The Development of Early Childhood Dysregulation and Psychiatric Comorbidity – Influences from Maternal Prenatal Depression and Child Genetic Susceptibility

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A dissertation submitted to McGill University in partial fulfillment of the requirements for the degree

Doctor of Philosophy in School & Applied Child Psychology

August 2019

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General Abstract

There is ample evidence that exposure to maternal prenatal stress, such as anxiety and depression, is associated with increased risk for child psychopathology. The mechanisms at play may include pathways such as gene by environment interactions. Prenatal stress has been associated with dysregulation, and dysregulation has been associated with psychopathology, however, evidence for these associations within a longitudinal model is scarce. Our knowledge is thus primarily based on theoretical frameworks. The aim of my dissertation was to describe the longitudinal course of early dysregulation from 3 months to 5 years of age, and its association with comorbid psychiatric disorders at 6 years of age. An additional aim was to examine whether exposure to maternal prenatal depression and child genetic susceptibility would interact to predict early dysregulation trajectories. I examined this across three studies using data from the the Maternal Adversity Vulnerability and Neurodevelopment longitudinal birth cohort (MAVAN; $N = 582$). In Study 1, I investigated whether maternal prenatal depression interacted with children's genotype for the serotonin transporter polymorphism (*5-HTTLPR*) to predict dysregulation at 3, 6, 18 and 36 months of age. In Study 2, I outlined early developmental trajectories of dysregulation from 3 to 60 months of age (i.e. 3, 6, 18, 36, 48 and 60 months) and examined whether maternal prenatal depression interacted with child serotoninergic and dopaminergic candidate genes (i.e. *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT*, and *COMT*) to predict the early dysregulation trajectories. In Study 3, I examined whether maternal prenatal depression interacted with child serotonergic and dopaminergic candidate genes and polygenic risk scores (PRS) to predict comorbid psychiatric disorders at 6 years of age. Further, I explored whether this association was mediated by children's early dysregulation trajectories from 3 to 60 months of age. Prenatal depression interacted with child *5-HTTLPR* to predict the development of early dysregulation, and early dysregulation predicted psychiatric

comorbidity at 6 years of age. However, prenatal depression did not interact with child genetic susceptibility to predict psychiatric comorbidity. These findings can be interpreted as potential evidence for the prenatal programming of postnatal plasticity hypothesis, whereby children exposed to prenatal distress may develop more susceptible phenotypes, such as dysregulation, that confer increased developmental plasticity to either favorable or unfavorable postnatal environments (Hartman & Belsky, 2018; Pluess & Belsky, 2011). From a differential susceptibility framework, prevention and early intervention of psychiatric comorbidity can begin as early as dysregulation problems first emerge, i.e. around 18 months of age.

Résumé Général

Il existe de nombreuses preuves que la détresse maternelle prénatale, telle que l'anxiété et la dépression, est associée au développement de la maladie mentale de l'enfant. Les mécanismes biologiques incluent les gènes et l'environnement et leurs interactions. Bien que le stress prénatal ait été associé au tempérament et à la psychopathologie de l'enfant, ces associations dans les données longitudinales sont rares et les conclusions finales reposent principalement sur des cadres théoriques. En tant que tel, le but de cette thèse était de tracer le développement de la dérégulation de 3 mois à 5 ans et de déterminer comment la dérégulation est associée à la comorbidité psychiatrique à l'âge de 6 ans. Le développement de la dérégulation a également été étudié en fonction de la dépression prénatale en interaction avec le génotype de l'enfant pour les gènes candidats sérotoninergiques et dopaminergiques et les scores de risque polygénique (PRS). Dans trois études fondées sur les données de la cohorte de naissance longitudinale prospective de l'étude MAVAN (N = 582), nous avons examiné: (1) la dépression prénatale en interaction avec le génotype de l'enfant pour *5-HTTLPR* dans la prévision de la dérégulation à 3, 6, 18, et 36 mois; (2) les trajectoires de la dérégulation de 3 à 60 mois (3, 6, 18, 36, 48 et 60 mois) et la dépression prénatale en interaction avec les gènes candidats de la sérotonine et de la dopamine (*5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT* et *COMT*) dans la prévision des trajectoires de la dérégulation; et, (3) la dépression prénatale en interaction avec les gènes candidats de la sérotonine et de la dopamine et PRS dans la prévision de la comorbidité psychiatrique à l'âge de 6 ans et la médiation de cette association par les trajectoires de la dérégulation de 3 à 60 mois. Dans l'ensemble, nous avons constaté que la dépression prénatale interagit avec les gènes de l'enfant pour prédire le développement d'une dérégulation élevée et que la dérégulation prédit la comorbidité psychiatrique. Cependant, la dépression prénatale en

interaction avec les gènes de l'enfant ne prédit pas la comorbidité psychiatrique. Nous interprétons ces résultats comme une preuve de la programmation prénatale de la plasticité postnatale, dans laquelle les enfants exposés à la détresse prénatale peuvent développer des phénotypes plus susceptibles, dans ce cas une dérégulation, qui confère une plasticité développementale accrue à des environnements postnatals favorables ou défavorables (Hartman & Belsky, 2018; Pluess & Belsky, 2011). Dans un cadre de susceptibilité différentielle, la prévention et l'intervention précoce de la comorbidité psychiatrique peuvent commencer dès que les problèmes de dérégulation peuvent être évalués et détectés, à partir d'environ 18 mois.

This doctoral dissertation is dedicated to my grand-parents, Mr. and Mrs. Babineau, whose love and support have been integral in all of my academic pursuits and life accomplishments.

Acknowledgements

First and foremost, I would like to acknowledge my gratitude for my thesis supervisor Dr. Ashley Wazana, whose guidance, support, and confidence in my abilities have been unwavering throughout the years and invaluable to both my academic and professional development. A special acknowledgement also goes to my co-supervisor Dr. Jacob Burack, who provided me with the foundation, opportunity, and support to complete a multidisciplinary doctoral training under his supervision in the Department of Educational and Counselling Psychology and under the supervision of Dr. Wazana in the Department of Psychiatry. I would also like to thank the late Dr. Klaus Minde, a mentor whose insights were never short of fundamental to my work. Further, I would like to acknowledge how fortunate I have been to share this experience with my lab mates Cathryn (Katie) Gordon Green and Alexia Jolicoeur-Martineau. Katie – we worked side by side in the same labs, shared supervisors, traveled to many conferences, studied in the same clinical program, and built an incredible friendship along the way – I am endlessly grateful that we got to share our journey through grad school together – thank you for always being supportive and fun. Alexia – thank you for the countless hours and patience you have put forward in helping me with my statistical analyses, and for sharing so many thoughtful insights along the way – I am and always will be inspired by your brilliance. I would also like to acknowledge all members of the Maternal Adversity Vulnerability and Neurodevelopment (MAVAN) team, especially those with whom I have had the pleasure of working with at the Jewish General Hospital, including Dr. Eszter Szekely, Sahar Balvardi, Leonora King, and Dr. Noriyeh Rahbari. I would also like to thank my colleagues in the School/Applied Child Psychology program at McGill University for their ongoing support throughout the years during

course work and clinical training, many of whom became good friends including Bianca Levy, Jillian Stewart, and Keeley White.

The MAVAN team, located at the Douglas Mental Health University Institute and at the Jewish General Hospital in Montreal, at St. Joseph's Healthcare Hamilton in Hamilton, and at the Centre for Addiction and Mental Health (CAMH) in Toronto, includes many individuals to be thanked. In no particular order, I would like to thank the principle investigators who established research protocols and obtained funding to support the MAVAN, including Drs Stephen Matthews, Marla Sokolowski, Leslie Atkinson, Robert Levitan, Meir Steiner, Alison Flemming, John Lydon, Ellen Moss, James Kennedy, Roberto Sassi, Ashley Wazana, Sherif Karama, Patricia Silveira, Kieran O'Donnell, Susan Goldberg, Alan Evans, and Michael Meaney. I would also like to thank the research coordinators Hélène Gaudreau, Susan Goldman, Amber Rieder, Dawn Gore, Patricia Szymkow, and Carmen MacPherson; the various research assistants who helped with participant recruitment, data collection and data coding, including Sandra Das Neves, Magdalena Zdebik, Angelika Moroch, Estelle Lawrence, Mariana Sola, Diana Nicolici, Reagan Magwood, Eriona Kruja, Kelsey Campbell, Erica Tatham, Brittany Doody, Anita Andoh, Brittany Horodecki, Hilary Grenville, Danielle Berlingieri, Leanna McGrimmond, Carly McLeod, Alissa Papadopoulos, Francine Brochu, Isabelle Royal, Sara Colalillo, Katherine O'Donnell, Andrée-Anne Bouvette-Turcot, Pascale Dumouchel, Fellah Mercier, Myriam Roussel-Bergeron, Diane Racine, Erika Piercy, Valérie Aubut, Lyne Duchaine, Danielle Duchesne, Jade Corriveau, Camille Gagnon-Trudeau, Jessica Bernard, Cassandre Carpentier-Laberge, Christine Laganière, Dana El Saleh, Jade Vandenbossche-Makombo, Shireen Sindi, Zayna Aston, Caroline Boivin, Jennifer Lys-Grenier, and Paula Gonzalez; the computer programmers and statisticians who helped with data management, programming and

analysis, including Abdoul Karim Sow, Salim Mohamed Tadili, Vincent Jolivet, Jonhathan Deslauriers, Etienne Léger, Pablo Moreno, Nicolas Brossard, David Brownlee, Irina Pokvisneva, Lisa Barbosa; the lab technicians, managers and principle investigators who collaborated in the process of salivary DNA storing, extraction, sequencing and batch corrections, including Marg Coote, Natalie Freeman, and Kieran O'Donnell; and the numerous volunteers over the years. Additionally, I would like to extend an immense thank you to all of the families who participated in the MAVAN study from the prenatal period to childhood and ongoing – without their dedication and commitment to our project, none of what we do would be possible.

I would also like acknowledge the funding sources that made our research possible, including a Canadian Institutes of Health Research (CIHR) trajectory grant (191827) to Drs Meaney and Matthews, as well as multiple CIHR grants to Drs Levitan, Meaney, Wazana, Kennedy, Silveira, and Flemming. Private support was also received from the Faculty of Medicine at McGill University, the Norlien Foundation, the Woco Foundation, and the March of Dimes Foundation. I have also been fortunate to receive personal funding and scholarships to pursue my graduate studies, including a Doctoral Training Grant from Fonds de Recherche du Québec - Santé (FRQS) in collaboration with the Foundation of Stars, a Canada Graduate Scholarship to Honour Nelson Mandela from CIHR, and a Vanier Canada Graduate Scholarship from CIHR.

I would also like to thank Ed Potts, Brian Small and Dr. Helen Egger from Duke University Medical Center for their training and support throughout our administration of the *Preschool Age Psychiatric Assessment* (*PAPA*) and *Child and Adolescent Psychiatric Assessment* (*CAPA*), and Marie-Julie Béliveau and Suzanna Lépin for translating these measures into French.

I would also like to extend a special thank you to my mentors and collaborators Drs Tim Oberl ander and Sherryl Goodman – thank you for inspiring my work, encouraging my endeavors, and welcoming me to your field of research.

Last and certainly not least, I want to thank my friends and community for their ongoing support in both my professional and personal aspirations, especially Lyndsay Bedard, Evan Winstanley, Adam Breault, Hans Jean-Charles, and Sarah Kizuk.

Contribution of Authors

The present thesis includes three manuscripts which together represent my doctoral work and dissertation under the supervision of Drs Ashley Wazana and Jacob Burack. The data reported is from a larger research program that was conducted by the MAVAN. The research design and protocol of the MAVAN, which are the foundations of studies 1, 2, and 3, were written by principle investigators Drs Stephen Matthews, Marla Sokolowski, Leslie Atkinson, Robert Levitan, Meir Steiner, Alison Flemming, John Lydon, Ellen Moss, James Kennedy, Roberto Sassi, Ashley Wazana, Sherif Karama, Susan Goldberg, Alan Evans, and Michael Meaney. Dr. Wazana and I were responsible for writing the specific design of studies 1, 2, and 3.

Hélène Gaudreau, Susan Goldman, Amber Rieder, Dawn Gore, Patricia Szymkow, and Carmen MacPherson coordinated participant recruitment and data collection. Although I did not take part in recruitment, I was responsible for the majority of data collection and coding of the *Preschool Age Psychiatric Assessment* (*PAPA*) used in Study 3, and contributed to the training and supervision of other graduate students in the administration and coding of the *PAPA*.

For each study, I conducted the literature searches and reviews, generated research questions, designed theoretical models, planned and conducted the statistical analyses and interpretation, and prepared the manuscripts for publication, all of which was done in close collaboration with Dr. Ashley Wazana (thesis supervisor), Dr. Jacob Burack (thesis co-supervisor), Dr. Eszther Szekeley (postdoctoral student), Cathryn Gordon Green (PhD candidate), and Alexia Jolicoeur-Martineau (biostatistician). All of the authors contributed to and approved the final papers. I also led the revise and resubmit process for Study 1, which was subsequently published in the *Journal of Child Psychology and Psychiatry.* Studies 2 and 3 are currently in preparation for submission.

Contribution of Original Knowledge

The present thesis involves several original contributions. One, we are the first group to attempt to outline the development of early childhood dysregulation as of infancy to school age (i.e. 3 to 60 months of age). Two, we are the first group to explore the biological and environmental interplay that leads to the development of early childhood dysregulation, including child genes and exposure to prenatal depression. Three, we are the first group to identify a link between early childhood dysregulation and the development of childhood psychiatric comorbidity at 6 years of age.

In Study 1, we found that dysregulation at 3, 6, 18, and 36 months of age was predicted by an interaction between child genes (e.g. *5-HTTLPR*) and exposure to prenatal depression, within a framework of differential susceptibility. Children with susceptible genotypes for *5-HTTLPR*, when exposed to greater prenatal depression, were more likely to develop greater dysregulation problems. However, children with susceptible genotypes for *5-HTTLPR*, when exposed to less or no prenatal depression, were the children who were most likely to have few or no dysregulation problems.

In Study 2, we outlined the development of dysregulation from 3 to 60 months of age (i.e. 3, 6, 18, 36, 48, and 60 months), and found that early childhood dysregulation follows two qualitatively distinct trajectories: persistently *low dysregulation* (94%), and *high dysregulation* (6%) that is initially low but increases over time as of 18 months of age. Further, we found that membership in the high dysregulation group was predicted by an interaction between child *5-HTTLPR* and exposure to prenatal depression, although other candidate genes explored were not associated with dysregulation.

In Study 3, we found that the high dysregulation group from Study 2 predicted the development of child comorbid psychiatric disorders at 6 years of age. Although prenatal depression and child genes did not interact to predict child psychiatric comorbidity, they had interacted to predict high dysregulation, and high dysregulation then predicted psychiatric comorbidity, which we interpreted as the prenatal programming of postnatal plasticity. Additionally, the children exposed to postnatal depression were more likely to develop psychiatric comorbidity.

General Introduction

Dysregulation is a pattern of neurobehavioural processes that interferes with adaptive development, and can lead to vulnerability for and maintenance of comorbid psychopathology from childhood to adulthood (Althoff, Verhulst, Rettew, Hudziak, & van der Ende, 2010; Calkins, 1994; Holtmann et al., 2011; Meyer et al., 2009). The term *dysregulation* is derived from *regulation*, which involves a broad rubric of loosely-related processes and strategies used to exert control over one's thoughts, emotions, and behaviours, such as maintaining, enhancing, or inhibiting reactions to the environment (Althoff et al., 2010; Kopp, 1982, 1989). Dysregulation is well-defined and extensively studied from childhood through adulthood (see Althoff et al., 2010; Calkins, 1994; Holtmann et al., 2011; Meyer et al., 2009). A next step in the study of dysregulation is to identify its early developmental pathways and origins, including biological and environmental factors, and determine whether early dysregulation in infancy to early childhood is the same as, or a developmental precursor to, dysregulation later in life. One way to investigate the latter is to determine whether early dysregulation also leads to the development of comorbid psychopathology. Identifying developmental pathways of dysregulation and determining whether early dysregulation leads to psychiatric comorbidity can inform new directions for prevention and early intervention (Carballo et al., 2014).

Defining dysregulation

The ability to regulate includes cognitive and neurophysiological processes that decrease distress and modulate behavioural responses to the environment (Cole, Michel, & O'Donnell Teti, 1994; Luciana, 2016). For example, regulation involves attention and inhibition, which enable monitoring, delaying, adjusting, and adapting to ongoing sensory input and environmental demands. When regulation strategies either are ineffective or interfere with adaptive developmental, the risk for psychopathology is increased (Carballo et al., 2014). For example,

difficulty regulating attention could lead to attention disorders, difficulty regulating the intensity and duration of emotion could lead to depression or anxiety, and difficulty regulating behaviour could lead to disruptive behaviour disorders. Children who display a combination of attention, emotion, and behaviour problems are described as having a dysregulation problem (Althoff, 2010), whereby dysregulation predicts severity and comorbidity of psychopathology over time (Althoff et al., 2010; Holtman et al., 2011; Meyer et al., 2009). Although the components of dysregulation can be studied separately (e.g. difficulties regulating attention, difficulties regulating emotion, or difficulties regulating behavior), it is really the combination of difficulties regulating attention, emotion and behavior, that defines the dysregulation profile in children (Althoff, 2010).

Dysregulation is studied as early as infancy, as well as in childhood and adulthood. In infancy and early childhood, the symptoms that are considered include problems with eating, sleeping, and sensory sensitivities, being highly irritable or difficult to sooth, having difficulties with inhibition as well as with focusing or shifting attention, and high intensity stimulation seeking (Briggs-Gowan, & Carter, 2007; Gartstein & Rothbart, 2003; Putnam, Gartstein, & Rothbart, 2006). In childhood, the symptoms that are considered include depression, anxiety, somatic complaints, and sleep problems, or a combination of attention problems, depression, anxiety, and aggression. Symptoms of dysregulation in childhood are associated with outcomes such as attention deficit hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD; Althoff et al., 2010; Degangi, Breinbauer, Roosevelt, Porges, & Greenspan, 2000; Kim et al., 2012). Dysregulation in childhood and adolescence is also associated with conditions in adulthood such as anxiety, mood and behaviour disorders, substance use problems, suicidal ideation, cluster B personality disorders (e.g. antisocial personality disorder, borderline

personality disorder), as well as with with comorbid psychopathology (Althoff et al., 2010; Holtmann et al., 2011; Meyer et al., 2009).

