *Psychological Methods*. 2012; 17:615–622. [PubMed: 22984788]
- Wong MY, Day NE, Luan JA, Chan KP, Wareham NJ. The detection of gene-environment interaction for continuous traits: Should we deal with measurement error by bigger studies or better measurement. *International Journal of Epidemiology*. 2003; 32:51–5. [PubMed: 12690008]

Key points

- Early childhood dysregulation is associated with maladaptive affect and behaviour, and with the development of lifelong comorbid psychopathology
- Prenatal adversity can modify the development of the foetus and is associated with postnatal development, as outlined by the theory of prenatal programming
- A polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) is associated with anxiety, depression and affective regulation
- In the present study, prenatal depression and 5-HTTLPR interact in a differential susceptibility model to predict dysregulation in the child throughout the first three years of life

Figure 1.

The prediction of regulation (standardized) at 3 (A), 6 (B), 18 (C) and 36 (D) months of age from the interaction of prenatal depression and child 5-HTTLPR genotype (confirmatory analyses).

Table 1

Descriptive statistics of MAVAN mother and child at 36 months (N = 213 pairs)

	<u>Montreal</u>		<u>Hamilton</u>	
	<i>M(SD)</i>	%	<i>M (SD)</i>	%
<u>Children</u>				
Sex – Female		50.4		43.1
BSID-II MDI *	95.5 (11.8)		101 (9.42)	
BSID-II PDI *	98 (11.8)		108.6 (10.7)	
Dysregulation				
3 months	1.1 (3.7)		-.1 (3.4)	
6 months	.4 (3.2)		.2 (3.2)	
18 months	0 (4.4)		.5 (4.4)	
36 months	.2 (4.4)		.7 (4.2)	
5-HTTLPR genotype				
S/S, S/L _G , S/L _A , L _G L _G , L _A L _G		66.7		74.5
L _A L _A		33.3		25.5
<u>Women</u>				
Age at delivery *	29.1 (4.5)		31.7 (4.5)	
In a partnership		91.9		93.1
Prenatal depression score	11 (8.2)		13.1 (11)	
Depression cutoff		21.6		31.4
Education				
High school		9		4.9
Some college		8.1		10.8
College graduate		30.6		37.2
University graduate		52.3		47.1
Annual household income				
<15 000		6.3		3.9
15 000 to <30 000		13.5		5.9
30 000 to <50 000		23.4		21.6
50,000 to <80 000		25.2		30.4
80 000		31.5		38.2

Note: Regulation scores are the aggregation of standardized subscales. Mother education and income categories as per Kramer et al. (2009). In analyses, education categories “ High school”, “Some college” and “College graduate” are collapsed into one category and compared with “ University graduate”.

* Significant site difference at $p < .05$

Table 2

The prediction of dysregulation from the interaction of prenatal depression and child 5-HTTLPR genotype:
Confirmatory analyses for Strong Differential Susceptibility and Strong Diathesis-Stress models

Scale	Strong Differential Susceptibility				Strong Diathesis-Stress			
	3M	6M	18M	36M	3M	6M	18M	36M
Intercept	.45	-.04	-.58	0	.7	.62	.48	.94
Cross-over point	6.53	15.85 **	19.27 **	14.24 **	-	-	-	-
Interaction	-.08 *	-.08 **	-.12 **	-.14 ***	-.07 *	-.05 *	-.05	-.08
AIC	410.79	505.12	615.92	643.49	429.22	509.6	622.19	648.31

Notes: AIC, Akaike information criterion. In the Strong Differential Susceptibility model at 3 months $R^2 = .04$, $F(2, 169) = 3.69^*$, at 6 months $R^2 = .05$, $F(2, 212) = 5.57^{**}$, at 18 months $R^2 = .05$, $F(2, 208) = 5.07^{**}$, and at 36 months $R^2 = .06$, $F(2, 218) = 7.05^{**}$. In the Strong Diathesis-Stress model at 3 months $R^2 = .04$, $F(1, 170) = 6.78^*$, at 6 months $R^2 = .03$, $F(1, 213) = 5.56^*$, at 18 months $R^2 = .01$, $F(1, 209) = 1.75$, and at 36 months $R^2 = .03$, $F(1, 219) = 7.09^{**}$.

* $p < .05$,

** $p < .01$,

*** $p < .001$