Raising Twins: Studies on Maternal Well-Being and Characterization of Twins' Early Brain and Behavioural Development

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A Thesis

In the Department

Of

Psychology

Presented in Partial Fulfilment of the Requirements

For the Degree of

Doctor of Philosophy (Psychology, Research Option)

Concordia University

Montréal, Québec, Canada

July, 2023

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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Abstract for Ph.D.

Raising Twins: Studies on Maternal Well-Being and Characterization of Twins' Early Brain and Behavioural Development

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Concordia University, 2023

The early environment which includes the *in-utero* and the postnatal period, plays a role in brain and behavioural development. Twin designs provide us with the unique opportunity to study non-shared environmental contributions while controlling for genetic effects and the shared environment. However, there is some evidence to suggest that parents of twins also face unique stressors. The main objective of this thesis is to better understand maternal mental health in mothers of twins and behavioural and preliminary brain outcomes in the twins. The first chapter is a meta-analysis that included 14 articles to quantify the differences in depressive symptoms in mothers of singletons and mothers of twins. The second chapter is a latent class analysis in mothers of twins three, six, and nine months postpartum. We identified three possible trajectories for mothers of twins' depressive symptoms. We tested the hypothesis that mothers with a trajectory of increasing symptoms over time were not associated with behavioural outcomes in the twins at 24 months. The third chapter of this thesis is a case report paper about five families that agreed to participate in a neuroimaging study when the twins were four years old. We showed the feasibility of doing brain imaging in twins at the preschool age and obtained data regarding the twins' experiences in the MRI. This dissertation highlights the need for additional resources for mothers of twins. Additionally, the resources should be offered more than immediately postpartum. We conclude that neuroimaging in a pediatric population of identical twins is feasible.

Acknowledgements

As I sit here reflecting on this section, I realize it will be the hardest to write. To properly acknowledge everyone for their support and contributions to the completion of this dissertation would take a book of its own.

To my supervisor Dr Linda Booij, whom I have had the pleasure of working with since my undergraduate degree, your guidance and support have gotten me to where I am. Your patience and kindness as a supervisor allowed me to grow as a researcher and were by far one of the most rewarding aspects of my graduate career. The SNEL lab, all of which the members were handpicked by you, are some of the greatest colleagues I could have asked for.

To my committee members, Drs. Stack and Steele, thank you for your encouragement and support in the several ways this project has changed. A special thank you for your immeasurable guidance for this dissertation.

The memories I have garnered during my time as a graduate student would not have been possible without Édith, Lucy, Melissa, and Naomi. All of whom have been my academic support system and my greatest encouragers in moments I needed it the most. Thank you all so much.

To my mom, dad, and sisters, thank you for supporting me in my endless pursuit of education. I think I have gotten to where I wanted to be now – finally.

To Jesse thank you for believing in me. Your unconditional love and support in the last five years is the only way I was able to make it through this. My dearest sweet Benji, the definition of pet therapy. Without you, I would not be so well adjusted.

To Kyle, my other greatest accomplishment. You're the best detour I took in the completion of this dissertation. I love you to the moon and back.

Contribution of Authors

The current dissertation consists of three manuscripts for publication:

Dr Linda Booij and I were responsible for the design of Study 1. I was responsible for the literature review and article screening, conducted the analyses and drafted the manuscript. Dr. Catherine Herba critically reviewed the manuscript and provided additional expertise in maternal health to the project. Rachel Dufour participated in the quality screening of the articles and provided further feedback on the manuscript. Dr. Booij participated in the quality screening of the articles, supervised the statistical analyses, provided edits to the manuscript, and oversaw the work.

Study 2 and study 3 were conducted in the Projet des Ressemblances entre Jumeaux Monozygotes (PDIMOZY) cohort. The longitudinal cohort of monozygotic twins was initiated by Dr. Richard E. Tremblay, in collaboration with Dr. Linda Booij, Dr. Sylvana Côté and other collaborators across Quebec. Data collection until the twins were 2 years of age was supported by a grant from the Canadian Institutes of Health Research (PI: Tremblay). In 2019, Dr. Booij obtained a CIHR grant as PI to further expand the cohort and to support the MRI epigenetic, and additional behavioural data-collection in the PDIMOZY cohort as of 3 years of age, and she has been leading the PDIMOZY cohort since then.