The neurobiology of dysregulation

From a neurobiological perspective, the human capacity to regulate involves reciprocal interactions between the prefrontal cortex (PFC) and subcortical brain structures, such as the amygdala, hypothalamus, nucleus accumbens, and brainstem nuclei (Arnsten & Rubia, 2012; Banks, Eddy, Angstadt, Nathan, & Phan, 2017). For example, in directed efforts to suppress emotions, the PFC exerts "top down" control over the amygdala, a brain structure critical to emotion (Seo, Patrick, & Kennealy, 2008). Greater functional connectivity between the PFC and amygdala is associated with decreased negative affect, which highlights the role of PFC-amygdala interactions during emotion regulation (Banks et al., 2017). Neurochemical systems involved in this functional connectivity include serotonergic and dopaminergic pathways, which are also implicated in mood and behaviour regulation (Chang et al., 2018; Ruhé, Mason, & Schene, 2007; Seo et al., 2008). Abnormalities within these neurobiological structures and regulatory systems are highly associated with psychopathology, such as mood disorders, impulsive aggression, substance use disorder, suicidality, and their comorbidity (Seo et al., 2008). The factors that influence the development of these structures and systems likely predispose individuals to develop dysregulation problems and comorbid psychopathology.

Prenatal programming

In the 1980s, the epidemiologist David Barker's finding that lower birth weight (i.e. an indicator of intrauterine environment) was associated with subsequent risk for cardiovascular disease, led to two hypotheses – the developmental origins of health and disease (DOHaD) hypothesis and the prenatal programming hypothesis (Barker, 2004; Barker, Osmond, Margetts,

& Sommond, 1989; van den Bergh et al., 2017). According to the DOHaD hypothesis, there are critical periods in development when environmental factors can have lasting effects on biological systems and subsequent plasticity (Barker, 2004). Similarly, according to the prenatal programming hypothesis, maternal prenatal stress, such as depression or anxiety, can "program" postnatal plasticity and susceptibility to environmental effects (Hartman & Belsky, 2018). Exposure to maternal prenatal stress, such as anxiety and depression, can influence fetal and infant brain development and subsequent child development (Monk, Lugo-Candelas, & Trumpff, 2019; Pearson et al., 2013). Although clear links to dysregulation have yet to be established, maternal prenatal stress has been associated with cognitive, affective, and behavioural outcomes throughout infancy and childhood (Monk et al., 2019). In infancy, outcomes include elevated cortisol levels, fussiness, sleep problems, greater negative behavioural reactivity to novelty, and slower rate of behavioural stress-response recovery (Davis, Glynn, Waffarn, & Sandman, 2011; Davis et al., 2004; Field et al., 2004). In childhood, outcomes include attention disorders, depression, anxiety, and behavioural disorders (Luoma et al., 2004; Monk et al., 2019; Pearson et al., 2013). Of particular relevance in the process of prenatal programming is adversity in the third trimester of pregnancy, during which time the neural connectivity between brain regions associated with affect and behaviour regulation (e.g. limbic and cortical regions) are undergoing rapid development (Geva & Feldman, 2008). Aversive events during this period can, therefore, modify the connectivity between these regions (Barker, 2004).

Genetic susceptibility

Exposure to prenatal adversity, such as maternal prenatal depression or anxiety, does not necessarily lead to the development of psychopathology. The outcome of psychopathology depends on how vulnerable one is to the adverse environment, whereby vulnerability is

influenced by, among other factors, allelic variation in gene expression (i.e. genetic variants; Bock at al., 2015). According to the *differential susceptibility* hypothesis, biological or genetic factors that lead to greater vulnerability (i.e. risk) under adverse conditions can also promote adaptive development under more favourable conditions. In this context, genetic *risk* variants are reframed as *susceptibility* variants, whereby carriers (i.e. children who carry a specific genetic variant) are more susceptible to either adverse or enriched environments (Pluess & Belskey, 2011; Pluess, Belsky, & Neuman, 2009). For example, carriers will show a significantly greater response to intervention (i.e. greater symptom reduction) as compared to non-carriers (Eley et al., 2012). In a meta-analytic study, the "risk" allele of the serotonin transporter gene-linked polymorphism (*5-HTTLPR*) was demonstrated to serve instead as a susceptibility allele, given that carriers were not only more vulnerable to negative environments than non-carriers, but also benefited more from positive environments (van IJzendoorn, Belsky, & Bakermans-Kranenburg, 2012).

Advances in molecular and genetic research have lead to the identification of mechanisms by which gene expression can be influenced by the environment, leading to psychiatric outcomes (Tsankova, Renthal, Kumar, & Nestler, 2007). Referred to as epigenetic mechanisms, these processes include components or regions of genes that are specifically sensitive to the environment and proximal to the coding region of the gene (e.g. DNA methylation and histone modification). Methylation processes, which can alter how genes are expressed, do not alter the underlying DNA sequence (Tsankova et al., 2007). There is evidence that maternal pre- and postnatal stress can modify gene expression, leading to changes in neurodevelopmental pathways (e.g. hypothalamic-pituitary-adrenal axis) associated with cognition, behaviour, and affect regulation (Meaney, Szyf, & Seckl, 2007; Weaver et al., 2004).

Genetic variants that influence serotonin and dopamine neurotransmission are of particular interest in the development of dysregulation, as they are thought to contribute to mood and behaviour regulation (Chang et al., 2018; Ruhé et al., 2007; Seo et al., 2008). Gene variants for the following genes have been identified: *5-HTTLPR* (Gutknecht et al., 2015; Karg, Burmeister, Shedden, & Sen, 2011), *HTR1A*, *HTR1B* and *HTR2A* (Ciobanu et al., 2016), *BDNF* (Hünnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007), *DRD4* and *DRD2* (Mota et al., 2013a; Mota et al., 2013b), *DAT* (Daly, Hawi, Fitzgerald, & Gill, 1999), and *COMT* (Eisenberg et al., 1999). Genetic variants for these genes have previously been associated with depression (Ciobanu et al., 2016; Gutknecht et al., 2015; Karg, Burmeister, Shedden, & Sen, 2011), ADHD (Daly et al., 1999; Eisenberg et al., 1999), conduct disorder (CD), drug use, alcohol dependence (Mota et al., 2013a), and anxiety and depression related personality traits (Hünnerkopf et al., 2007). Multiple genes are of interest in the prediction of dysregulation, given that dysregulation is associated with a wide range of internalizing and externalizing disorders. However, a limitation of including multiple genes in the prediction of such a large endophenotype is that associations may become more difficult to identify. Although it may be possible to find associations between individual disorders and genes, when multiple neurodevelopmental processes are involved, such as in the development of comorbid psychiatric disorders, the identification of component biological correlates may become more complex to disentangle.

In addition to candidate genes, advances in genomics have led to large-scale screening of the genome and the development of polygenic risk scores (PRS), which can be shared across studies and research groups. The PRS indicate an individual's genetic susceptibility to traits and disorders, among which depression and ADHD are the most studied (Chen et al., 2018). PRS are computed based on previous genome-wide association studies (GWAS) meta-analyses. For the

purpose of studying dysregulation, relevant PRS created thus far include the PRS from the Cross Disorder Group of the Psychiatric Genomics Consortium (PRS Cross Disorder; Cross Disorder Group of the Psychiatric Genomics Consortium, 2013), and the PRS from the EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium (PRS Total Problems; Neumann et al., in preparation). The PRS Cross Disorder reflects genetic susceptibility to autism spectrum disorder, ADHD, schizophrenia, bipolar disorder, and major depressive disorder in 33,332 adult cases that are compared to 27,888 adult control cases (Cross Disorder Group of the Psychiatric Genomics Consortium, 2013). The PRS Total Problems reflects genetic susceptibility to childhood psychological problems such as attention problems, anxiety, depression, and insomnia among 29,446 children aged 5 to 16 years old, and is described in detail elsewhere (Neumann et al., in preparation).

Aims and hypotheses

The overall aim of this dissertation is to investigate the development of early childhood dysregulation from 3 months to 5 years of age as predicted by an interaction between maternal prenatal depression and child genetic susceptibility, and whether early childhood dysregulation predicts comorbid psychiatric disorders at 6 years of age. Across three separate studies, the data from this dissertation can inform the origins and early developmental pathways of childhood dysregulation. The aim of the first study is to investigate prenatal depression in interaction with child genotype for *5-HTTLPR* in the prediction of dysregulation at 3, 6, 18 and 36 months of age. The aim of the second study is to outline a trajectory of dysregulation with time points 3, 6, 18, 36, 48 and 60 months of age, and to investigate prenatal depression in interaction with child serotonergic and dopaminergic genotypes (i.e. *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT*, and *COMT*) in the prediction of the outlined trajectory of dysregulation.

The aim of the third study is to determine whether the trajectory of dysregulation as outlined in the second study (i.e. from 3 to 60 months of age) predicts child comorbid psychiatric disorders at 6 years of age as measured by the *Preschool Age Psychiatric Assessment* (*PAPA*; Egger, Ascher, & Angold, 1999), and to investigate prenatal depression in interaction with child serotonergic and dopaminergic genotypes and PRS in the prediction of child comorbid psychiatric disorders.

For the first study, the hypothesis is that prenatal depression and *5-HTTLPR* will interact to predict dysregulation at 3, 6, 18 and 36 months of age. More specifically, children with susceptible genotypes are expected to be more dysregulated as a result of exposure to greater prenatal depression, and less dysregulated as a result of low or absent prenatal depression. Within the initial and exploratory nature of outlining dysregulation from infancy to early childhood, the second study involves no specific hypothesis as per the direction or number of dysregulation groups that will result. The hypothesis for the second study is that prenatal depression and child genetic susceptibility will interact to predict severity of dysregulation as per the trajectories created in the second study. For the third study, the hypothesis is that the dysregulation trajectories identified from the second study will predict the outcome of child comorbid psychiatric disorders at 6 years of age. Further, prenatal depression and child genetic susceptibility are expected to interact to predict child comorbid psychiatric disorders, as moderated by the trajectories of dysregulation from the second study.

Study 1

Prenatal Depression and *5-HTTLPR* Interact to Predict Dysregulation from 3 to 36 Months – A Differential Susceptibility Model

By Vanessa Babineau[1], Cathryn Gordon Green[1], Alexis Jolicoeur-Martineau[2], Andrée-Anne Bouvette Turcot[3], Klaus Minde[3], Roberto Sassi[4], Martin St-André[5], Normand Carrey[6], Leslie Atkinson[7], James L. Kennedy, John Lydon, Meir Steiner, Hélene Gaudreau[3], Robert Levitan, Michael Meaney[1], & Ashley Wazana[8] for the MAVAN project

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Babineau, V., Gordon Green, C., Jolicoeur-Martineau, A., Bouvette-Turcot, A. A., Minde, K., Sassi, R., … & Wazana, A. (2015). The joint influence of prenatal depression, 5-HTTLPR and maternal education on the development of regulation from 3 to 36 months. *Journal of Child Psychology and Psychiatry, 56,* 21-29.

Link to published paper: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5398894/

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 of 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 depression and *5-HTTLPR* interacted to predict dysregulation from 3 to 36 months, within a model of strong differential susceptibility. **Conclusion** Children with 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

Prenatal Depression and *5-HTTLPR* Interact to Predict Dysregulation from 3 to 36 Months – A Differential Susceptibility Model

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 at 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 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 & Belskey,

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 moderateded 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 (see 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 (α = .73) and 6 months of age (α = .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 (α = .69) and 36 months of age (α = .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):

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

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

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 cross-over 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 ($\beta_1 \neq 0$ 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 with prenatal depression or child dysregulation, and child *5-HTTLPR* genotype was not significantly associated with 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 cross-over 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-HTTPLR 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 behaviours (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 dysegulation 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).

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Tables

Table 1 Descriptive Statistics of MAVAN Mother and Child at 36 Months (*N* = 213 pairs)

	Montreal		Hamilton	
	M(SD)	%	*M (SD)*	%
Children				
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_GL_G, L_AL_G		66.7		74.5
L_AL_A		33.3		25.5
Women				
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

	Strong Differential Susceptibility				Strong Diathesis-Stress			
	3M	6M	18M	36M	3M	6M	18M	36M
Scale								
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, $F(1, 219) = 7.09$**. *$p < .05$, **$p < .01$, ***$p < .001$

Figures

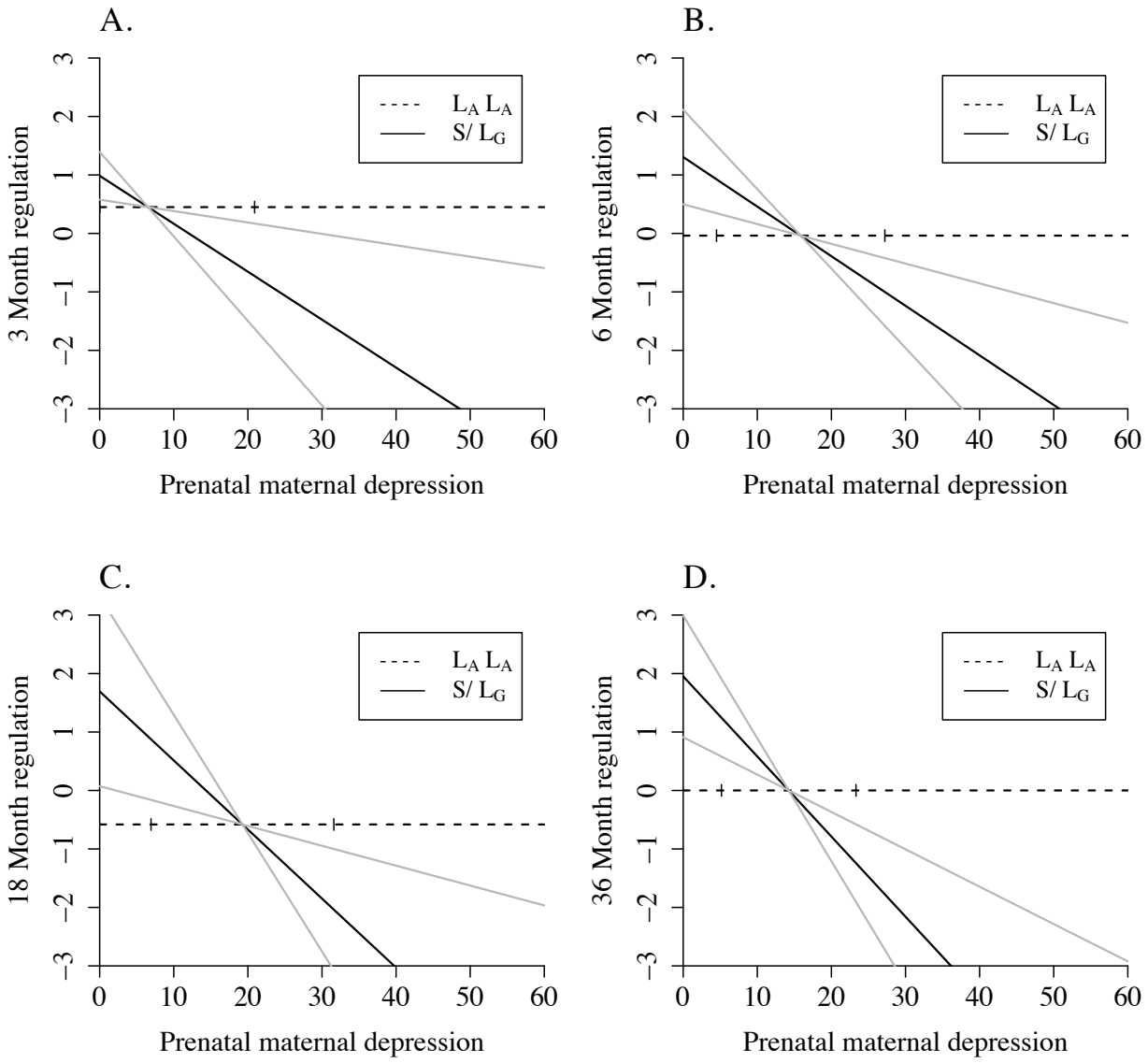


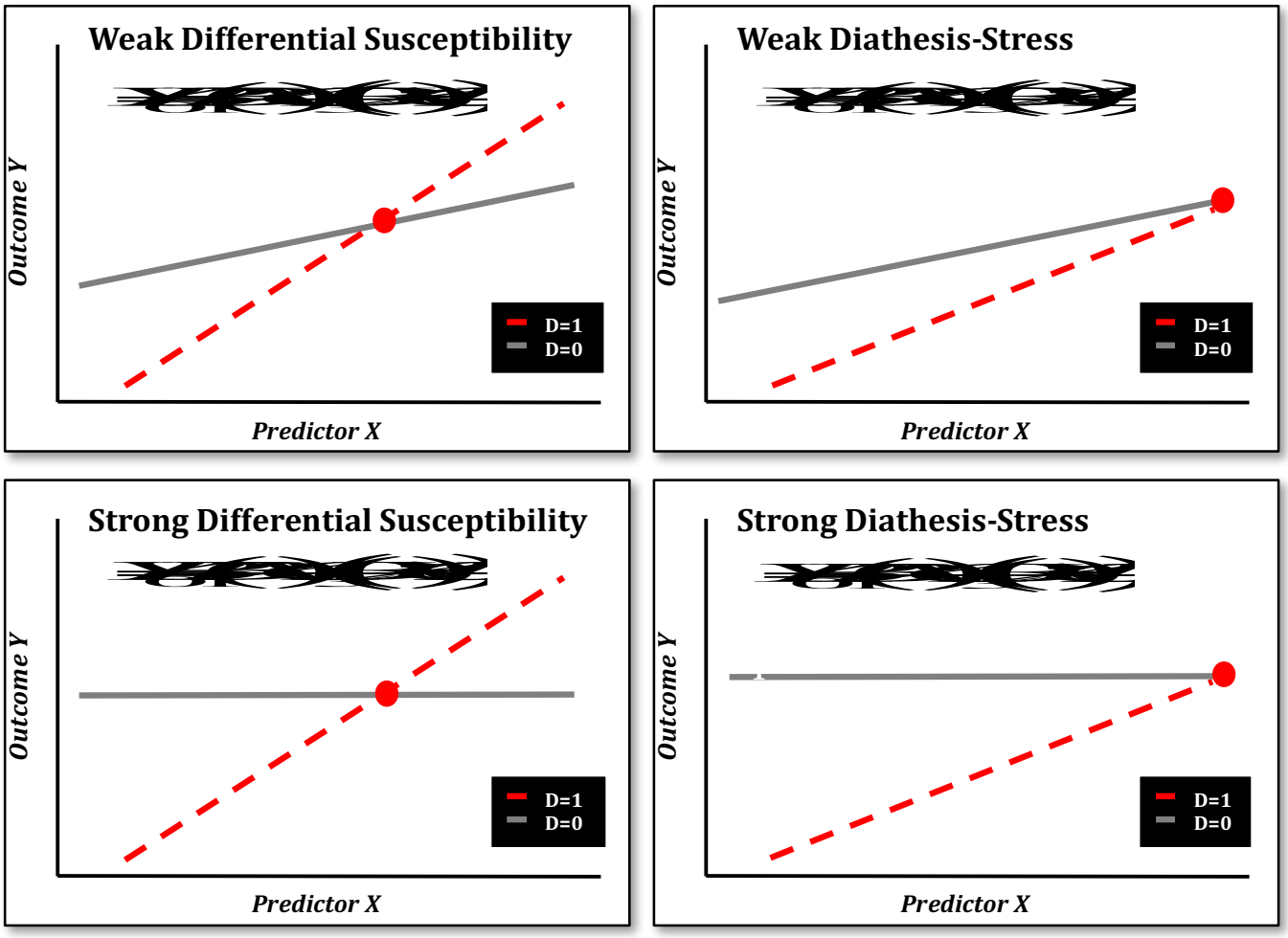
Figure 1. The prediction of regulation (standardized) at 3 (A), 6 (B), 18 (C) and 36 (D) months of age from the interaction of prenatal depression and child *5-HTTLPR* genotype (confirmatory analyses).

Appendix 1

ECBQ-R Factor Loadings at 18 Months (N=405) and 36 Months (N=370)

	Negative Affectivity		Surgency-Extraversion		Regulation	
Scale	18M	36M	18M	36M	18M	36M
Activity Level			.76	.60		
Attention Focusing					.39	.40
Attention Shifting					.58	.70
Cuddliness					.52	.54
Discomfort	.74	.71				
Fear	.78	.64				
Frustration	.52	.55				
High-Intensity Pleasure			.58	.62		
Impulsivity	-.33			.58		
Inhibitory Control			-.49	-.32	.39	.47
Low-Intensity Pleasure					.69	.75
Motor Activation	.59	.52				
Perceptual Sensitivity	.39	.53			.52	.41
Sadness	.60	.56				
Shyness	.47	.39		-.44		
Sociability				.50	.35	.35
Soothability	-.51	-.31			.32	.51

Appendix 2



Schematic representation of the four models tested by confirmatory analysis (courtesy, Pluess (2013)).

Appendix 3

Confirmatory Models of Strong Differential Susceptibility Using Prenatal Depression and 5-HTTLPR as Predictors of Regulation

Predictors	3 Months β	6 Months β	18 Months B	36 Months B
Intercept	1.22	.46	.43	-2.03
Cross-over point	9.18	15.75**	20.69*	14.6*
Interaction	-.09**	-.1**	-.09*	-.1*
Covariates				
Maternal postnatal depression	-	.02	-.08*	-.08**
Gender	-.19	.15	-.04	-.32
Maternal age	-	-	-	.09
Maternal education	-	-	-	-
College graduate	-.5	-.83	-.39	1.27
University graduate	-1.23	-.76	.39	.49

Note: At 3 months $R^2 = .07$, $F(5, 161) = 2.33$*, at 6 months $R^2 = .06$, $F(5, 195) = 1.96$, at 18 months $R^2 = .09$, $F(5, 190) = 3.19$**, and at 36 months $R^2 = .1$, $F(5, 213) = 3.69$***
*$p < .05$, **$p < .01$, ***$p < .001$

Bridge to Study 2

In Study 1, we provided support for the hypothesis that maternal prenatal depression interacts with child *5-HTTLPR* to predict the development of dysregulation at 3, 6, 18 and 36 months of age. We found that greater scores of maternal prenatal depression led to higher scores of dysregulation, specifically among children with susceptible genotypes for *5-HTTLPR*. These findings led to questions about the stability or developmental course of dysregulation from infancy to childhood, and whether or not other susceptible genotypes associated with mood and behaviour regulation would also interact with prenatal depression in the prediction of early childhood dysregulation.

In addition to maternal report of child dysregulation at 3, 6, 18, and 36 months of age, we included 48 and 60 month time points in Study 2. With six time points across the first five years of life, we can begin to outline the early developmental course of dysregulation with a trajectory analysis. Once the developmental trajectories are created, we can determine whether or not they are predicted by maternal prenatal depression in interaction with child *5-HTTLPR*. Further, we can explore whether other serotonergic and dopaminergic genes (e.g. *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT*, and *COMT*), which are associated with emotional and behavioural regulation, are also involved in the prediction of early dysregulation.

Although the trajectory analysis is explorative in nature, we expect to find that the trajectories will be predicted by an interaction between maternal prenatal depression and child genotype, whereby greater prenatal depression will lead to higher dysregulation among children with susceptible genotypes.

Study 2

Developmental Trajectories of Childhood Dysregulation from 3 Months to 5 Years of Age: The Influence of Prenatal Depression and Child Genetic Susceptibility

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Abstract

Background Childhood dysregulation is a combination of attention, emotion and behaviour problems associated with lifelong psychopathology. Multiple studies outline trajectories of dysregulation from childhood to early adulthood, however, none have outlined trajectories from infancy to childhood. Predictors of such trajectories are also unknown. Here, we 1) outline developmental trajectories of dysregulation as of infancy to 5 years of age and 2) examine whether prenatal depression interacts with child serotoninergic and dopaminergic genes to predict these trajectories. **Method** Our sample is a prospective birth cohort of $N = 582$ mother-child pairs from the MAVAN. Mothers rated their children's dysregulation at 3 and 6 months (*IBQ-R)*, at 18 and 36 months (*ECBQ*), and at 48 and 60 months (*CBCL-DP*). Mothers rated their depressive symptoms at 24-36 weeks' gestation and 12 months postnatal (*CES-D*). Child serotonergic and dopaminergic genes included *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT1*, and *COMT*. Covariates were child sex, education and postnatal depression. Analyses included latent class mixed models and *LEGIT*. **Results** Two qualitatively distinct dysregulation trajectories were found between 3 months and 5 years of age: persistently *low dysregulation* (94%), and *high dysregulation* (6%) that is initially low but increases over time as of 18 months. Prenatal depression was moderated by *5-HTTLPR*, whereas maternal postnatal depression was moderated by child genetic score, to predict high dysregulation. Males and children whose mothers had low to mid level education backgrounds were twice as likely to have high dysregulation. **Conclusion** This is an initial attempt to outline the course of dysregulation as of infancy. Our findings indicate that clinical stability of dysregulation emerges as of 18 months of age and can be traced from specific biological and environmental influences. Targets for

intervention include maternal pre- and postnatal depression, especially among males or children from households with lower education backgrounds.

Developmental Trajectories of Childhood Dysregulation from 3 Months to 5 Years of Age: The Influence of Prenatal Depression and Child Genetic Susceptibility

Childhood dysregulation can manifest as difficult temperament in infancy and as difficulty regulating thoughts, emotions, and behaviours throughout childhood. In infancy, indicators include problems with eating, sleeping, sensory processing, and soothability (Briggs-Gowan & Carter, 2007). In early childhood, they include depression, anxiety, somatic complaints, and sleep problems, while in later childhood they involve inattention, aggression, anxiety and depression (Degangi, Breinauer, Roosevelt, Porges, & Geenspan, 2000; Kim et al., 2012). In adolescence and early adulthood, they manifest as a combination of affective and substance use problems, suicidal ideation, and personality disorders (Althoff et al., 2010; Holtmann et al., 2011). Developmental trajectories of dysregulation from childhood to early adulthood are generally stable and indicate comorbid disorders (Holtmann et al., 2011; Meyer et al., 2009). Conversely, effective regulation skills are associated with favourable outcomes throughout life, including greater academic achievement, increased physical health, lower substance use, better personal finances, and fewer criminal offences (McClelland, Acock, Piccinin, Rhea, & Stallings, 2013; Moffit et al., 2011; Sawyer et al., 2015).

In an initial study on early trajectories of dysregulation in children aged 4 to 9.5 years, Winsper and Wolke (2014) identified four groups ranging from *low* to *very high* dysregulation. The groups were stable and strongly associated with regulatory problems at 6 to 30 months. Montroy, Bowles, Skibbe, McClelland, and Morrison (2016) identified three trajectories of regulation from 3 to 7 years, ranging from *early developers* to *later developers*, where girls and children with highly educated mothers were more likely to be early developers. In contrast, Wanless et al. (2016) found that trajectories of regulation around 4 years of age during an 18-

month period led to two groups – children who develop at a regular rate and slow developers. However, little is known about predictors of these trajectories.

Prenatal stress is often considered as a predictor of early development (Field, 2011). The most common forms of prenatal stress are depression and anxiety, which are highly comorbid (Barker, Jaffee, Uher, & Maughan, 2011). Prenatal depression is associated with a wide range of temperamental difficulties and psychopathology in the offspring (Davis, Glynn, Waffarn, & Sandman, 2011; Kochanska, Philibert & Barry, 2009). Additionally, prenatal depression is more prevalent than postnatal depression (Andersson, Sundström-Poromaa, Wulff, Aström, & Bixo, 2006), ranging from 20 to 38% among Canadian and American women respectively (Bowen & Muhajarine, 2006; Records & Rice, 2007), and strongly predicts postnatal depression (Edwards, Gallety, Semmler-Booth, & Dekker, 2008; Milgrom et al., 2008).

Disentangling the effects of maternal pre- and postnatal depression is difficult given that prenatal depression frequently precedes postnatal depression, and they are associated with similar outcomes (Murray, Fearon, & Cooper, 2015). Nonetheless, prenatal depression has been prospectively associated with child outcomes beyond the effects of postnatal depression (Barker et al., 2011; Lahti et al., 2017). In a complex statistical model, Pearson et al. (2013) demonstrated that the association between maternal postnatal depression and child depression was moderated by maternal education, whereas prenatal depression represented an independent risk factor that was not moderated by maternal education. These results provide indirect evidence for differential pathways of maternal pre- and postnatal depression.

Exposure to prenatal depression does not necessarily lead to negative outcomes. Susceptibility to prenatal affective stress can be moderated by variations in children's genetic makeup. For example, the risk allele of the serotonin transporter gene-linked polymorphism (*5-*

HTTLPR) exacerbates the negative effects of prenatal anxiety, leading to increased negative emotionality at 6 months of age (Pluess et al., 2011). We reproduced this interaction with prenatal depression in the prediction of infant dysregulation at 3, 6, 18 and 36 months of age (Babineau et al., 2015).

Genes implicated in serotonergic and dopaminergic signaling are significant to the exploration of dysregulation given their implication in the regulation of mood (Ruhé, Mason, & Schene, 2007) and behaviour (Chang et al., 2018). Relevant genes in the serotonergic system include *5-HTTLPR,* which moderates the experience of environmental stress in the development of depression (Gutknecht et al., 2015; Karg, Burmeister, Shedden, & Sen, 2011), and serotonin receptors *HTR1A, HTR1B* and *HTR2A*, which are associated with many psychiatric phenotypes including anxiety, depression and attention deficit hyperactivity disorder (ADHD; Norton & Owen, 2009). In the dopaminergic system, the brain-derived neurotropic factor (*BDNF*) interacts with other genes in the prediction of anxiety and depression-related personality traits (Hünnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007); receptors *DRD2* and *DRD4* are associated with externalizing behaviours and conduct disorder (Mota et al., 2013); while the transporter *DAT1* and *COMT* are linked to ADHD (Daly et al., 1999; Eisenbeg et al., 1999).

Aims

In the present study, we aim to (1) outline the developmental trajectories of dysregulation from 3 months to 5 years of age; (2) determine the influence of prenatal depression on these developmental trajectories; and (3) examine how children's serotonergic and dopaminergic genes moderate the effect of prenatal depression on trajectories of dysregulation.

Method

Participants

The participants are mother-child pairs from the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN), a Canadian community-based birth cohort (for details, see Babineau et al. 2015; O'Donnell et al., 2014). Eligibility criteria are ≥18 years of age at delivery and a singleton term pregnancy (≥ 37 weeks). The present study includes $N = 582$ mother-child dyads (see Table 1) for whom at least one measure of child dysregulation was available, which reduces to $N = 162$ when accounting for complete genetic data.

Measures

Prenatal depression. The *Center for Epidemiologic Studies Depression Scale* (*CES-D*; Radloff, 1977), a 20-item self-report measure of depressive symptomatology validated for pregnancy (e.g. Davis et al., 2011), was completed by the mothers at 24-36 weeks' gestation. The scores were centered.

Child dysregulation. The *Infant Behavior Questionnaire-Revised* (*IBQ-R*; Gartstein & Rothbart, 2003), a measure of child temperament, was completed by the mothers when the infants were 3 and 6 months of age. The *IBQ-R* leads to the three factors of Negative Emotionality, Surgency-Extraversion, and Regulation (*low regulation* is interpreted as *high dysregulation*). Five subscales (Smiling/Laughter, Low-Intensity Pleasure, Cuddliness/Affiliation, Duration of Orienting, and Soothability) are standardized and aggregated to create a dysregulation score at 3 and 6 months of age (Gartstein, personal communication, May 15, 2012; Babineau et al., 2015).

The *Early Childhood Behavior Questionnaire* (*ECBQ*; Putnam, Gartstein, & Rothbart, 2006), the extension of the *IBQ-R* for toddlers, was completed by the mothers when the children

were 18 and 36 months old. The *ECBQ* leads to the same three factors (Negative Emotionality, Surgency-Extraversion, and Regulation) with 8 subscales (Attention Shifting, Low-Intensity Pleasure, Cuddliness, Attention Focusing, Inhibitory Control, Perceptual Sensitivity, Sociability, and Soothability) that are standardized and aggregated to create a dysregulation score at 18 and 36 months of age.

The *Child Behavior Checklist* (*CBCL*; Achenbach & Rescorla, 2000), a measure of child behaviour and symptomatology, was completed by the mothers when the children were 48 and 60 months of age. The *CBCL-Dysregulation Profile* (*CBCL-DP*; Althoff et al., 2010) is created from three subscales (Attention Problems, Anxious/Depressed, and Aggressive Behaviour) that are standardized and aggregated to create a dysregulation score at 48 and 60 months of age. The dysregulation scores were normally distributed across measures from 3 to 18 months, and became gradually more skewed to the right from 36 to 60 months (p's < .01).

Genotype. Child genetic score is a combination of multiple serotonergic and dopaminergic genes previously implicated in dysregulation. Nine genes are explored: *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT1*, and *COMT*. DNA was extracted from saliva obtained from buccal swabs with the TaqMan method on the ABI-7000 for Single Nucleotide Polymorphism markers and on the ABI-3100 for repeat polymorphisms. When genotypes were ambiguous they were discarded. To check for error rates (.5%), each 20th marker was re-genotyped. We coded each variant as genetic susceptibility = 1, versus non-susceptibility = 0 (see Table 1), with the exception of *COMT* which was coded as genetic susceptibility = 1, partial genetic susceptibility = 0.5 versus non-susceptibility = 0 (Nikolova, Ferrell, Manuck, & Hairi, 2011).

Covariates. Demographic covariates were obtained from the *Health and Well Being of Mothers and their Newborns* questionnaire completed by the mothers at 24-36 weeks' gestation (Kramer et al., 2009). Covariates identified *a priori* were retained when associated with both a predictor and the outcome (i.e., sex, maternal education, maternal postnatal depression). Maternal education was dichotomized as 'mid/low education' (i.e. ≤high school, some college/trade, and college/trade graduate) or 'high education' (≥university graduate; see Table 1), given the smaller frequencies of the mid and low categories. Maternal postnatal depression was assessed with the *CES-D* at 12 months postnatal. Variables not retained were maternal genotypes, mother's age at birth, family income, and child birth weight.

Statistical analyses

We fitted the developmental trajectories of dysregulation from 3 to 60 months ($N = 582$) with the *Extended Mixed Models using Latent Classes and Latent Processes (LCMM*; Proust-Lima, Philipps, & Liquet, 2017) package in R. Dysregulation scores at six time points (3, 6, 18, 36, 48 and 60 months) were entered into the model. We compared model fit across 2, 3, or 4 groups based on lowest BIC (Schwarz, 1978). We further conducted a chi-square test of independence to explore any group differences by sex.

In order to validate trajectories extracted from three different instruments, we compared an *Item Response Theory (IRT)* trajectory model of dysregulation (*Dysregulation-IRT)* which only included items from the dysregulation subscales of the *IBQ-R*, *ECBQ* and *CBCL-DP* comparable across all three instruments (see Appendix). The items which were similar across at least two instruments were selected independently by a graduate student and postdoctoral fellow (74% concordance rate), with consensus reached in cases of discordance. Good

concordance between the original model and the *IRT* model was established by correlation analysis, chi square test, paired t-test, and weighted Cohen's kappa.

The prediction of dysregulation group membership from the interaction of maternal pre- and postnatal depression and child genotype was modeled with the *Latent Environmental and Genetic InTeraction* (*LEGIT*) package in R (Jolicoeur-Martineau et al., 2018; Jolicoeur-Martineau et al., 2019). *LEGIT* fits a GxE model, where G is a weighted sum of the observed genetic variants and E is a weighted sum of the observed environmental variables. We constructed four separate models: (A) prenatal depression x *5-HTTLPR*; (B) postnatal depression x *5-HTTLPR*; (C) prenatal depression x genetic score (multiple serotonergic and dopaminergic genes); and (D) postnatal depression x genetic score. Models A and C were adjusted by postnatal depression, and B and D by prenatal depression. Models A and B extended our previous findings (Babineau et al., 2015).

Results

Trajectory analysis

The trajectory analysis identified a 2-classs model as the best fit (BIC: 4193.23; see Figure 1). Children in the low dysregulation group (94%) remained persistently low on dysregulation over time, whereas those in the high dysregulation group (6%) were initially low and became increasingly high on dysregulation over time. Group differences between low and high dysregulation became apparent as of 18 months. Although not significant, males (OR = 2.22, p = .11) and children with mothers in the low/mid education category (OR = 2.15, p = .11) were slightly more likely to be in the high dysregulation group. More males (23 vs. 12) were in the high dysregulation group ($\chi^2(1) = 7.72$, $p < .01$).