For Study 2, I was responsible for the literature review and article screening, conducting the analyses and drafting the manuscript. Dr. Édith Breton contributed her invaluable knowledge of SAS and the Proc Traj program, conducted the preliminary analyses, and read and provided feedback on the manuscript. Dr. Booij supervised the work and provided edits to the chapter. Both Dr. Côté and Tremblay provided expertise in child development to the project.

For Study 3, I was responsible for the data collection. I received assistance in data collection from Naomi Azar and Kaniza Salam. I drafted the case reports. Dr. Kevin Casey, RA in the lab, processed the data. My supervisor Dr. Linda Booij secured funding for the project, designed the study, supervised data collection, and statistical analyses, and provided edits to the writing of the chapter. The familiarization protocol was designed by Dr. Miriam Beauchamp. Dr. Sylvana Côté and Dr. Richard E. Tremblay contributed to the expertise in the design of the study.

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Chapter 1: General Introduction

The early environment which includes the *in-utero* environment and the postnatal period, has been suggested to play a role in brain, behaviour and cognitive outcomes in children who experience suboptimal early life environments. However, given that not all children are affected the same way by potentially negative early life experiences, it is pertinent to identify the mechanisms behind the association between early life adversity and negative outcomes. For example, it is difficult to determine whether or not the children differ genetically or due to their non-shared environmental factors (Chiarella et al., 2015). For this reason, comparing individual child to individual child is not ideal. Monozygotic (MZ) twin designs provide researchers with the unique opportunity to study non-shared environmental contributions while controlling for genetic effects and the shared environment without experimental manipulation (Vitaro et al., 2009). This is due to MZ twins sharing nearly all of their genetic sequence and having the same shared environment. Barring the uniqueness of twins, what is equally of interest is twin parenthood. Mothers of twins also experience different stressors than mothers of singletons which may be associated with poorer emotional well-being (Kehoe et al., 2016; Wenze & Battle, 2018). Poor maternal mental health may also be associated with negative early life experiences in some cases, which may be associated with child outcomes (Goodman et al., 2011; Farewell et al., 2021). The present thesis includes studies with both a parent and a child focus. Specifically, it includes two studies on the maternal psychological well-being of mothers of twins (Studies 1 and 2). Next, pilot data of an MRI study in twins at the preschool age is described (Study 3). The general introduction provides an overview of the early environment including, the importance of the *in-utero* and postnatal period and the association it may have with cognitive, behavioural, and

brain outcomes in children. Furthermore, it provides an overview of twin designs and mental health in mothers of twins.

Early Environment

The early environment is a critical period of development for children and includes the *in-utero* and postnatal periods. The developmental origins of health and disease framework (DOHaD) have been used to study the cause of physiological diseases and have now been extended to include mental health conditions (Suzuki, 2018). Past research using a DOHaD has shown an association between perinatal adversity and future mental health problems (Newman et al., 2016; Van Den Bergh, 2011). According to DOHaD, adverse events in early development can lead to enduring changes in physiology and metabolism, which can have long-term effects on adult health, leading to increased risk of diseases such as coronary heart disease (Barker & Osmond, 1986), hypertension (Eriksson et al., 2007), and type 2 diabetes (Hales et al., 1991). The framework postulates that this enduring effect occurs due to the fetal and early life programming (Lewis et al., 2014; Lieshout & Krzeczkowski, 2016). Programming is about the influence of a specific environmental factor at a critical point in development, which may cause a long-term consequence that may affect outcomes in the future (Lewis et al., 2014). The theory began by only focusing on the fetal period of development; however, it has now evolved to include the entire developmental period from the earliest generation of cell division *in-utero*, which is known as early life programming (Suzuki, 2018). This framework has also expanded to include the etiology of mental illness (Lewis et al., 2014). Adversity experienced in early life has been associated with unfavourable mental health and cognitive development issues in the future, likely due to the effect of early-life programming (Lieshout & Krzeczkowski, 2016). Therefore,

the *in-utero* and postnatal period has increasingly become of interest in the etiology of mental health problems. Contrary to a disorder-centered approach, the importance of the *in-utero* environment in differences in brain, cognitive, and behavioural problems would indicate that adopting a broad developmental perspective is necessary (Luyten et al., 2008).

Importance of *In-utero* Environment

Research on *in-utero* risk factors includes lifestyle factors, maternal mental health, and teratogenic and neurotoxic exposures (Lewis et al., 2014). Lifestyle factors may consist of maternal nutrition and exercise, both pre-and during pregnancy (Lewis et al., 2014). Maternal mental health has been prominent in the literature as an *in-utero* risk factor. For example, maternal depression, stress and anxiety have negative effects on the fetus both cognitively and behaviourally (Newman et al., 2016; Robinson et al., 2015; Van Den Bergh, 2011). In addition, teratogenic and neurotoxic exposures *in-utero* may compromise fetal development. Examples of teratogenic and neurotoxic exposures include maternal smoking, drinking and drug use, as well as, exposure to substances such as lead, or chemicals with endocrine-disrupting properties (Lewis et al., 2014). Adversity *in-utero* and in the early postnatal period have been associated with risk for mental health problems throughout the life-span, including but not limited to attention deficit hyperactivity disorder (ADHD; Galéra et al., 2011), anxiety disorders (Côté et al., 2009) and conduct disorders (Tremblay, 2010). Elucidating the specific mechanisms between *in-utero* environment and future mental health problems would be fundamental in developing early interventions before onset and identifying which brain regions specifically play a role. All these factors can create a suboptimal *in-utero* environment that may affect future behavioural, cognitive and brain development outcomes.

Importance of psychosocial environment in early life

Exposure to adverse psychosocial events in early life can be associated with future mental health issues in children in the future (Berens et al., 2017). Adverse psychosocial experiences in early life include maltreatment or abuse of the child, exposure to depression or stress, exposure to domestic violence, and exposure to a violent community (Berens et al., 2017). Experiencing some adversity in early life is not always indicative of problematic child outcomes in the future (Cicchetti & Rogosch, 1996). Yet, it remains important to develop certain interventions to reduce the risk. For example, physicians providing care to patients during pregnancy should provide resources regarding stress and depressive symptoms both during pregnancy and postnatally (Wenze & Battle, 2018). Several studies have found that maternal depression or stress can be associated with behavioural outcomes in some children in the future. For example, maternal depression was associated with an increased incidence of internalizing (g= .21, p <.001) and externalizing (g= -.05, p <.001) behaviours in some children, as well as an increased incidence of general psychopathology (Goodman et al., 2011).

Low-Birth Weight

The quality *in-utero* environment can have multiple proxies, such as head circumference, birth weight, birth length and ponderal index (Krishna et al., 2019). Low birth weight (< 1500g) is a proxy for the quality of the *in-utero* environment, and may correlate with malnutrition, maternal depression and anxiety, and exposure to toxins (Lewis et al., 2014; Martinussen et al., 2005; Skranes et al., 2013). Therefore, studies conducted with low-birth-weight individuals may allow for investigation of the association between *in-utero* adversity, behavioural and cognitive development, and brain development. A cohort of individuals with extremely low birth weight (501 – 1000g; Boyle et al., 2011) were more likely to have symptoms of ADHD reported by

parents as early as 8 years of age, and a higher likelihood of experiencing depression-like symptoms at 14 years of age compared to normal birth weight controls (Saigal et al., 2003). Following this, at 23 years of age those of extremely low birth weight had higher levels of depressed mood, anxiety, social withdrawal, and poor self-esteem in adulthood compared to normal birth weight controls (Boyle et al., 2011). This study suggested that differences in behavioural outcomes between extremely low birth weight children and normal birth weight controls begin early in childhood and persist into adulthood.