The prediction of dysregulation trajectories

In Model A, prenatal depression and *5-HTTLPR* interacted to predict the probability of being in the high dysregulation group, such that exposure to greater prenatal depression resulted in a greater likelihood of high dysregulation for children with the susceptible genotypes (S/L_G). Males, low/mid maternal education, and exposure to postnatal depression also increased the likelihood of being in the high dysregulation group (see Table 2-A and Figure 3-A).

In Model B, neither main effects nor interaction effects were found for maternal postnatal depression or *5-HTTLPR*. However, all covariates were significant, with males, low/mid maternal education and exposure to prenatal depression as significant predictors of being in the high dysregulation group (see Table 2-B and Figure 3-B).

In Model C, *child genetic score* was determined by a bi-directional stepwise (starting with *5-HTTLPR*) search that resulted in the retention of five of nine genes chosen for analysis (*5-HTTLPR, HTR1A, HTR2A, COMT* and *DAT1*). A main effect of child genetic score, but not of prenatal depression, was found. A higher genetic score was linked to a greater probability of high dysregulation (Figure 3). The susceptibility variants of *5-HTTLPR*, *HTR1A*, and *COMT* predicted a greater probability of being in the high dysregulation group, whereas susceptibility variants of *HTR2A* and *DAT1* predicted a greater probability of being in the low dysregulation group (Table 2-C and D for genetic weights). No interaction effects were found. Male sex and low/mid maternal education were predictors of being in the high dysregulation group, while postnatal depression had trend-level significance (Table 2-C and Figure 3-C).

In Model D (Table 2-D and Figure 3-D), the bidirectional step-wise search retained four of nine genes (*5-HTTLPR, HTR2A, COMT* and *DAT1*). Maternal postnatal depression and child genetic score interacted to predict greater likelihood of being highly dysregulated. Genes in

Model D had a similar influence on dysregulation as did genes in Model C. Further, male sex and maternal low/mid education were predictors of high dysregulation, whereas prenatal depression was not.

When comparing effect size across *LEGIT* Models A-D (see Table 2), we took into consideration that Models A and B were single candidate gene models, whereas Models C and D were multi-genetic gene models. A 5.7x increase in effect size was found when comparing Models A and B to Models C and D, as a result of the flexibly weighted multi- genetic score (see Jolicoeur-Martineau et al., 2018 for a detailed understanding of *LEGIT*). Further, no differences in effect size were found when comparing Model A to B, or Model C to D.

Discussion

This is an initial attempt to outline developmental trajectories of childhood dysregulation as early as infancy. These trajectories are grounded in complementary measures of mother and child across pre- and postnatal environments, repeated measures of dysregulation over the first five years of life, and multiple genetic variants. Whereas the study of genetic moderation of prenatal depression on child neurobehavioural outcomes has previously relied on single candidate gene studies, our multi-gene profile has the potential to further the understanding of the etiology of mental health (Abbott, Gumusoglu, Bittle, Beversdorf, & Stevens, 2018). The use of repeated measures is validated within our second set of analyses using Item Response Theory. Further, the prevalence rates and biological sex differences within our trajectories align with those from other studies of child dysregulation (Montroy et al. (2016). Together, these trajectories provide a foundation for the developmental study of dysregulation from infancy to childhood and throughout the lifespan.

In our trajectory model, we found two distinct developmental trajectories of dysregulation from 3 months to 5 years of age – children with stable *low dysregulation*, and children with increasingly *high dysregulation*. The shape of the trajectories indicated that the two groups followed a similar course between 3 and 18 months of age with marked differences emerging as of 18 months, potentially indicating the clinical stability of dysregulation in this group. Children with high dysregulation accounted for 6% of our sample, which is consistent with previous estimates of 4 to 11% of children in community-based samples (Althoff, Rettew, Faraone, Boomsma, & Hudziak, 2006; Kim et al., 2012).

We hypothesized that prenatal depression and children's genetic score would interact to predict a greater probability of high dysregulation from 3 months to 5 years of age. Multiple serotonergic and dopaminergic genes were of interest given their role in mood and behaviour regulation, including social functioning, attention and behaviour problems. Our findings suggest that child *5-HTTLPR* moderated prenatal depression, but not postnatal depression, in predicting high dysregulation. However, when genes from the dopaminergic network were also considered, the child genetic scores moderated maternal postnatal depression, but not prenatal depression in predicting high dysregulation. More specifically, a child's probability of being highly dysregulated increased as a result of being exposed to greater maternal postnatal depression among children with susceptible genotypes for *5-HTTLPR* and *COMT*. However, children appeared to benefit from susceptible genotypes for *HTR2A* and *DAT1* which made them less likely to be dysregulated. Although susceptible genotypes for *HTR2A* and *DAT1* are typically associated with adverse mental health outcomes, they may operate differently when in interaction with maternal stress and in conjunction with other genes. For example, the inclusion of other genotypes may actually modify the usual direction of the effect for *HTR2A* and *DAT1*,

not to mention even notable negative findings for the mediation of maternal pre- and postnatal depression by *5-HTTLPR* or *DAT1* in the prediction of ADHD subtypes (Park et al., 2014). Further investigation is warranted to determine the moderating role of serotonin and dopamine related genes in the development of child mental health.

From this pattern of results, maternal pre- and postnatal depression appeared to have unique and separate contributions in the development of dysregulation, even when considering other factors (i.e. covariates) such as sex and maternal education. While maternal pre- and postnatal depression both predicted the probability of being highly dysregulated when moderated by genetic factors, they did so through distinct pathways. Where prenatal depression was moderated by *5-HTTLPR* as a single gene, postnatal depression was not, and where postnatal depression was moderated by child genetic score (i.e. a combination of serotonin and dopamine related genes), prenatal depression was not. Different moderators can be interpreted as indirect evidence for separate influences of maternal pre- and postnatal depression, beyond the continuum of environmental exposure from the pre- to the postnatal period (Pearson et al., 2013). For example, prenatal depression may transcend to the fetus via placental mechanisms, whereas postnatal depression may be shared in the day-to day interactions (e.g. maternal sensitivity and care during activities such as play, meal time, bedtime; maternal modeling of mood and self-regulation; dynamic between mother and child during interactions leading to shared mood). Despite complex statistical models, it remains that prenatal stress frequently precedes postnatal stress, both of which are associated with similar outcomes for children exposed (Murray et al., 2015).

Additional factors considered in all of our analyses were sex and maternal education. Children who were male or from a household where mothers were less highly educated were at

significantly greater risk of being highly dysregulated when exposed to either pre- or postnatal depression, even when genetic makeup was not considered. These findings are similar to those of Montroy et al. (2016) who demonstrated that children who were female or whose mother was in the highest education category were among those who demonstrated the most rapid beneficial gains along developmental trajectories of regulation. Additionally, two thirds of children in our high dysregulation group were male. These findings may be attributed to the fact that that dysregulation has a similar profile to disorders that are more common in males than in females prior to puberty, such as ADHD (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015) and oppositional defiant disorder (ODD; Demmer, Hooley, Sheen, McGillivray, & Lum, 2017). Disruptive mood dysregulation disorder, a disorder with similar features to the *CBCL-DP*, is highly comorbid with ADHD, ODD, depression and anxiety in children 3 to 6 years of age (Dougherty et al. 2015). Additionally, our findings of sex differences may be attributed to the fact that males are more sensitive and at greater risk of developing neurodevelopmental diseases as a result of exposure to prenatal adversity than females (Bale, 2016). As for maternal education, this factor has previously been identified as an important variable in family socioeconomic status and resources, including access to material and social resources, as well as access to recreational and learning material from infancy to adolescence (Bradley & Corwyn, 2002; Mercy & Steelman, 1982).

Limitations

A primary limitation of our study is that we are the first to use the *IBQ-R* and *ECBQ* measures dysregulation along the same continuum as the *CBCL-DP* – while the *ECBQ* is considered to be an upward extension of the *IBQ-R* (Putnam, Gartstein, & Rothbart, 2006), the *CBCL* is a separate measure. Nevertheless, all three measures are parent report questionnaires of

child behaviour with dysregulation subscales that are based on aspects such as mood, attention, play behaviours, and aggression. Further, the *IBQ-R* and *ECBQ* have been found to predict the *CBCL* (Gartstein & Bateman, 2008; Gatstein, Putnam & Rothbart, 2012).

A second limitation is our reliance on maternal self-report measures. For example, maternal mood may influence observation and response style across measures (Atella, DiPietro, Smith, & James-Roberts, 2003). However, questionnaires allow for a longer observation period, multiple time points with repeated measures, and a reduction of bias given the specific and recent occurring behaviours being reported (Gartstein & Rothbart, 2003).

A third limitation is that, as compared to other genetic studies, we have a relatively small number of participants with genetic data available for analysis. Thus, our work can be considered preliminary and will require replication and validation from larger samples.

Implications

We would like to align our longitudinal study of dysregulation from infancy to early childhood with previous longitudinal studies of dysregulation from childhood to early adulthood (e.g. Althoff et al., 2010; Holtmann et al., 2011). Accordingly, we provide an initial attempt to outline the early development of dysregulation from as early as infancy, and are able to show that the clinical stability of dysregulation emerges around 18 months of age. Importantly, risk for dysregulation appears to be predicted by specific biological and early environmental influences.

Similar to the Research Domain Criteria (RDoC) framework which states that diagnostic categories are less predictive of treatment response as compared to refined full range dimensions, our trajectories include a dimensional approach regarding the development of dysregulation. Subgroups at risk of high dysregulation became apparent, which might be key for the creation of preventative interventions (Insel et al., 2010). We identified differential pathways that support

separate effects of maternal pre- and postnatal depression, which suggests that women and their children can benefit from interventions as early as the prenatal period. Given that links between childhood dysregulation and the development of psychopathology have been established, (Caspi et al., 1995; Gartstein & Bateman, 2008; Pitzer, Jennen-Steinmetz, Esser, Schmidt, & Laucht, 2011; Ullsperger, Nigg, & Nikolas, 2015), our findings support clinical research that investigates early intervention targets such as maternal pre- and postnatal depression, especially among children who are male or from households with lower education backgrounds.

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Tables

Table 1 Descriptive Statistics of MAVAN Mother and Child (*N* = 582 pairs)

	M (*SD*)	%
Mothers		
Age at delivery	30.58 (5)	
In a partnership at delivery		94.8
Prenatal depression	12.44 (9.7)	
Depression cut-off (*CES-D*≥16)		28.5
Education		
≤High School		10.7
Some College/Trade		9
College/Trade Graduate		31.1
≥University Graduate		49.2
Annual household income		
<15 000		6.8
15 000 to <30 000		12.6
30 000 to <50 000		20
50 000 to <80 000		24.2
≥80 000		36.4
Children		
Sex – Female		46.2
Genetic susceptibility		
5-HTTLPR		69
(SS, SL_G, L_GL_G, L_AL_G vs L_AL_A)		
HTR1A		28
(GG vs GC, CC)		
HTR1B		44
(CC, CG vs GG)		
HTR2A		79
(CC, CT vs TT)		
BDNF		68
(AA, AG vs GG)		
DRD4		38
(presence vs absence of 7 or 8)		
DRD2		37
(AA, AG vs GG)		
DAT1		50
(10-10 vs 9-9, 9-10)		
COMT		73*
(AA vs GA vs GG)		

Education and income categories as per Kramer et al. (2009). In analyses, the categories ≤High School, Some College/Trade, and College/Trade Graduate are combined as the "Low/Mid Education" group, and compared to ≥University Graduate which is the "High Education" group.
*25% with partial genetic susceptibility and 48% with full genetic susceptibility

Table 2 Regression Analysis Results Demonstrating the Probability of *High Dysregulation* from 3 to 60 Months as Influenced by Maternal Pre- and Postnatal Depression in Interaction with Child *5-HTTLPR* Status and Child Genetic Score (*5-HTTLPR, HTR1A, HTR2A, COMT, DAT1*)

	5-HTTLPR		Genetic Score	
	(A) Prenatal $N = 268$	(B) Postnatal $N = 268$	(C) Prenatal $N = 162$	(D) Postnatal $N = 164$
	ß	ß	ß	ß
Intercept	-.29	-.26	-.36	-.37
Covariates				
Sex – male	.59**	.39*	.17***	.18***
Education – high	-.49*	-.48*	-.77**	-.73*
Maternal depression	.03*	.02*	$.03^{t}$	.01
Predictors				
Gene(s)	$.62^{t}$	.03	.56***	.52***
Maternal depression	-.02	.02	-.01	.01
Genes x maternal depression	-.05*	-.01	.09	.22*
Genetic weights[a]				
5-HTTLPR	$.62^{t}$	.03	.19***	.2***
HTR1A			.08*	
HTR2A			-.3***	-.3***
COMT			.25***	.3***
DAT1			-.18***	-.2***
R^2	.04	.04	.23	.23

***$p < .001$, **$p < .01$, *$p < .05$, $^{t}p < .1$

Model A, B, C and D coincide with Figure 2-A, B, C and D respectively

Note[a]: Negative genetic weights lead to a lower probability of high dysregulation, while positive genetic weights lead to a greater probability of high dysregulation.

Figures

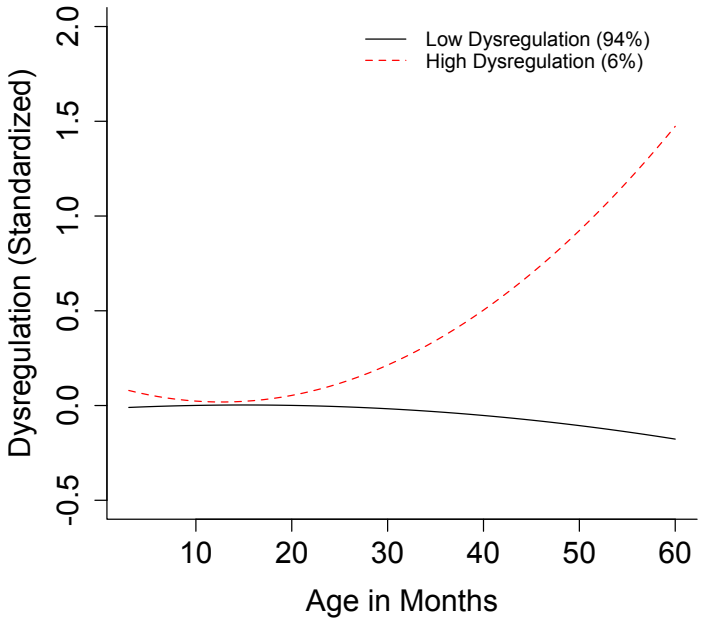

Figure 1. Trajectory of dysregulation from 3 to 60 months.

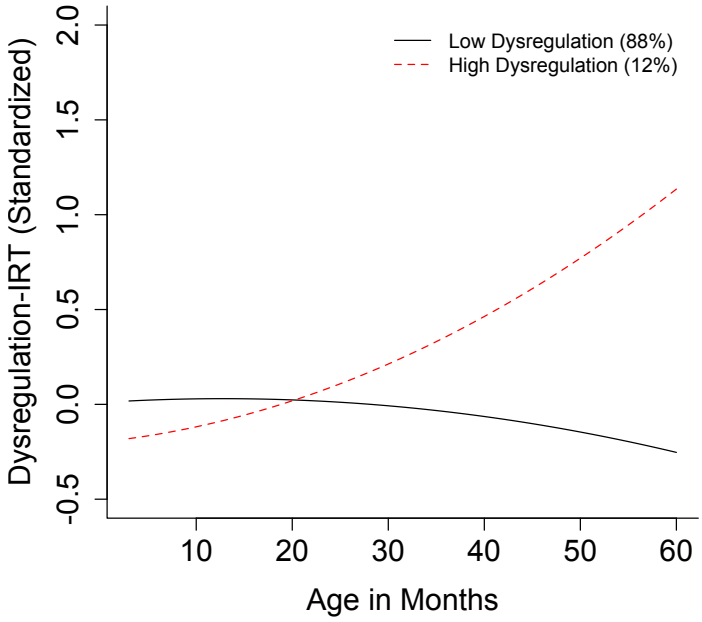

Figure 2. Trajectory of *Dysregulation-IRT* from 3 to 60 months.

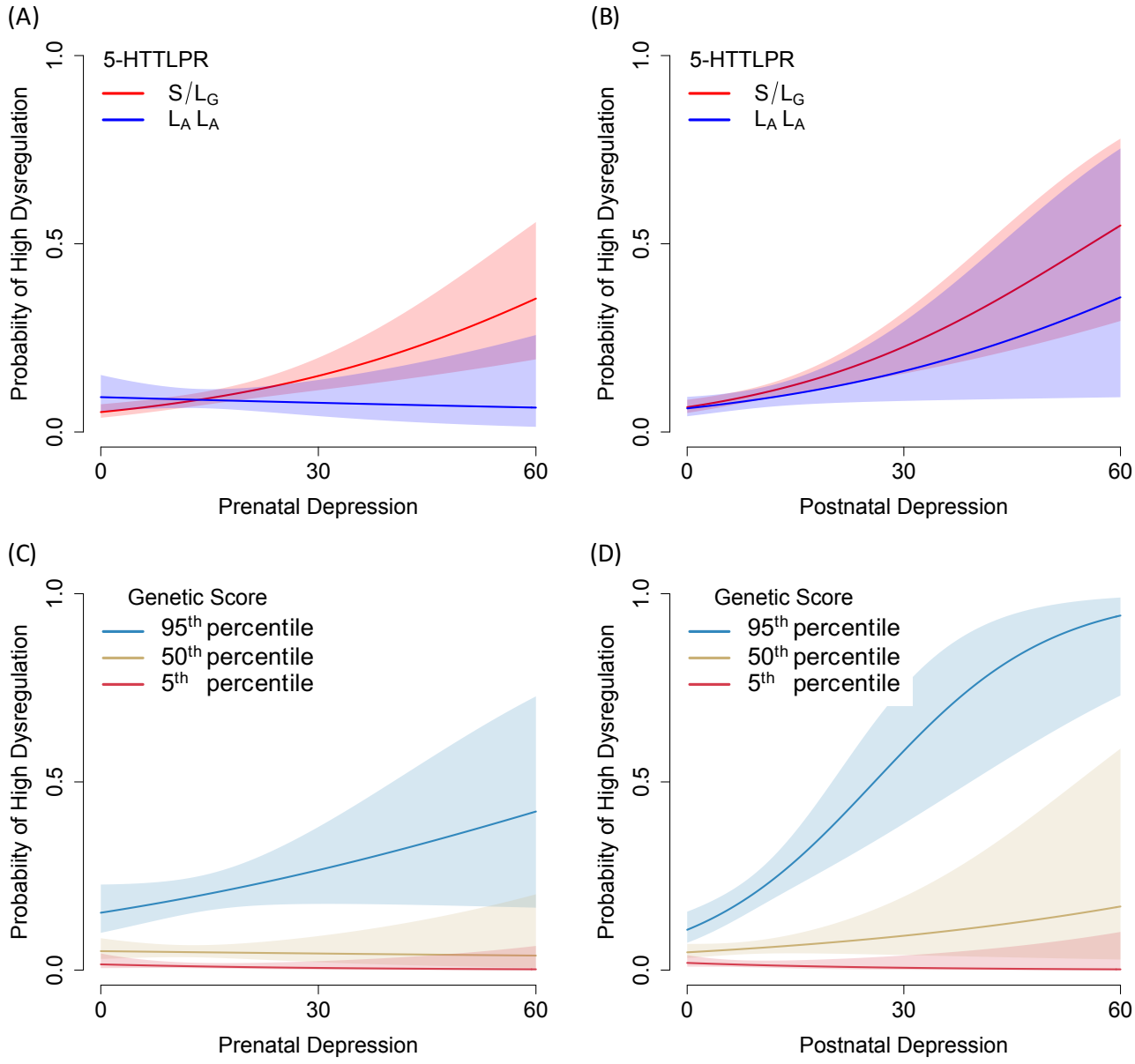

Figure 3. Results from regression analysis: (A) Probability of high dysregulation – Prenatal depression x *5-HTTLPR*, (B) Probability of high dysregulation – Postnatal depression x *5-HTTLPR*, (C) Probability of high dysregulation – Prenatal depression x genetic profile, (D) Probability of high dysregulation – Postnatal depression x genetic profile.

Appendix

Dysregulation-IRT

In the *Dysregulation-IRT* model, 57% of the *IBQ-R* dysregulation items were retained, 55% of the *ECBQ* dysregulation items were retained, and 29% of the *CBCL-DP* items were retained (see Table S1). Dysregulation measures were highly correlated at each time point when comparing our original measures of dysregulation to our *Dysregulation-IRT* measures (see Table S2).

The *Dysregulation-IRT* trajectory analysis, similar to the initial trajectory analysis, identified a 2-class model as the best fit (BIC: 2417.87; Figure 2): a persistently low dysregulation group (88%), and an increasingly high dysregulation group (12%) who were initially low and then increased in dysregulation over time. Group differences became apparent as of 18 months of age. However, 7% of children initially in the low dysregulation group and 14% of children initially in the high dysregulation group were then misclassified in the *Dysregulation-IRT* trajectories ($\chi^2(1) = 177.42$, $p < .001$). There was also a 6% difference between probability of group membership for the initial trajectories as compared probability of group membership for the *Dysregulation-IRT* trajectories ($t(495) = 8.71$, $p < .001$). Nevertheless, reliability between the initial trajectories and the *Dysregulation-IRT* trajectories was .83 (weighted kappa).

Table S1 Items Retained for the *Dysregulation-IRT* Analysis

Scale (%Retained)	*IBQ-R Dysregulation* (57)	*ECBQ Dysregulation* (57)	*CBCL-DP* (28)
	30. When it was time for bed or a nap and your baby did not want to go, how often did s/he whimper or sob?		10. Clings to adults or too dependent
	31. When it was time for bed or a nap and your baby did not want to go, how often did s/he become tearful?		10. see above item
	34. When being dressed or undressed during the last week, how often did the baby smile or laugh? 36. When put into the bath water, how often did the baby smile? 40. When face was washed, how often did the baby smile or laugh? 43. When hair was washed, how often did the baby smile?		67. Seem unresponsive to affection
	46. How often during the last week did the baby look at pictures and/or magazines for 2-5 minutes at a time?	126R. When playing alone, how often did your child become easily distracted? 167R. While looking at picture books on his/her own, how often did your child stay interested in the book for 5 minutes or less? 169R. While looking at picture books on his/her own, how often did your	5. Can't concentrate, can't pay attention for long 6. Can't sit still, restless, or hyperactive 59. Quickly shifts from one activity to another 95. Wanders easily

	child become easily distracted? 196R. When playing alone, how often did your child move from one task or activity to another without completing any? 197R. When playing alone, how often did your child have trouble focusing on a task without guidance?	
47. How often during the last week did the baby look at pictures in books and/or magazines for 5 minutes or longer at a time?	167R. see item above 169R. see item above	5. see item above 6. see item above 59. see item above 95. see item above
48. How often during the last week did the baby stare at a mobile, crib bumper or picture for 5 minutes or longer?	126R. see item above 196R. see item above 197R. see item above	5. see item above 6. see item above 59. see item above 95. see item above
49. How often during the last week did the baby play with one toy or object for 5-10 minutes?	90R. When engaged in an activity requiring attention, such as building with blocks, how often did your child move quickly to another activity? 92R. When engaged in an activity requiring attention, such as building with blocks, how often did your child tire of the activity relatively quickly?	5. see item above 6. see item above 59. see item above 95. see item above

	127. When playing alone, how often did your child play with s set of objects for 5 minutes or longer at a time?	
50. How often during the last week did the baby play with one toy or object for 10 minutes or longer?	91R. When engaged in an activity requiring attention, such as building with blocks, how often did your child stay involved for 10 minutes or more?	5. see item above 6. see item above 59. see item above 95. see item above
55. How often during the last week did the baby pay attention to your reading during most of the story when looking at picture books?		5. see item above 6. see item above 59. see item above 95. see item above
59. How often during the last week did the baby enjoy being sung to?	29. During daily or evening quiet time with you and your child, how often did your child enjoy just being quietly sung to?	
60. How often during the last week did the baby enjoy being read to?	31. During daily or evening quiet time with you and your child enjoy just being talked to?	
64. How often during the last week did the baby enjoy lying quietly and examining his/her fingers or toes?	12. While playing outdoors, how often did your child enjoy sitting quietly in the sunshine?	
71. How often during the last week did the baby enjoy listening to a musical toy in a crib?	30. During daily or evening quiet time with you and your child smile at the sound of words, as in nursery rhymes?	
72. When playing quietly with one of her/his favorite toys, how often did your baby show pleasure?	193. While playing outdoors, how often did your child enjoy sitting down and playing quietly?	
73. When playing quietly with one of	49R. When engaged in play with his/her favorite	

her/his favorite toys, how often did your baby enjoy lying in the crib for more than 5 minutes?	toy, how often did your child play for 5 minutes or less?	
74. When playing quietly with one of her/his favorite toys, how often did your baby enjoy lying in the crib for more than 10 minutes?	50. When engaged in play with his/her favorite toy, how often did your child play for more than 10 minutes?	
91. How often during the last week did the baby when in a position to see the television set, look at it for 2 to 5 minutes at a time?	158R. When interrupted during a favorite TV show, how often did your child not finish watching the program?	5. see item above 6. see item above 59. see item above 95. see item above
92. How often during the last week did the baby when in a position to see the television set, look at it for 5 minutes or longer?	157. When interrupted during a favorite TV show, how often did your child immediately return to watching the TV program?	5. see item above 6. see item above 59. see item above 95. see item above
100. How often during the last week did the baby look at children playing in the park or on the playground 5 minutes or longer?		5. see item above 6. see item above 59. see item above 95. see item above
101. How often during the last week did the baby watch adults performing household activities (e.g., cooking, etc.) for more than 5 minutes?	179. While you were talking with someone else, how often did your child easily switch attention from speaker to speaker?	5. see item above 6. see item above 59. see item above 95. see item above
105R. When being held, how often did the baby pull away or kick?	159. While held on your lap, how often did your child pull away and kick?	20. Disobedient
107. When being held, how often did the baby	18. When your child was carried, how often did	

seem to enjoy him/herself?	s/he snuggle up next to you?	
108R. When being held, how often did the baby squirm?	16R. When your child was carried, how often did s/he squirm?	
123. When rocked or hugged, in the last week, did your baby seem to enjoy him/herself?	32. During daily or evening quiet time with you and your child enjoy rhythmic activities, such as rocking or swaying?	
124R. When rocked or hugged, in the last week, did your baby seemed eager to get away?	80R. When being gently rocked or hugged, how often did your child seem eager to get away?	
125R. When rocked or hugged, in the last week, did your baby make protesting noises?	81R. When being gently rocked or hugged, how often did your child make protesting noises?	
129. When being carried, in the last week, how often did the baby seem to enjoy him/herself?	14. When your child was carried, how often did s/he like to be held?	
130R. When being carried, in the last week, how often did the baby push against you until put down?	15R. When your child was carried, how often did s/he push against you until put down?	88. Uncooperative
131. When sitting in your lap how often did your baby seem to enjoy her/himself?	103. During daily or evening quiet time with you and your child, how often did your child want to be cuddled?	
174. When rocking your baby, how often did he/she soothe immediately?	188. When s/he was upset, how often did your child become easily soothes?	
175. When rocking your baby, how often did he/she not soothe immediately, but in the first two minutes?	46. When s/he was upset, how often did your child change to feeling better within a few minutes?	
176R. When rocking your baby, how often did he/she take more than 10 minutes to soothe?	47R. When s/he was upset, how often did your child soothe only with difficulty?	44. Angry moods

179R. When singing or talking to your baby, how often did s/he take more than 10 minutes to soothe?	44. Angry moods
182R. When walking with the baby, how often did s/he take more than 10 minutes to soothe?	44. Angry moods
185R. When giving him/her a toy, how often did the baby take more than 10 minutes to soothe?	44. Angry moods
188R. When showing the baby something to look at, how often did s/he take more than 10 minutes to soothe?	44. Angry moods
191R. When patting or gently rubbing some part of the baby's body, how often did s/he take more than 10 minutes to soothe?	44. Angry moods

Table S2 Correlation Matrix – *Dysregulation-IRT* Scales and Original Dysregulation Scales

	Dysregulation-IRT					
	IBQ-R 3 months	*IBQ-R* 6 months	*ECBQ* 18 months	*ECBQ* 36 months	*CBCL-DP* 48 months	*CBCL-DP* 60 months
IBQ-R 3months	0.93***					
IBQ-R 6 months		0.91***				
ECBQ 18 months			0.89***			
ECBQ 36 months				0.88***		
CBCL-DP 48 months					0.90***	
CBCL-DP 60 months						0.92***

***$p < .001$

Bridge to Study 3

In Study 1, we found that maternal prenatal depression interacted with child genotype for *5-HTTLPR* to predict dysregulation at 3, 6, 18 and 36 months of age. In Study 2, we identified two distinct developmental trajectories of early dysregulation from 3 to 60 months of age (i.e. 3, 6, 18, 36, 48, and 60 months time points); persistently *low dysregulation*, and *high dysregulation* that is initially low but increases over time as of 18 months of age. The high dysregulation group was predicted by an interaction between maternal prenatal depression and child *5-HTTLPR.* In studies 1 and 2, greater prenatal depression led to greater dysregulation for children with susceptible genotypes for *5-HTTLPR.* New questions that emerge based on our findings from studies 1 and 2 are whether or not dysregulation from 3 to 60 months of age predicts child psychiatric comorbidity, and whether or not psychiatric comorbidity is predicted by an interaction between maternal prenatal depression and child genetic susceptibility, as moderated by high dysregulation.

As dysregulation has previously been demonstrated to be stable from school age to adulthood, and associated with severe and comorbid psychopathology (Althoff et al., 2010; Meyer et al., 2009), Study 3 is designed to determine whether children with increasingly high dysregulation between 3 to 60 months of age are more likely to develop comorbid psychiatric disorders at 6 years of age. In childhood, as per the *CBCL-Dysregulation Profile* (*CBCL-DP*; Althoff, 2010), dysregulation is a combination of attention problems, aggression, anxiety, and depression. Therefore, in Study 3, we assess children's comorbid psychopathology at 6 years of age by including disorders that are specifically characteristic of child dysregulation, such as ADHD, CD, ODD, anxiety and depression. Further, we examine whether the development of comorbid psychopathology is influenced by an interaction between maternal prenatal depression

and child candidate genes implicated in mood and behaviour regulation (i.e. *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT*, and *COMT*). In addition to the above candidate genes, and with the availability of genome-wide data in MAVAN, we also include children's polygenic risk scores (PRS) for psychopathology as an additional measure of child genetic susceptibility.

We expect to find that the developmental trajectory of high dysregulation will predict comorbid psychiatric disorders at 6 years of age. We further expect to find that psychiatric comorbidity will be predicted by an interaction between prenatal depression and child genetic susceptibility, as moderated by high dysregulation.

Study 3

A Longitudinal Study of Early Childhood Dysregulation and Psychiatric Comorbidity – The Influence of Maternal Prenatal Depression and Offspring Genetic Susceptibility

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Abstract

Background Childhood dysregulation is a combination of attention, emotion and behaviour problems. Dysregulation in childhood and adolescence predicts psychiatric comorbidity up to adulthood. In the present study, we hypothesized that infant and early childhood dysregulation would similarly be associated with psychiatric comorbidity as of 6 years of age. We further hypothesized that psychiatric comorbidity would be predicted by an interaction between maternal prenatal depression and child genetic susceptibility and that early dysregulation would partly mediate this relationship. **Method** Our sample was a prospective birth cohort of $N = 234$ mother-child pairs from the MAVAN. The dysregulation trajectories (low vs. high) were based on maternal report of child dysregulation at six time-points: 3 and 6 months (*IBQ-R)*, 18 and 36 months (*ECBQ*), and 48 and 60 months (*CBCL-DP*). Child psychiatric disorders and comorbidity were based on the *Preschool Age Psychiatric Assessment* (*PAPA*) administered to mothers by research assistants when children were 6 years old. Women rated their depressive symptoms at 24-36 weeks' gestation and 6, 12, 24, 36, 48, 60 and 72 months postnatal (*CES-D*). Child genes included serotonergic and dopaminergic candidate genes (i.e. *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT1*, and *COMT*) and two polygenic risk scores (PRS): a cross disorder phenotype and a childhood total psychological problems phenotype. **Results** Children with high dysregulation trajectories were significantly more likely to have ADHD, CD, ODD, anxiety, depression, and psychiatric comorbidity. Maternal prenatal depression did not interact with child genetic susceptibility to predict comorbidity. An interaction between maternal prenatal depression and child genes did not lead to an indirect effect on comorbidity through high dysregulation group membership. **Discussion** Our findings support the conclusion that early childhood dysregulation from 3 to 60 months of age is a predictor of comorbid psychiatric

disorders as early as 6 years of age, and a likely phenotype of severe and lifelong psychopathology. Although prenatal depression and child genes did not predict psychiatric comorbidity, high dysregulation did predict comorbidity, which we interpret as potential evidence for the prenatal programming of postnatal plasticity.

A Longitudinal Study of Early Childhood Dysregulation and Psychiatric Comorbidity – The Influence of Maternal Prenatal Depression and Offspring Genetic Susceptibility

Childhood dysregulation has been longitudinally associated with the development of various psychiatric disorders and comorbidity of disorders up to adulthood (Althoff, Verhulst, Rettew, Hudziak, & van der Ende, 2010). More specifically, *dysregulation problems*, as assessed by parent- and self-report of day-to-day affective, behavioural, and attention problems, have been associated with outcomes such as mood and anxiety disorders, attention deficit hyperactivity disorder (ADHD), suicidality, substance use problems, and cluster B personality disorders (e.g. antisocial personality disorder, borderline personality disorder), as well as with increased severity and comorbidity of these disorders (Althoff et al., 2010; Holtmann et al., 2011; Meyer et al., 2009). Convergent evidence across studies indicates that childhood dysregulation can predict the development of lifelong comorbid psychiatric impairment. However, most longitudinal studies of dysregulation are focused on school age children and adolescents (e.g. Holtmann et al., 2011; Meyer et al., 2009), with no known studies prior to 4 years of age (see Althoff et al., 2010) This leaves a gap in the literature from 0 to 3 years of age and how this period might also predict psychiatric comorbidity, and act as a potential target for earlier intervention than what is currently known. Although psychopathological correlates of early childhood dysregulation have been investigated which are important for identifying dysregulation, we are aware of few studies on the prediction of early childhood dysregulation. Predictors likely include complex developmental pathways between environmental and biological factors, eventually leading to the development of comorbid psychopathology. More precise information of and evidence for these pathways would additionally inform intervention in the development of early childhood dysregulation.

Psychopathological correlates of childhood dysregulation and the prediction psychiatric comorbidity have been studied as early as 3 years of age. In a single time-point study, Kim et al. (2012) found that dysregulation at 3 years of age was concurrently associated with depression, anxiety, ADHD, and oppositional defiant disorder (ODD). In a 3-year follow-up study, Dougherty et al. (2015) found that dysregulation at 3 years of age was predictive of disruptive mood dysregulation disorder (DMDD) at 6 years of age. DMDD was diagnosed in 8.2% of children in their sample, and 60.5% of these children concurrently met criteria for comorbid emotional or behavioural disorders such as depression, anxiety, ADHD, and ODD (Dougherty et al., 2015). In a longitudinal study, Althoff et al. (2010) found that dysregulation as early as 4 years of age predicted anxiety disorders, mood disorders, behaviour disorders, and substance use problems in adulthood.

As for the prediction of dysregulation, both environmental and biological factors are likely contributors. According to the developmental origins of health and diseases (DOHaD) hypothesis, there are critical or sensitive periods throughout development during which environmental factors can have long lasting effects on developing biological systems, including subsequent plasticity (Barker, 2004). For example, exposure to early environmental adversity occurring as early as gestation, such as exposure to prenatal stress, can have long lasting effects on the child's cognitive, behavioural, and affective development (Monk, Lugo-Candelas, & Trumpff, 2019; Pearson et al., 2013). The effect of prenatal environmental adversity is not uniform but can be modified by biological mechanisms to shape neurobiological function of specific brain regions and circuits, which in turn influence neurobehavioural outcomes such as cognitive, behavioural, and emotional regulation (van den Bergh et al., 2017). Brain regions of particular interest that are affected by prenatal stress and related to regulation abilities and to

problems of dysregulation include the limbic system (hippocampus, amygdala) and prefrontal cortex, and the connections between these regions (van den Bergh et al., 2017). During pregnancy, the brain of the fetus undergoes rapid growth in neuron production, migration, connections, and differentiation, and thus fetal development is considered a specifically sensitive period for exposure to maternal stress (Monk et al., 2019). The identification of candidate genes and candidate gene networks which interact with prenatal stress to predict long term psychopathology further support an understanding of brain regions susceptible to stress during fetal brain development (Abbott, Gumusoglu, Bittle, Beversdorf, & Stevens, 2018).