Extremely low or low birth weight has also been associated with differences in cognitive outcomes and brain development. In a sample of preterm 15-year-old extremely very low birth weight (<1500g) adolescents, cortical surface areas were reduced in the ventrolateral, prefrontal, temporal, and parietal regions (Skranes et al., 2013). Intelligence Quotient (IQ) test scores were correlated with reduced cortical area in the inferior frontal gyrus and parahippocampal gyrus in very low birth weight preterm adolescents compared to controls (Skranes et al., 2013). Previous research has also shown an association between birth weight and brain volume, specifically cortical grey matter (Martinussen et al., 2005). Adolescents with very low birth weight (<1500g) and adolescents that were smaller for gestational age (lower average weight) showed smaller total brain volume, with very low birth weight adolescents also showing reduced cortical grey matter as a proportion of brain volume (Martinussen et al., 2005). As reflected in the research, children with low birth weights appear to have smaller brains and altered cortical morphology in adolescence compared to controls. These studies, taken together, suggest that lower birth weight is at times associated with differences in future behavioural and cognitive outcomes and differences in brain structures. However, it becomes difficult to disentangle whether this association is driven by genes or by early environmental effects.

Twin Designs

Twin designs are ideal when attempting to elucidate mechanisms behind complex associations that can become confounded with genetic and environmental differences. When utilizing singleton study designs, it becomes difficult to control for the effect that genes may play versus the role of the environment. To illustrate, children who are born and have experienced *in-utero* or early life adversity will differ from their peers who did not experience adversity both genetically and environmentally (Chiarella et al., 2015). For this reason, employing a monozygotic (MZ) twin design is an ideal study design for attempting to elucidate mechanisms behind complex associations. Specifically, MZ twins share nearly all of their genetic baggage, this uniquely allows researchers to control for the effect of the non-shared environment (Chiarella et al., 2015). Additionally, since MZ twins share the same environment, this also allows researchers to control for the effect that the shared environment may have in an association. Longitudinal MZ twin designs are ideal to measure the changes over time and elucidate the potential mechanisms that could be driving this change.

Birth Weight Discordance in Twins

As a proxy for *in-utero* adversity in a twin design, birth weight discordance within MZ twins is utilized (Salafia et al., 2008). Birth weight discordance within a twin pair has been suggested to be associated with differences in behavioural and cognitive outcomes in the future (Chiarella et al., 2015). Birth weight discordance is a widely used and accepted proxy as an index of adversity *in-utero* or early in life. Whereby, the twin with the lower birth weight may suggest a less optimal *in-utero* environment compared to the higher birth weight cotwin. This method, referred to as the discordant MZ twin, is a method for determining a probable causal

pathway between the non-shared environment and outcomes in the twins' behaviour, cognitive, emotional, and brain development due to the control over potential confounding variables (Vitaro et al., 2009). By employing a longitudinal discordant MZ twin design, researchers can further expand on the potential association between the non-shared environment and behavioural outcomes as it may predict increases in differences in MZ twins' behaviours over time (Vitaro et al., 2009).

This discordant MZ twin design as described above is not without limitations. Specifically, when studying discordances, the outcome variable being assessed mustn't be entirely explained by genetics, as discordances would be lower due to the high heritability (Vitaro et al., 2009). The outcome measure should preferably be continuous rather than categorical, as dichotomous or categorical variables do not allow for a range (Vitaro et al., 2009). Due to their similarities, MZ twins are usually also rated similarly on measures of self-report. Despite this, it remains a strong methodological design to study the role of the non-shared environment in the development of unfavourable outcomes in behaviour in the future.

Birth Weight Discordance Association with Behaviour and Cognitive Outcomes.