In an initial study, we (Babineau et al., 2015) demonstrated that dysregulation, over the first three years of life (3 to 36 months) as assessed by the *IBQ-R* and the *ECBQ*, appears to be influenced by an interaction between maternal prenatal depression and child genotype for the risk allele of the serotonin transporter gene-linked polymorphism (*5-HTTLPR*). In a follow-up study, we (Babineau et al., in preparation) outlined the development of dysregulation over the first five years of life (3 to 60 months) as assessed by the *IBQ-R*, *ECBQ*, and *CBCL*, and the trajectories resulted in two groups: high and low dysregulation. The likelihood of being in the high dysregulation group was predicted by an interaction between prenatal depression and child genotype for *5-HTTLPR* genotype, indicating that children with susceptible genotypes exposed to prenatal depression were more likely to develop dysregulation problems across the first five years of life.

Genes that regulate serotonergic and dopaminergic neurotransmission are of particular interest because of their role in regions of the brain, such as the limbic system and prefrontal cortex, with implications for mood and behaviour regulation (Chang et al., 2018; Ruhé, Mason, & Schene, 2007; Seo, Patrick, & Kennealy, 2008). Genes involved in these biological systems

and mechanisms have previously been associated with depression (Ciobanu et al., 2016; Gutknecht et al., 2015; Karg, Burmeister, Shedden, & Sen, 2011), ADHD (Daly, Hawi, Fitzgerald, & Gill, 1999; Eisenberg et al., 1999), externalizing behaviours such as conduct disorder (CD) and drug and alcohol dependence (Mota et al., 2013a), and anxiety and depression related personality traits (Hünnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007). Based on this evidence, candidate genes considered in the present study were *5-HTTLPR* (Gutknecht et al., 2015; Karg et al., 2011), *HTR1A*, *HTR1B* and *HTR2A* (Ciobanu et al., 2016), *BDNF* (Hünnerkopf et al., 2007), *DRD4* and *DRD2* (Mota et al., 2013a; Mota et al., 2013b), *DAT* (Daly et al., 1999), and *COMT* (Eisenberg et al., 1999).

In addition to the candidate gene studies, advances in genomics have led to large-scale screening of the genome and the development of polygenic risk scores (PRS), which indicate an individual's genetic susceptibility to a given trait or disorder (Chen et al., 2018). Among the most studied are the PRS associated with single disorders such as Depression and ADHD, developed for example by the Psychiatric Genomics Consortium (2013). Since both dysregulation and psychiatric comorbidity affect multiple psychological systems (i.e. affect, behaviour, attention), PRS specifically designed to reflect genetic susceptibility for psychopathology in a more general sense would seem more likely to predict comorbidity. This would include the PRS Cross Disorder from the Psychiatric Genomics Consortium (Cross Disorder Group of the Psychiatric Genomics Consortium, 2013), which reflects overlapping genetic susceptibility of five major psychiatric disorders (i.e. autism spectrum disorder, ADHD, schizophrenia, bipolar disorder, and major depressive disorder). Also pertinent would be the PRS Total Problems from the EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium

(Neumann et al., in preparation), which reflects genetic susceptibility for childhood psychological problems.

The present study

The purpose of this study was to outline a developmental pathway from prenatal stress in interaction with genetic susceptibility to early age dysregulation and preschool comorbid psychopathology. First, we examined the prediction of preschool psychiatric comorbidity from early childhood dysregulation. Disorders of interest in the present investigation of child psychiatric comorbidity were ADHD, CD, ODD, depression, and anxiety, based on their association with dysregulation (i.e. attention, behavior, and emotion problems). Second, we examined whether predictors of early childhood dysregulation, namely maternal prenatal depression and child genetic susceptibility (from individual genes and a PRS), would also predict child psychiatric comorbidity. Third, we examined whether early childhood dysregulation would mediate the association between prenatal depression and child genetic susceptibility to predict child psychiatric comorbidity. These three foci were intended to expand on our previous findings that prenatal depression and child *5-HTTLPR* status interacted to predict 1) dysregulation over the first three years of life (Babineau et al., 2015); and 2) the probability of being highly dysregulated over the first five years of life (Babineau et al., in preparation).

Method

A more detailed description of methodology is available in Babineau et al. (2015), Babineau et al. (in preparation), and O'Donnell et al. (2014).

Participants

The participants were mother-child pairs from the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN), a Canadian community-based birth cohort. The eligibility criteria

were ≥18 years of age at delivery and a singleton term pregnancy (≥ 37 weeks). The present study included $N = 234$ (see Table 1) mother-child dyads for whom we had information on psychiatric disorders when children were 6 years of age.

Measures

Prenatal depression. The *Center for Epidemiologic Studies Depression Scale* (*CES-D*; Radloff, 1977), a 20-item self-report measure of depressive symptomatology validated for pregnancy (e.g. Davis, Glynn, Waffarn, & Sandman, 2011), was completed by the mothers at 24-36 weeks' gestation. The scores were centered around zero.

Child dysregulation. The mothers were administered the *Infant Behavior Questionnaire-Revised* (*IBQ-R*; Gartstein & Rothbart, 2003) when their children were 3 and 6 months of age, the *Early Childhood Behavior Questionnaire* (*ECBQ*; Putnam, Gartstein, & Rothbart, 2006) when their children were 18 and 36 months of age, and the *Child Behavior Checklist* (*CBCL*; Achenbach & Rescorla, 2000) when their children were 48 and 60 months of age (for more details, please refer to Babineau et al., 2015; Babineau et al., in preparation).

Early trajectories of child dysregulation from 3 to 60 months were computed previously (Babineau et al., in preparation). According to this, we found two distinct developmental trajectories of early dysregulation. The children with stable *low dysregulation* accounted for 94% of the sample, whereas the children with increasingly *high dysregulation* accounted for 6% of the sample, with marked differences emerging as of 18 months.

Child psychiatric comorbidity. The *Preschool Age Psychiatric Assessment (PAPA*; Egger, Ascher, & Angold, 1999) is a structured diagnostic interview to assess parent-reported psychiatric disorders in children up to 8 years of age. The *PAPA* is the downward extension of the *Child and Adolescent Psychiatric Assessment* (Angold & Costello, 2000) for children 9 to 18

years of age. The mothers from the MAVAN were interviewed by trained research assistants when their children were 6 years of age. In order to generate child psychiatric diagnoses according to the *DSM-IV* (American Psychiatric Association, 2000), information such as onset date, duration, frequency, and intensity of symptoms up to 3 months prior to the interview was collected. Factors such as social, personal, and academic impairment were also considered. Symptoms occurring up to 3 months prior to the interview are rated to maximize response accuracy, and initial onset dates are elicited when they precede the 3-month primary period. The interviewer served as a guide to determine whether a symptom was present based on the information collected from the mother. Possible outcomes for child comorbid disorders were classified according to "no disorder", "one disorder" (i.e. no more than one psychiatric diagnosis), or "comorbid disorders" (i.e. two or more psychiatric diagnoses). The possible disorders were ADHD, CD, ODD, anxiety (i.e. separation anxiety, social phobia, generalized anxiety, panic attacks, or selective mutism), and depression (i.e. major depression or dysthymia).

Candidate genes. The nine genes that were explored were *5-HTTLPR, HTR1A, HTR1B, HTR2A, BDNF, DRD4, DRD2, DAT1*, and *COMT*. Information on DNA was obtained by buccal swabs using the TaqMan method on the ABI-7000 for Single Nucleotide Polymorphism markers and on the ABI-3100 for repeat polymorphisms. If a genotype was ambiguous, it was discarded, and each 20th marker was re-genotyped to check for error rates (.5%). Each variant was coded for genetic whereby susceptibility = 1, and non-susceptibility = 0 (see Table 1), with the exception of *COMT* which was coded as genetic susceptibility = 1, partial genetic susceptibility = 0.5 versus non-susceptibility = 0 (Nikolova, Ferrell, Manuck, & Hairi, 2011). Please refer to Table 1 for genotype distribution across candidate genes.

PRS. The PRS Cross Disorder and the PRS Total Problems were computed based on previous independent genome-wide association studies (GWAS) meta-analyses. The PRS Cross Disorder from the Psychiatric Genomics Consortium includes shared genetic effects for five major psychiatric disorders in 33,332 adult cases compared to 27,888 adult controls, and is described in detail elsewhere (Cross Disorder Group of the Psychiatric Genomics Consortium, 2013).

Given that the PRS Cross Disorder is based on adult cases only, we also used the PRS Total Problems. The PRS Total Problems is derived from a GWAS meta-analysis by the EAGLE consortium for total childhood psychological problems and includes ADHD, anxiety, depression, and insomnia among 29,446 children between the ages of 5 to 16 years from 16 population-based cohorts (Neumann et al., in preparation). The psychiatric problems were assessed by parent-rated questionnaires, including the *CBCL* (Achenbach & Rescola, 2000), Strengths and Difficulties Questionnaire (*SDQ*; Goodman, 1997), Multidimensional Peer Nomination Inventory (Pulkkinen, Kaprio, Rose, & Peers, 1999), *Rutter Children*' *Behaviour* Questionnaire (Rutter, 1967), A-TAC (Hansson et al., 2005), and items derived from a health examination survey (Wells, 1980).

Genome wide genotyping, imputation strategies, and quality control procedures for the MAVAN are described elsewhere (see Silveira et al., 2017). The population structure of the MAVAN cohort was evaluated using a principle component analysis of all autosomal SNPs that passed the quality control (Price et al., 2006; Silveira et al., 2017). The first three principle components derived were used as covariates in further analyses including PRS, as these were proven to be the most informative of the population structure in the MAVAN cohort (Silveira et al., 2017).

Covariates. Further covariates included maternal postnatal depression, education, and age at delivery, and child biological sex. Maternal postnatal depression was assessed with the *CES-D* at 6, 12, 24, 36, 48, 60 and 72 months postnatal. All of the other information was obtained at birth by demographic questionnaire.

In order to control for maternal postnatal depression across time, we computed trajectories of maternal postnatal depression from 6 to 72 months postpartum (N = 582 mothers) with the *Extended Mixed Models using Latent Classes and Latent Processes (LCMM*; Proust-Lima, Philipps, & Liquet, 2017) package in R. Postnatal depression at seven time points (6, 12, 24, 36, 48, 60, and 72 months) was entered into the model. We compared model fit across 2, 3, or 4 groups based on lowest BIC (Schwarz, 1978).

The trajectory analysis identified a 3-class model as the best fit (BIC: 20380.21; see Figure 1). The mothers in the Class 1 (82.36%) had persistently low depressive symptoms that were below the clinical threshold for depression at all time points and were labelled the "No Depression" group. The mothers in Class 2 (8.82%) had persistently high depressive symptoms that were chronically above clinical threshold and labelled the "Ongoing Depression" group. The mothers in Class 3 (8.82%) had a spike of higher depressive symptoms at 6 months postpartum followed by decreased yet persistent depressive symptoms above clinical threshold from 12 to 72 months postpartum and were labelled the "Postpartum Depression" group. Trajectory groups were entered as categorical (i.e. dummy coded) covariates throughout analyses.

Statistical analyses

Question 1: Does early childhood dysregulation predict psychiatric comorbidity? We conducted a series of descriptive analyses in R to determine how the dysregulation trajectories (i.e. low vs. high dysregulation from 3 months to 5 years of age) were associated with child

psychiatric disorders (i.e. ADHD, CD, ODD, anxiety, and depression) and comorbidity (two or more diagnoses vs. a single diagnosis or no diagnosis) at 6 years of age. Logistic regressions were used to compare the likelihood of psychiatric disorders in the low and high dysregulation groups.

Question 2: Does prenatal depression interact with child genetic susceptibility to predict psychiatric comorbidity? To determine whether an interaction between prenatal depression and child candidate genes predict psychiatric comorbidity, we used the *Latent Environmental and Genetic InTeraction* (*LEGIT)* package in R (Jolicoeur-Martineau et al., 2018; Jolicoeur-Martineau et al., 2019; for details see Babineau et al., in preparation). Gene selection for *LEGIT* models was conducted with the *Rank-One Natural Evolution Strategy* (*R1NES*; Sun, Gomez, Schaul, & Schmidhuber, 2011) function from the *LEGIT* package. The *R1NES* search is an additional step outside of the standard *LEGIT* method and was conducted in interest of retaining statistical power within our small sample size.

To determine whether an interaction between prenatal depression and PRS Cross Disorder or PRS Total Problems would predict comorbidity, we conducted regression analyses with the *General Linear Model* (*GLM*) function in R. PRS were derived at multiple p-value thresholds (.0001, .001, .01, .05, .1, .2, .3, .4, .5). The best threshold to use in the main analyses was chosen based on the largest R^2 change between the baseline model (multiple linear regression model including only the covariates and principle components) and the model that additionally included the PRS at a given p-value threshold. Separate models were run for prenatal depression x PRS Cross Disorder and prenatal depression x PRS Total Problems. The covariates included maternal postnatal depression trajectories, maternal education, maternal age, child biological sex, and the first three principal components from the GWAS derived in MAVAN.

Question 3: Does early childhood dysregulation mediate an association between prenatal depression and child genetic susceptibility to predict psychiatric comorbidity? We conducted moderated mediation analyses with the *Average Casual Mediation Effects* (*ACME)* package in R (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) and the *Latent Variable Analysis (LAVAAN)* package in R (Yves, 2012). The moderated mediation analyses were used to determine whether a prenatal depression by child genes interaction effect on psychiatric comorbidity would be mediated by early dysregulation. A total of four moderated mediation analyses were conducted. The first two analyses examined whether prenatal depression would be moderated by either flexibly weighted or equally weighted candidate genes. First, the candidate genes were flexibly weighted by entering each gene with a weighted term (i.e. beta coefficient) based on their fit from the *LEGIT* models performed in Babineau et al. (in preparation) for dysregulation. Second, the candidate genes were equally weighted by averaging the genes (i.e. equivalent to assuming equal weights, which is more conservative and less likely to be over-fitting). The candidate genes were chosen based on previously established associations to dysregulation (Babineau et al., in preparation), namely, *5-HTTLPR, HTR1A, HTR2A, COMT,* and *DAT*. In the third and fourth analyses, the PRS Cross Disorder and PRS Total Problems, respectively, were used as the genetic component to test for moderated mediation effects.

Results

The prediction of co-morbidity from the dysregulation trajectory

The children with high dysregulation as compared to the children with low dysregulation were significantly more likely to have ADHD, CD, ODD, anxiety, and depression, and significantly more likely to have a psychiatric diagnosis (i.e. single diagnosis compared to no diagnosis) or comorbid disorders (i.e. two or more diagnoses compared to none and single

diagnosis; see Table 2 and Figure 2). More specifically, the children with high dysregulation were *nine times* more likely than the children with low dysregulation of having comorbid disorders as compared to one disorder or no disorder combined, four times more likely of having comorbid disorders compared to the no disorder only group, and three times more likely of having one disorder compared to no disorder (see Table 2). Among the children with low dysregulation, 22.38% had one disorder only and 11.43% had comorbid disorders. Among the children with high dysregulation, 25% had one disorder only and 54.17% had comorbid disorders (see Figure 2).

Prenatal depression x candidate genes to predict comorbidity

The best *LEGIT* solution retained only *DRD4* based on the *R1NES* variable selection procedure for further analysis. However, neither main effects for prenatal depression or child *DRD4* genotype nor prenatal depression-by-child *DRD4* genotype interaction effects were significant.

Prenatal depression x PRS to predict comorbidity

The best threshold for the PRS Cross Disorder was $p < .3$. However, neither significant main effects nor interaction effects emerged for prenatal depression and PRS Cross Disorder on comorbidity. Among the covariates, ongoing postnatal maternal depression had a significant effect on comorbidity (ß = 1.35, $p < .05$; $R^2 = .46$).

The best threshold for the PRS Total Problems was $p < .2$. Similar to the findings with the PRS Cross Disorder, no significant main effects or interaction effects emerged for prenatal depression and PRS Total Problems on comorbidity. Ongoing postnatal maternal depression also had a significant effect on comorbidity status (ß = 1.33, $p < .05$; $R^2 = .46$).

Moderated mediation to predict comorbidity

Although no significant interaction effect were found between prenatal depression and child genes in the prediction of comorbidity, there remained the possibility of testing for an indirect interaction effect between prenatal depression and child genes as mediated by early dysregulation to predict comorbidity (see Hayes, 2013), but the results were not significant (see Appendix for details).

Discussion

The focus of this study was the study of the trajectory of dysregulation with a developmental model of prenatal and genetic influences to late preschool psychopathology and comorbidity. This study is strengthened by repeated and cohesive measures of dysregulation across the first 6 years of life, and the administration of a semi-structured diagnostic interview. Additionally, it is rooted in a gene by environment model with data from a longitudinal prospective birth cohort. We found strong support that early dysregulation spanning infancy and early childhood predicted childhood psychiatric comorbidity. Our finding is consistent with studies with older children suggesting that dysregulation in school age and adolescence predicts comorbid psychopathology up to adulthood (Althoff et al., 2010; Holtmann et al., 2011; Meyer et al., 2009). In this study, the children with high levels of early dysregulation were more likely to have ADHD, CD, ODD, anxiety, and depression, and as much as nine times more likely to have two or more disorders (i.e. psychiatric comorbidity) than the children with low levels of dysregulation.

In a similar study to ours with children at 3 years of age who did or did not meet criteria for a dysregulation profile as defined and measured by the *CBCL-DP*, Kim et al. (2012) found that the children in the dysregulation group had significantly higher concurrent symptoms of ADHD, ODD, anxiety and depression as assessed by the *PAPA* (the CD scale was not used in

their study) as compared to the children who were not in the dysregulation group. The findings from that study and our study together support an understanding of childhood dysregulation as a combination of attention, behaviour and emotion problems that predict a greater likelihood of developing comorbid psychiatric disorders. Although the children in the low dysregulation group also develop a range of psychiatric disorders, those in the high dysregulation group are much more likely to go on to develop comorbid psychiatric disorders.