Within a twin pair, the twin with the lower birth weight is presumed to have had a suboptimal *in-utero* experience compared to their higher birth weight twin. This would place the lower birth weight twin at a higher risk of experiencing negative outcomes both cognitively and behaviourally. Past research has found an association within a twin pair, where the twin with a lower birth weight shows an increase in internalizing and externalizing behaviours (Van Os et al., 2001). This correlates with previous research in singleton low birth weight children in comparison to average birth weight controls. An example of this in MZ twins comes from a

population-based study that studied the evolution of ADHD symptoms over time. ADHD was assessed at two time points, one when the twins were eight to nine years old, and the other when the twins were thirteen to fourteen years old (Hultman et al., 2007). Overall, the researchers found that the lower birth weight twin scored higher on the symptoms of ADHD assessments at both time points compared to the higher birth weight twin (Hultman et al., 2007). This would suggest that regarding hyperactivity and impulsivity, within a twin pair, it is the lower birth weight twin that may have more problematic behaviours. In addition, it also suggests that this association is enduring, in that their behaviour remained more problematic than their cotwin after several years. This finding has also been replicated in another population-based study that recruited all parents of twins in Sweden when they were between nine and twelve years old, whereby reduced birth weight was associated with increased ADHD symptoms (Pettersson et al., 2015). There is literature to also suggest that this association exists outside of ADHD symptoms. In the United Kingdom, 960 twins were recruited to participate in a study to examine psychological development from birth until five years old (Tore et al., 2018). Of the 960 twins that were recruited 354 were MZ twin pairs, Child Behaviour Checklist (CBCL) scores from when the twins were approximately 3 years of age were analyzed to determine if birth weight differences existed within a twin pair in regards to birth weight discordance (Tore et al., 2018). The researchers observed a difference in total CBCL scores, internalizing symptoms, emotional reactivity, and sleep problems in lower birth weight twins (Tore et al., 2018). The results here suggest that not only may low birth weight be associated with externalizing symptoms, but also with internalizing symptoms.

Past research has also shown associations with low birth weight and cognitive outcomes within MZ twin pairs. Specifically, within a MZ twin pair, it has been suggested that lower birth

weight twins have decreased scores on IQ tests in comparison with their high birth weight cotwin (Newcombe et al., 2007). Another study found that twins at three years of age with discordant birth weights performed differently on intelligence measures despite no longer having a weight discordance at three years of age. They found that the lower birth weight twin performed less well on measures of intelligence than their higher birth weight cotwin (Ross et al., 2012). Furthermore, birth weight has been associated with verbal intelligence scores in seven-year-old MZ twin pairs with birth weight discordances. Specifically, within twin pairs the lower birth weight twin had lower scores on verbal IQ but in this study, there was no difference in performance IQ within the twin pair (Edmonds et al., 2010). These differences in behaviour and cognition appear to endure from childhood into adulthood (Van Os et al., 2001). The association between birth weight and behavioural and cognitive outcomes is still found even if the twins' birth weight is considered to be within a normal range (Kramer, 1987; Van Os et al., 2001).

Birth Weight Discordance Association with Brain Development.

MZ twin designs are also ideal for studying brain development differences as confounds are controlled for (i.e., genes and shared environment) and any differences in brain development may be the result of the non-shared environment which can assist in early detection of unfavourable mental health outcomes in the future.

In research conducted in our lab with participants recruited from the Quebec Newborn Twin Study (QNTS), we have found that in approximately 108 MZ twins birth weight discordance is associated with differences in brain development in adolescent monozygotic twin pairs, where the lower birth weight twin shows differences in cortical volume and differences in connectivity compared to their higher birth weight cotwin (Casey et al., 2017; Hayward et al.,

2020; Levesque et al., 2015). Specifically, the greater the birth weight discordance within the twin pair, the greater the discordance observed in total grey and white matter volumes (Levesque et al., 2015). A reduction in total grey and white matter as well as decreased cortical surface area and increased cortical thickness, suggests that while controlling for genetic effects and shared environments, twins with a lower birth weight experience neurodevelopmental differences compared to their higher birth weight twin (Casey et al., 2017; Levesque et al., 2015). In both of these studies, it is suggested that the differences in neurodevelopment were larger within twin pairs that had larger birth weight discordances (Casey et al., 2017; Levesque et al., 2015). More recently, we observed differences in limbic network connectivity in MZ adolescent twins. Where, the lower birth weight twin had reduced connectivity in the limbic network, specifically in the right hippocampus and amygdala than their high birth weight cotwin (Hayward et al., 2020). This study suggests that within twin pairs, controlling for genetics and shared environment, the unique *in-utero* environment may also affect limbic connectivity demonstrating once again neurodevelopmental differences within twin pairs, for the lower birth weight twin (Hayward et al., 2020). Previous research from other research groups has found that in monozygotic twin pairs between the ages of three and thirty, birth weight discordances predicted differences in cognitive outcomes and cortical surface area (Raznahan et al., 2012). The twin with the lower birth weight scored lower in cognitive outcomes and had a smaller cortical volume compared to their higher birth weight cotwin (Raznahan et al., 2012).

The current literature on brain development in critical periods is lacking (Gilmore et al., 2018). A critical period where brain imaging would be particularly relevant is during the preschool years because neuronal pruning has not yet begun, and would allow for a unique examination of connectivity (Stiles & Jernigan, 2010). However, due to the difficulty in

acquiring brain imaging in children during their preschool years, the literature is scarce. For example, the study described above by Raznahan et al., had a large age range from three to thirty years old, this is due to attempting to recruit children at a young age, but being unable to meet sample size requirements to observe an effect (2012).

Although MZ twin research is of interest due to the unique ability to control for genetic effects and the shared environment, mothers of twins are also of interest. Given that research shows that maternal depression is a risk factor for unfavourable mental health outcomes in children in the future, studying the mental health of mothers is pertinent. Maternal mental health may be a contributing environmental factor behind differences in behaviour, cognitive, and brain development outcomes in association with birth weight differences.

Maternal Mental Health

Postpartum depression (PPD) is a mental health issue that can be experienced in the first year following the birth of a child. The diagnostic criterion is major depressive disorder, however, there is a specifier that the episode occurs during pregnancy or after four weeks after the birth of a child (American Psychiatric Association, 2013). Symptoms of PPD include difficulties with sleep and appetite, loss of interest in things that once brought joy, feeling worthless or guilty, lack of concentration, loss of energy, and suicidal ideation (American Psychiatric Association, 2013). PPD is becoming an increasing concern for mental health professionals, as if left untreated could lead to two tragic consequences: infanticide or maternal suicide (Cook et al., 2023). However, it is important to note that not all symptoms of depression do reach clinical level and there is some normality to experiencing depressive symptoms following childbirth. Postpartum blues can be experienced by 15-85% of women, and the

symptoms are cycled through rather rapidly (Pearlstein et al., 2009). Despite this, experiencing depressive symptoms even though not clinical does place women in a precarious position to experience mental health issues.

Severity of Depressive Symptoms

PPD is the most common mental health disorder in women in the first three to four months postpartum, and sometimes symptoms are present during pregnancy (Shorey et al., 2018). Once diagnosed, intervention becomes necessary to reduce the risk of child behavioural or cognitive issues and more tragically infanticide or maternal suicide (Cook et al., 2023); therefore, identifying women who are at higher risk for PPD is necessary. This can be done by recruiting from community samples and identifying women with high levels of depressive symptoms. In a recent latent profile analysis, researchers found that women at high risk for PPD showed high levels of depressive and anxiety symptoms in the first four months postpartum (Carona et al., 2023). Higher levels of symptoms of depression in the postnatal period may suggest an increased risk of developing clinically significant symptoms (Putnick et al., 2020; Verreault et al., 2014). Although clinically significant levels of depression may be associated with child behavioural and cognitive outcomes, it has been found that even nonclinical levels of depressive symptoms may be associated with child outcomes (van der Waerden et al., 2015). Therefore, given that elevated levels of symptoms of depression during pregnancy and early postpartum could predict the later onset of a depressive disorder, focusing on nonclinical populations may help identify mothers who are at greater risk and develop timely interventions.