Contrary to expectations, we did not find the prenatal or genetic antecedents of dysregulation (Babineau et al., 2015; Babineau et al., in preparation) to also predicted psychiatric comorbidity. The association of individual genes with psychiatric comorbidity might have been limited by the complexity and heterogeneity of comorbidity as an outcome. Although genetic risk scores associated with cross disorder and total problems were included specifically to increase our ability to detect a gene or a gene-by-environment effect, these gene scores did not yield any further associations. We interpret our findings as potential evidence for the prenatal programming of postnatal plasticity (see Figure 3). For example, prenatal depression and genes did not directly influence the outcome of child psychiatric comorbidity, although they did predict the development of high dysregulation which in turn predicted psychiatric comorbidity. According to the theory of prenatal programming of postnatal plasticity, exposure to prenatal stress leads to the development of greater behavioural and physiological reactivity which are phenotypes that confer increased susceptibility or developmental plasticity to either favorable or unfavorable postnatal environments, culminating in positive or negative developmental outcomes respectively (Hartman & Belsky, 2018; Pluess & Belsky, 2011). In this case, the development of high dysregulation may have made children more susceptible to the effect of maternal postnatal depression, leading to the development of comorbid psychiatric disorders.

Conversely, the absence of maternal postnatal depression may explain why not all of the children with susceptible genotypes exposed to prenatal depression went on to develop comorbid psychiatric disorders. More specifically, these children may have been susceptible both for better and for worse, allowing for two separate trajectories. Children with susceptible genotypes exposed to prenatal depression might have developed a high dysregulation phenotype, which may have rendered them more susceptible to (1) maternal postnatal depression, leading to the development of psychiatric comorbidity, or to (2) the absence of maternal postnatal depression, leading to the absence of psychiatric comorbidity. Based on this interpretation, a direct path from prenatal depression and susceptible genotypes in the prediction of psychiatric outcomes would be obfuscated by the development of susceptible phenotypes to the postnatal environment. For example, given that only 28.5% of women met criteria for depression, as expected from a community based sample, it is possible that there was insufficient variability of mood symptoms in order to detect an interaction with genes to predict comorbidity.

Limitations

A primary limitation of our study is our small sample size, and the constraints that this imposed on our ability to conduct and interpret analytic tests for the high dysregulation group. Although we would have liked to provide more precise statistical analyses to support our interpretation of the prenatal programming of postnatal plasticity, our sample size was too small to do so (i.e. not enough cases in the high dysregulation group for a statistically meaningful analysis). A future direction would be the confirmations of this theory within a larger sample size. As dysregulation is present in 4-11% of children in community-based samples, a larger sample would confer a larger group of children with dysregulation problems, leading to greater analytic power and a more precise investigation of pre- and postnatal factors. Our high

dysregulation group included only 27 participants, and, therefore, we could not conduct these additional analyses to further interpret the phenotypic susceptibility in the presence or absence of postnatal depression.

Our reliance on maternal report of child dysregulation is also a limitation, as maternal mood may have influenced response style across the questionnaires (Atella, DiPietro, Smith, & James-Roberts, 2003). However, the questionnaires used allowed for a longer observation period, multiple time points with repeated measures, and a reduction of bias due to questions that probe the mother to respond on the frequency of recent occurring behaviours (Gartstein & Rothbart, 2003).

Given that the Cross Disorder GWAS was found to be specifically predictive of adult-onset disorders such as bipolar disorder, major depressive disorder, and schizophrenia, and less so predictive of autism spectrum disorder and ADHD (Cross Disorder Group of the Psychiatric Genomics Consortium, 2013), our likelihood of finding significant results in the prediction of childhood psychiatric comorbidity at 6 years of age may have been reduced. Nevertheless, we were able to use the PRS Total Problems, which was specially focused on child psychiatric problems, such as those reported on *CBCL* (Achenbach & Rescola, 2000) and *SDQ* (Goodman, 1997).

Implications

Our longitudinal study of early childhood dysregulation from age 3 to 60 months converges with previous longitudinal studies of dysregulation from age 3 years to adulthood (e.g. Althoff et al., 2010; Holtmann et al., 2011). In addition to converging on a consistent picture that dysregulation is associated with the development of psychiatric comorbidity, our findings

highlight that the development of early childhood dysregulation predicts of comorbid psychiatric disorders including ADHD, CD, ODD, anxiety and depression.

These findings can be interpreted within the context of entry to elementary school, whereby the higher demands placed on the child (e.g. social, emotional, behavioural demands) reveal the extent to which dysregulation problems can increase risk for impairment. For example, difficulty regulating mood could lead to depression or anxiety, difficulty regulating behaviour could lead to disruptive behaviour, and difficulty regulation cognition could lead to attentional problems (Carballo et al., 2014). However, the extent to which the child is affected by the increase in environmental demands likely depends on a range of interacting factors from the pre- to the postnatal period, including biological factors such as genetics. Understanding prenatal and genetic factors involved can further inform the early identification of children at risk for the development of psychopathology.

Within a differential susceptibility framework, a treatment model to prevent the development of psychiatric disorders and comorbidity can begin as early as dysregulation problems first emerge. Children with dysregulation problems who were initially exposed to prenatal depression and continue to be exposed to maternal depression in the postnatal period may be at greatest risk for developing psychiatric disorders and comorbidity, and may also have the greatest likelihood of benefiting from treatment due to their biological susceptibility both for better and for worse to environmental factors. For these children, once dysregulation has been detected in combination with a history of prenatal depression, and in the presence of postnatal depression, an effective intervention could include addressing both the child's symptoms of dysregulation and the mother's current symptoms of depression. By reducing or eliminating the mother's postnatal depression, the child might be especially likely to benefit from the

intervention (i.e. positive environmental exposure). As such, screening for dysregulation in early childhood may be warranted as a starting point to prevent the development of disorders such as ADHD, CD, ODD, anxiety and depression, as well as comorbidity of these disorders, especially among children exposed to pre- and postnatal depression. Given that stability in diagnoses is greater in late (e.g. 8-10 years old) as compared to early childhood (e.g. 4-6 years old; Wichstrøm, Belsky & Steinbekk, 2017), continuous measures of early dysregulation that retain stability over time (Carranza, González-Salinas, & Ato 2013; Gartstein, Slobodskaya, Putnam, & Kinsht, 2009; Kosmi et al., 2006; Putnam, Rothbart, & Gartstein, 2008) are likely to be more informative for early treatment and prevention than diagnostic categories, a future direction to be verified by clinical research.

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Tables

Table 1 Descriptive Statistics of MAVAN Mother and Child (N = 234)

	M (*SD*)	%
Mothers		
Age at delivery (years)	30.46 (4.78)	
In a partnership at delivery		97.84
Prenatal *CES-D* score	12.16 (9.85)	
(*CES-D*≥16)		27.36
Education		
≤High School		10.47
Some College/Trade		8.9
College/Trade Graduate		36.65
≥University Graduate		43.98
Annual household income (CAD)		
<15 000		6
15 000 to <30 000		14
30 000 to <50 000		20
50 000 to <80 000		25
≥80 000		35
Children		
Sex – Female		48
Genetic susceptibility		
5-HTTLPR		73.86
(SS, SL_G, L_GL_G, L_AL_G vs. L_AL_A)		
HTR1A		28.7
(GG vs. GC, CC)		
HTR1B		46.12
(CC, CG vs. GG)		
HTR2A		77.57
(CC, CT vs. TT)		
BDNF		68.5
(AA, AG vs. GG)		
DRD4		36.15
(presence vs. absence of 7 or 8)		
DRD2		30.37
(AA, AG vs. GG)		
DAT1		50.61
(10-10 vs. 9-9, 9-10)		
COMT		73.5*
(AA vs. GA vs. GG)		

Notes. Education and income categories as per Kramer et al. (2009). In analyses, the categories ≤High School, Some College/Trade, and College/Trade Graduate are combined as the "Low/Mid Education" group, and compared to ≥University Graduate which is the "High Education" group. *49% with partial genetic risk and 24.5% with full genetic risk, for a total of 73.5% genetic risk.

Table 2 Disorder Prevalence and Odds Ratios Among Children with Low Dysregulation versus High Dysregulation ($N = 234$)

	Low (n=210)	High (n=24)	OR (95% CI)
	(n)%	(n)%	
ADHD	(16) 7.62	(11) 45.83	10.26 (3.96-26.56)
CD	(11) 5.24	(4) 16.67	3.62 (1.05-12.42)
ODD	(16) 7.62	(10) 41.67	8.66 (3.32-22.58)
Anxiety	(49) 23.33	(11) 45.83	2.78 (1.17- 6.6)
Depression	(13) 6.19	(6) 25	5.05 (1.71- 14.89)
Comorbidity	(24) 11.43	(13) 57.17	9.16 (3.69-22.72)
Comorbidity vs One Disorder[a]	(24) 33.8	(13) 68.42	4.24 (1.43-12.56)
One Disorder vs No Disorder[b]	(47) 25.27	(6) 54.54	3.55 (1.04-12.170)

OR – Odds Ratio

[a]Children with no disorders removed

[b]Children with comorbidity removed

Figures

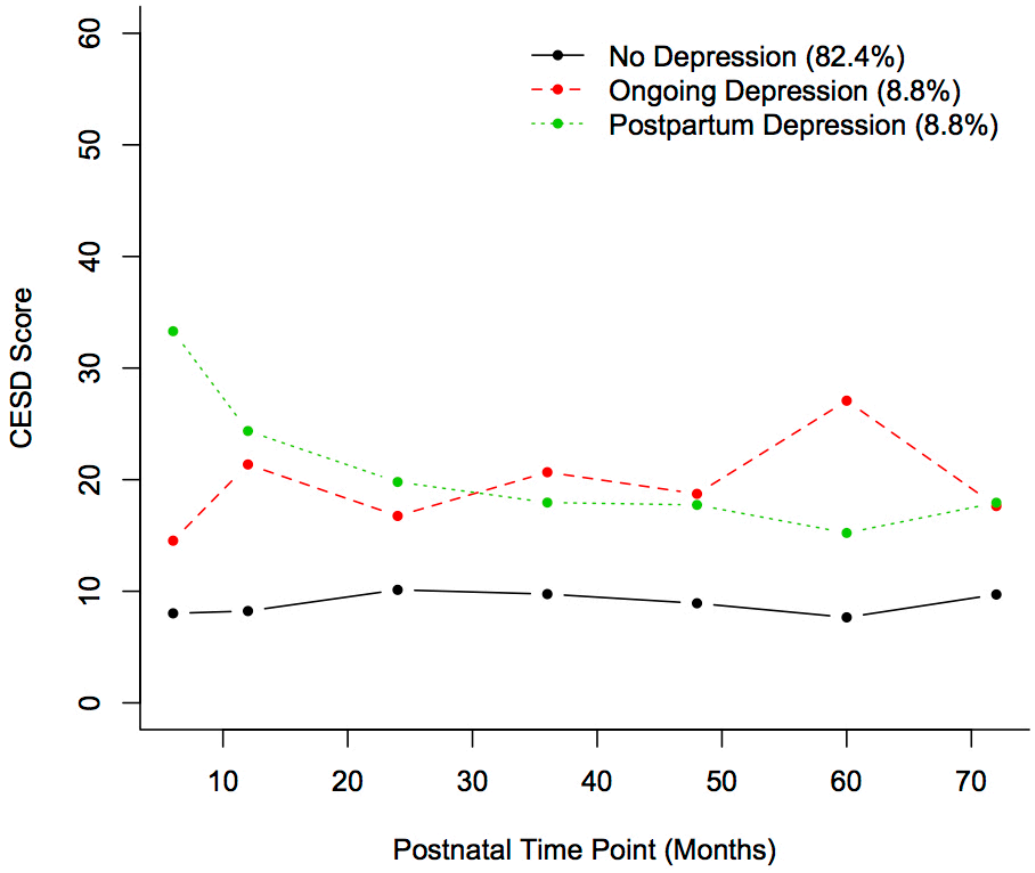

Figure 1. The trajectory of postnatal depression from 6 to 72 months.

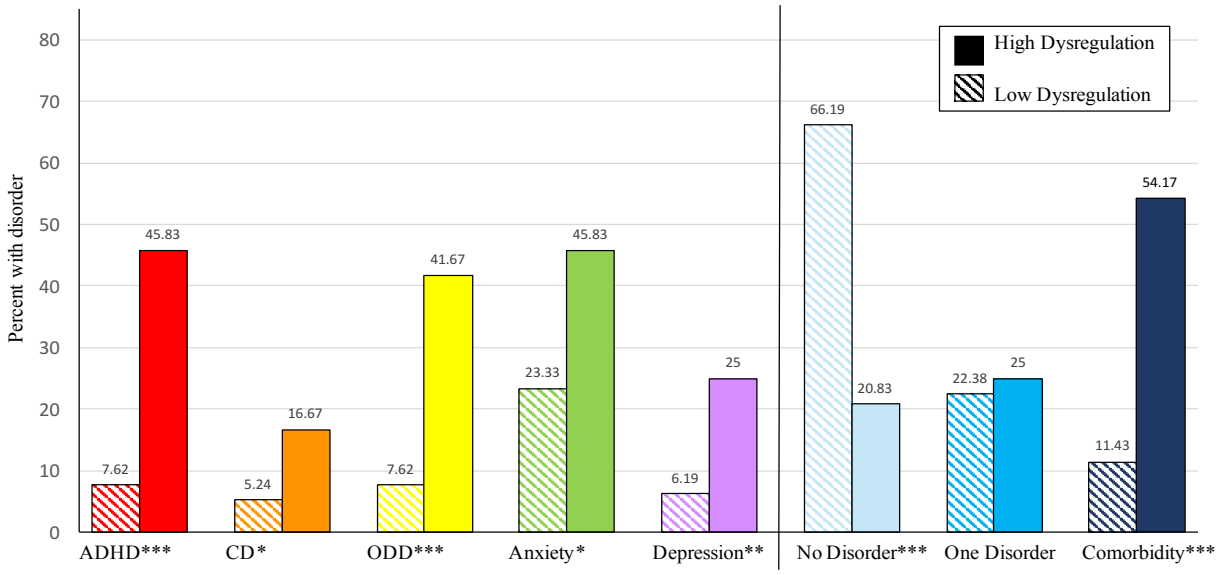

Figure 2. The prevalence of disorders according to low dysregulation and high dysregulation group. Children with high dysregulation were 10.26 times more likely to have attention deficit hyperactivity disorder (ADHD), 3.62 times more likely to have conduct disorder (CD), 8.66 times more likely to have oppositional defiant disorder (ODD), 2.78 times more likely to have an anxiety disorder, 5.05 times more likely to have a depressive disorder, and 9.16 times more likely to have comorbid disorders.
$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ for χ^2 Test

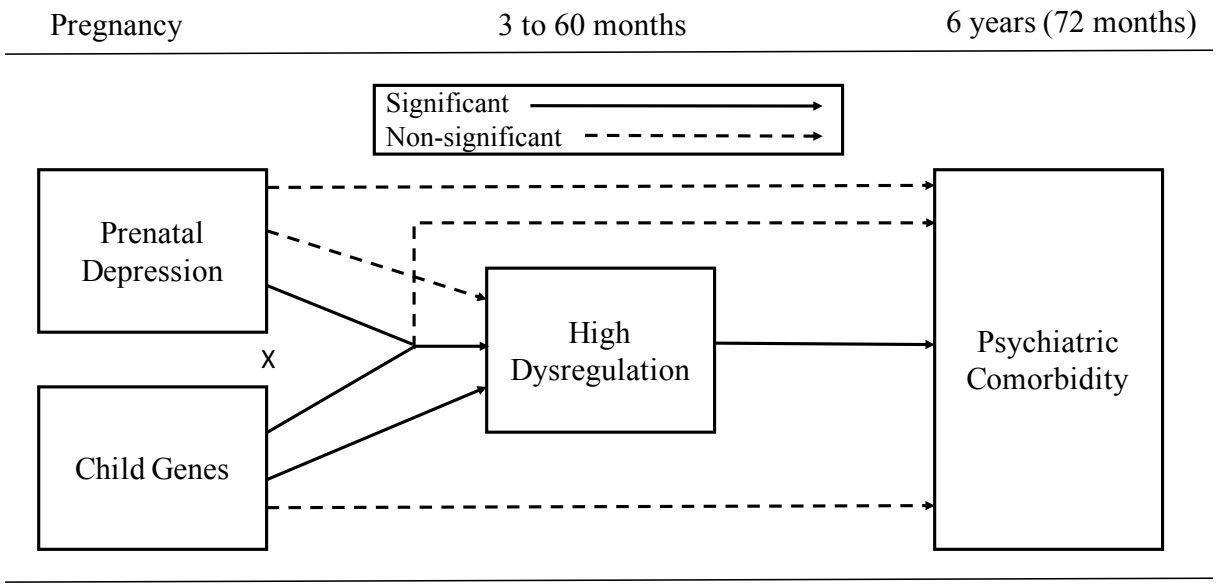

Figure 3. Summary model for the developmental pathway of dysregulation to comorbidity.

Appendix

We conducted moderated mediation analyses with the *Average Casual Mediation Effects* (*ACME)* package in R (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) and the *Latent Variable Analysis (LAVAAN)* package in R (Yves, 2012). Due to methodological constraints with *ACME* where we could not enter prenatal depression as a continuous term and could only use precise *CES-D* values, we explored models whereby *CES-D* =16 and *CES-D* =30. In these *ACME* models, genes were entered at low, medium and high levels for comparison, and explored according to 1) flexibly weighted terms, 2) equally weighted terms, 3) the PRS Cross Disorder and 4) the PRS Total Problems. Due to methodological constraints with the *LAVAAN* whereby we could not enter prenatal depression as a continuous term but rather as a dichotomous term, we first explored models whereby *CES-D* <12, *CES-D* ≥12, *CES-D* <50, and *CES-D* ≥50. In these *LAVAAN* models, genes were entered according to their assigned genetic risk (i.e. dichotomously) for candidate genes, and as the PRS Cross Disorder and the PRS Total Problems. The results were insignificant across all analyses, whereby a moderated mediation was not supported. For illustrative purposes, Figure S1 is a representation of a *LAVAAN* moderation mediation model whereby *CES-D* ≥12 in interaction with Total Problems PRS, as moderated by high dysregulation to predict psychiatric comorbidity.

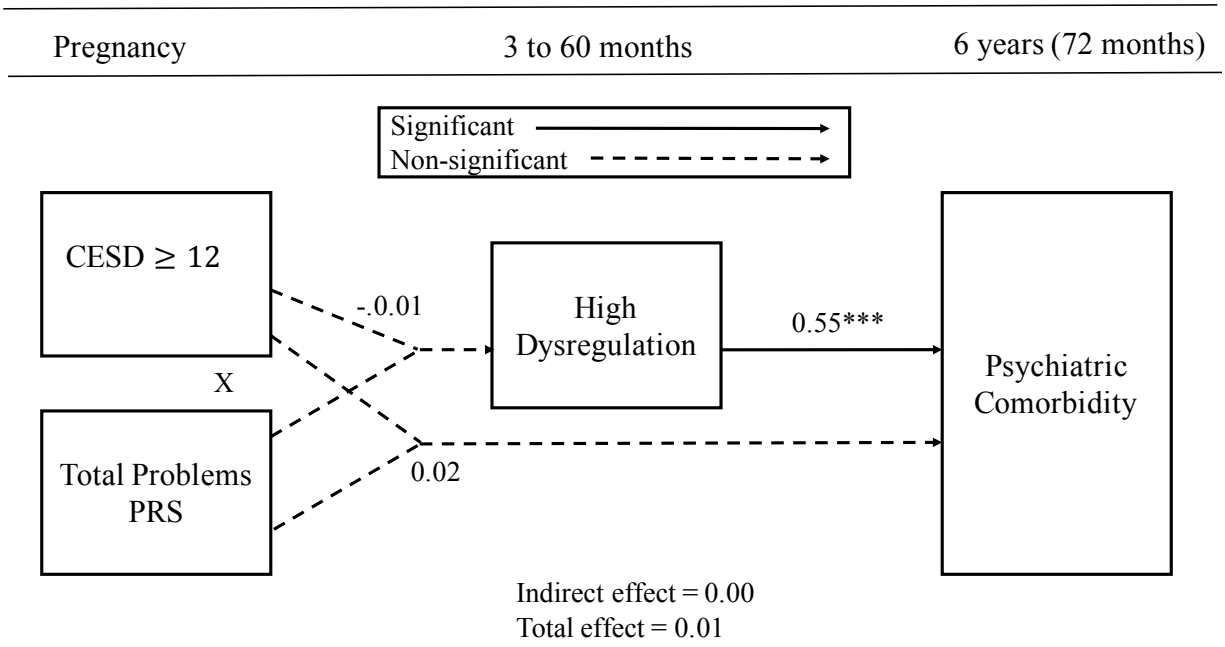

Figure S1. Moderated mediation in the prediction of psychiatric comorbidity.
***$p < .001$

General Discussion

Across studies 1, 2 and 3, our overall findings confirmed that the development of early childhood dysregulation from 3 to 60 months of age is predicted by an interaction between maternal prenatal depression and child genetic susceptibility. Our findings also confirmed that early childhood dysregulation is a predictor of comorbid psychiatric disorders at 6 years of age, including attention deficit hyperactivity disorder (ADHD), conduct disorder (CD), oppositional defiant disorder (ODD), anxiety and depression, and thus a phenotype for the development of severe psychopathology.

In Study 1, we found that early dysregulation at 3, 6, 18 and 36 months of age was predicted by an interaction between maternal prenatal depression and child genetic susceptibility for *5-HTTLPR*. We found a pattern of differential susceptibility, whereby children with susceptible genotypes exposed to greater prenatal depression were more likely to develop greater dysregulation problems. However, when exposed to low levels of or the absence of prenatal depression, children with susceptible genotypes were most likely to show the lowest level of dysregulation problems, or, good regulation abilities.

In Study 2, we outlined the development of dysregulation from 3 to 60 months of age (i.e. 3, 6, 18, 36, 48 and 60 months), which resulted in a low dysregulation trajectory and a high dysregulation trajectory. The trajectories were distinct from one another as of 18 months of age and onward. The high dysregulation trajectory in Study 2 was predicted by an interaction between maternal prenatal depression and child genetic susceptibility for *5-HTTLPR*. Further, males and children whose mothers were from low to mid education backgrounds were twice as likely to have high dysregulation problems.

In Study 3, we found that the high dysregulation trajectory from Study 2 was a predictor of comorbid psychiatric disorders at 6 years of age. However, psychiatric comorbidity itself was not predicted by an interaction between maternal prenatal depression and child genetic susceptibility. We also found that ongoing maternal depression in the postnatal period, which was a covariate of the study, further predicted psychiatric comorbidity. The lack of support for a gene by environment effect in the development of psychiatric comorbidity might be a consequence of attempting to predict such a large endophenotype. Although the predication of individual disorders is common, attempting to predict multiple disorders combined might not be an effective approach, whereby biological correlates become more complex to identify. Further, the lack of variability in our predictor of prenatal depression may also have led to this negative finding, given that the study relied on a community based sample where only 28.5% of women met criteria for depression.

Implications

Our primary research goal was to bridge a gap in the literature on the early developmental trajectory of dysregulation. Whereas previous longitudinal research includes support for development of dysregulation from school age to adulthood, we have identified dysregulation trajectories throughout infancy to school age. Further, this information is relevant to both the developmental trajectories of dysregulation and their psychiatric outcomes, and to the underlying biological mechanisms and environmental factors.

We provide evidence that *5-HTTLPR* interacts with prenatal depression to predict the development of early childhood dysregulation, however, genes and prenatal depression do not appear to interact in the prediction of childhood comorbid psychopathology. We also provide evidence that dysregulation predicts psychiatric comorbidity. As such, we propose that genetic

susceptibility and exposure to prenatal depression are predictors that scaffold towards comorbid psychiatric disorders. Within this model, dysregulation is not necessarily separate from but rather a developmental precursor of comorbid psychopathology. As such, we agree with Althoff et al. (2010), Holtmann et al. (2011), and Meyer et al. (2009) who suggest that dysregulation is a marker of severity or comorbidity of psychopathology. If high dysregulation, which can be detected as of 18 months of age, is a precursor of psychiatric comorbidity at 6 years of age and onward, this finding would strongly support prevention and early intervention of comorbid disorders. The need for prevention and early intervention is further supported by our finding that the children in the high dysregulation group were nine times more likely to develop comorbid disorders.

Similar to the RDoC framework, our research integrates many levels of information (e.g. genes, prenatal adversity, behavioural outcomes) to explore a basic dimension (i.e. dysregulation) along a continuum of functioning (e.g. low to high) with the goal of establishing a better understanding of mental illness in terms of varying degrees of functioning in psychological and biological systems (Insel et al., 2010). Within this thesis, we first investigated developmental trajectories of dysregulation from 3 months to 5 years of age according to degree of severity along a continuum of dysregulation. We then created subgroups of dysregulation according to low and high dysregulation problems and aligned these groups with the likelihood of developing a range of psychiatric disorders and comorbidity. As such, we provide information to support clinical research efforts in the development of measures to screen for and detect dysregulation problems throughout early childhood. The development of clinical measures that identify early dysregulation problems could lead to prevention and early intervention for children at risk for the development of comorbid psychiatric disorders. For example, future clinical

research may aim to clinically validate the *CBCL-DP* for use in clinical settings, given that this measure is only used in research settings, and may have the potential as a screening measure to identify children with early dysregulation at risk for comorbidity and in need for prevention and intervention services.

The clinical aims of this research were to inform preventative healthcare and early intervention to support the mental health of mothers and their children. The findings from this dissertation provide information on the pathophysiology of dysregulation, which as previously mentioned, is associated with severe and lifelong psychiatric comorbidity. With precise information on risk factors for the development of dysregulation and comorbid psychiatric disorders, and on the early developmental course of dysregulation from the prenatal period throughout the first six years of life, concrete advances in prevention and intervention are possible. For example, we were able to pinpoint associated factors such as the higher rate of dysregulation among males and among children whose mother is from a low to mid education background, and higher rates of comorbidity in the presence of ongoing maternal postnatal depression, which can inform the development of future psychological and psychiatric assessment tools and interventions.

From a nosological classification perspective, there is a possibility that the *high dysregulation* profile identified in this research is measuring the same phenotype as disruptive mood dysregulation disorder (DMDD). Further, high dysregulation in early childhood, or DMDD, may not be separate from, but rather the same as, psychiatric comorbidity. This is one way of interpreting the finding that children with high dysregulation were up to nine times more likely of developing comorbid psychiatric disorders. Other researchers who have studied the development dysregulation from childhood to adulthood have also postulated that dysregulation

might be an early measure of comorbidity (Althoff et al., 2010). Given that diagnoses from the *PAPA* relied on the *DSM-IV*, and that DMDD is a new disorder in the *DSM-V*, a variable for DMDD could not be constructed or compared to the high dysregulation profile in Study 3. DMDD has an 8.3% prevalence rate in community based samples and a 60% comorbidity rate with disorders such as depression, anxiety, ADHD, and ODD (Dougherty et al., 2015). In comparison, the high dysregulation profile had 6% prevalence rate (Study 2), and a 54.17% comorbidity rate with disorders such as depression, anxiety, ADHD, ODD, and CD (Study 3). Whether or not DMDD and the dysregulation profile in this research are the same phenomena has yet to be determined. However, comparisons of scores on the *CBCL-DP* with clinical ratings of DMDD indicates that DMDD is especially associated with outcomes such as anxiety and depression, whereas the *CBCL-DP* is associated with a greater range of disorders including anxiety, depression, and disruptive behavior disorders, as well as with overall greater general impairment (Guth & Althoff, 2015).

The findings that being biologically male, or having a mother with low to mid education background, increases risk for developing early dysregulation when exposed to either pre- or postnatal depression are similar to findings by Montroy et al. (2016) who demonstrated that children who were biologically female or whose mother was from a high education background exhibited more rapid gains in their development of regulation abilities. Findings such as these can be interpreted within the context that dysregulation is highly associated with disorders that are more common in boys than in girls prior to puberty, including ADHD (Arnett, Pennington, Willcutt, DeFries, & Olson, 2015) and ODD (Demmer, Hooley, Sheen, McGillivray, & Lum, 2017). Further, sex differences in the placenta have been demonstrated to produce sex-specific signals to the brain, which lead to a male-specific stress phenotype that may influence risk for

psychiatric disorders (Bale, 2016). As for maternal education background, throughout infancy to adolescence, maternal education is highly associated to family socioeconomic status and resources, including access to material resources, social resources, recreational resources, and learning materials (Bradley & Corwyn, 2002; Mercy & Steelman, 1982).

This research is also congruent with findings that maternal pre- and postnatal stress play a role in the modification of gene expression, which can lead to changes in brain development, and potentially result in altered cognitive, behavioral and affective regulation (Meaney et al., 2007; Weaver et al., 2004). In a series of three studies, this dissertation demonstrates that maternal prenatal depression interacts with or potentially modifies the expression of child *5-HTTLPR*, leading to the outcome of dysregulation, whereby dysregulation in turn predicts psychiatric comorbidity over time. Although changes in gene expression per se were not examined, the identification of specific genotypes susceptible to stress is consistent with molecular mechanisms that underlie neurodevelopment.

Limitations & future directions

A primary limitation of this work is the reliance on maternal report to measure both child dysregulation and maternal affective stress. This limitation is twofold, in that 1) mothers reported on their own symptoms of depression as well as on their child's symptoms of dysregulation, potentially leading to shared method variance, and 2) the influence of paternal affect was not considered. However, the effect of shared method variance should be attenuated by two factors. The first is the inclusion of multiple time points of dysregulation (i.e. 3, 6, 18, 36, 48, and 60 months of age) over time. The second is the administration of the *PAPA*, a semi-structured diagnostic interview, which was administered by research assistants who were well trained to clinically assess maternal reports of symptom onset, frequency and duration, within various

settings such as home, school and elsewhere. This method allowed the research assistants to make final decisions regarding coding of child psychopathology. Although the mother was the primary informant, the *PAPA* interview went beyond paper and pencil report measures. Nevertheless, father affect was not considered.

A second limitation of this research was small sample size. For example, certain gene by environment interactions may not have been detected due to small sample size, which in part was due to loss of DNA from unsuccessful genotyping, as well as lack of funding to obtain or fully process all genetic information. Nevertheless, a recently developed statistical method (*LEGIT*) was used that enables the simultaneous probing of interactions between multiple genes and environmental factors even within small samples (Jolicoeur-Martineau et al., 2018; Jolicoeur-Martineau et al., 2019). Additionally, the developmental trajectories of dysregulation that appear to diverge only as of 18 months of age may also be a result of small sample size. Although some children begin to show a dysregulation profile even as of 3 months of age, this is not apparent on average in the trajectories. The use of a small community based sample to investigate the early development of dysregulation may have limited or obscured the actual average point in time at which some children begin to develop a dysregulation profile. To address this methodical constraint and theoretical shortcoming, future research could investigate, for example, the construction and predictive power of trajectories of dysregulation as of 3 and 6 months of age only (i.e. *IBQ-R*) and perhaps up to 18 and 36 months of age only (i.e. *ECBQ*), and whether these early trajectories begin to diverge earlier in time (i.e. before 18 months) to predict dysregulation at 5 years of age and comorbid psychiatric disorders at 6 years of age (i.e. trajectories with the the removal of the 48 and 60 months time points derived from the *CBCL-DP* to predict outcomes on the *CBCL-DP* and *PAPA*).

A third limitation of this work is that prenatal depression in interaction with child genotype in the prediction of early dysregulation may be the result of a gene by environment correlation (rGE). For example, if the same genetic susceptibility for dysregulation also increased the likelihood of maternal depression, then what would seem to be an environmental exposure (prenatal stress) might actually be another manifestation of the genetic susceptibility inherited from the mother. However, across all three studies, prenatal depression was not associated with child genotypes, and maternal genotypes were not significant covariates in our models. Although prenatal stress can be considered as confounded by genetic transmission from mother to child, evidence from studies on prenatal stress as the result of natural disasters provides further evidence for findings beyond rGE. For example, infants exposed *in utero* to natural disasters develop poorer intellectual and language functioning at two years of age, which provides evidence for the influence of prenatal stress beyond rGE (see King & Laplante, 2005). Natural disaster studies provide the opportunity for an experimental design which otherwise would be unethical in human studies. More specially, women who are pregnant and exposed to the natural disaster can be compared to women who are pregnant and not exposed to the natural disaster. Participants can then be matched on all variables that influence child outcomes, such as familial genetic susceptibility for prenatal depression, socioeconomic status, maternal age at delivery, and social support, to determine the specific influence of prenatal stress on infant and child outcomes.

A final limitation of this work is that we did not account for prenatal exposure to maternal psychotropic medication. For example, 15% of mothers in our sample reported use of psychotropic medication either throughout or at some point during pregnancy. Although this information was gathered, we did not accounted for in our analyses. Potential influences include

differences in maternal depression symptoms, and differences in how child genetic status may have interacted with maternal adversity.

A future direction from a methodological and ecological point of view would be to explore environmental mediating factors beyond maternal mood. For example, these could include child attachment style, maternal care and sensitivity, and the amount of time the child spends in a daycare setting, with a focus on positive environmental factors as opposed to "negative" or aversive factors only. Protective factors that might mediate the development of dysregulation include a secure attachment style, and maternal care that is sensitive and attuned to her child's needs, both of which facilitate the infant or child's ability to cope with stress (Derryberry & Rothbart, 1984; Feldman & Eidelman, 2004; Schore, 2001). As for the amount of time spent in daycare, this needs to be considered within the context of the quality of the daycare setting as high quality daycares can lead to positive developmental outcomes and low quality daycares can lead to problems with mental health (Geoffrey, Côté, Parent, & Séguin, 2006; Leroy, Gadsden, & Guijarro, 2012). Future research might, therefore, explore not only the influence of maternal affect, but the additional mediating influence of parent-chid interaction factors such as attachment style, maternal care and sensitivity, and time spent in daycare.

An additional avenue of research may also be to explore the prenatal programming hypothesis (Barker, 2004) with consideration for the influence of a mismatch between the prenatal environment and the postnatal environment. For example, Sandman, Davis, and Glynn (2012) found that infants who were exposed to congruent levels of maternal depressive symptoms in the pre- and postnatal period, even when symptom levels were relatively high, thrived on dimensions of psychomotor and mental development, whereas infants whose mothers demonstrated a decrease in depressive symptoms from the pre- to the postnatal period were

relatively impaired in comparison. More specifically, infants with congruent pre- and postnatal environments fared better than infants with incongruent environments, lending support for the adaptive advantage among infants exposed to matching pre- and post-natal maternal depressive symptoms. When exploring outcomes of early dysregulation and psychiatric comorbidity, future researchers may want to consider whether infants or children experienced exposure to congruent or incongruent pre- and postnatal adversity.

Finally, future directions for this area of study could include additional biological factors in the development of dysregulation. For example, information on the biological mechanisms of transmission by which maternal prenatal affective stress transcends to the fetus would be of interest. A possible pathway may be through epigenetic changes in the placenta, whereby changes in glucocorticoid related genes may have implications for the neurodevelopment of the fetus and child (see Monk et al., 2016).

Conclusion

The lack of finding for a direct path between maternal prenatal depression in interaction with child genetic susceptibility to predict psychiatric comorbidity in part supports the theory of prenatal programming of postnatal plasticity. According to this theory, exposure to prenatal adversity can lead to the development of greater behavioural and physiological reactivity, which are endophenotypes that lead to increased susceptibility to environmental exposures (Hartman & Belsky, 2018; Pluess & Belsky, 2011). As such, exposure to maternal prenatal depression, in interaction with genetic susceptibility, may render children more dysregulated, which will in turn put them at greater risk for developing psychiatric comorbidity. As expected, we found that early dysregulation, which was predicted by an interaction between prenatal depression and child genetic susceptibility, led to a nearly tenfold increase in the likelihood of developing childhood

comorbid psychiatric disorders. This finding converges with previous evidence from longitudinal studies that childhood dysregulation as of school age is associated with psychiatric comorbidity up to adulthood, with implications for severe and lifelong psychopathology (Althoff et al., 2010; Holtmann et al., 2011; Meyer et al., 2009). Overall, we conclude that dysregulation can be detected as early as infancy, endures throughout early childhood, is highly associated with psychiatric comorbidity, and can be predicted by biological and environmental factors as of the prenatal period. Accordingly, our findings provide information that can shape nosology and our diagnostic understanding of comorbid psychopathology, and underscore the need for prevention and early intervention.

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