Women may experience depressive symptoms postpartum for several psychosocial and biological reasons. Past research has suggested that raising a child is one of the greatest joys in

life and brings immense happiness to the parents who embark on this journey (Gupta, 2023). However, in recent years there have been increasing changes to families' financial and psychosocial well-being where raising a child is not as necessary for lifetime fulfilment as was previously thought (Gupta, 2023). Career advancement has been prioritized for women which has led to considering having children older than in the past, and the decision to embark on parenthood could also cause a strain on career fulfilment (Gupta, 2023). Marital satisfaction may decrease following the birth of a child which puts additional strain on mothers (Gupta, 2023). Motherhood is a time of great change, both joyful and stressful. A recent qualitative study found that some mothers were internalizing an overwhelming amount of responsibility which led to feelings of inadequacy and depression (Johansson et al., 2020).

The joys and burdens of motherhood are experienced by most women who choose to have children; however, for mothers of twins, there is double the joy and perhaps an increase in the burden. Twin births have been on the rise, in North America, the twinning rate from 1980-1985 was 9.9, and from 2010-2015 it was 16.9, and in the United States in 2020, the rate was 31.2 (*FastStats*, 2023; Monden et al., 2021). This rise is presumed to be due to women having children at an older age which leads to increased use of artificial reproductive technology (ART; Damato, 2005; Monden et al., 2021). Previously, research on twins and twin births has been focused on obstetrical complications and the benefits of twins for research designs. Some studies identify mothers of twins at risk for mental health issues, however, despite this, they remain understudied and lack mental health resources (van den Akker et al., 2016; Vilska & Unkila-Kallio, 2010). The difference in depressive symptoms in mothers of twins and singletons conceived naturally or by ART has been studied in the past, and it has been found that it is specifically twin parenthood rather than the type of conception that puts mothers at risk for

unfavourable mental health outcomes (van den Akker et al., 2016; Vilska & Unkila-Kallio, 2010).

Mothers of Twins and Depressive Symptoms

The birth of more than one baby has been suggested as an adverse effect on mothers' emotional well-being (Kehoe et al., 2016). Past research on coping in mothers of twins has identified certain psychosocial factors that can instigate further difficulties in coping (e.g., socioeconomic status, sleep deprivation, and lack of social support) and several factors that can serve as a protective factor for parents (e.g., social support and family environment, Kehoe et al., 2016).

Socioeconomic status is an indicator of coping for parents of twins, as the cost of having a child is quite substantial in North America (Sarlo, 2013). Parents who are from higher socioeconomic status will have less pressure regarding the financial implications of having multiple babies. Twin births are frequently associated with obstetrical complications that can lead to preterm delivery and the need for neonatal intensive care, which creates larger healthcare service bills for parents. All necessities increase two-fold in parents of twins, therefore lower socioeconomic status may very well be a predictor of poorer coping once the babies are born (Leonard & Denton, 2006).

Social support is also an important factor when studying the association between twin motherhood and depressive symptoms. A lack of social support may contribute to more adverse coping in mothers of twins, whereas high-quality social support may contribute to better adjustment following the birth of twins. The more help that mothers of twins may have the more likely they will be able to better cope with the immense life changes that come from having a

child. Having supportive families, friends, and partners has been found in past research to be a protective factor against depressive symptoms (Hughes et al., 2020; Milgrom et al., 2019). Social support may also moderate the association between depressive symptoms changes in intimacy and lack of personal time. In this vulnerable period, it is important for new mothers to feel supported and secure, and to be able to trust other people who can help care for their children (Benute et al., 2013).

In addition to psychosocial risk factors, some biological factors also exist when discussing the risk of depressive symptoms. Throughout pregnancy, several physiological changes occur to the body to sustain a pregnancy, as well as prepare for childbirth and breastfeeding (Skalkidou et al., 2012). During pregnancy with singletons and twins, there are several hormones (estrogen, progesterone, testosterone, corticotropin-releasing hormone, and cortisol) that are produced at a level that would be considered pathological in non-pregnant people (Skalkidou et al., 2012). Following delivery, these hormones drop rather dramatically and can be one biological explanation of elevated depressive symptoms in mothers. Indeed, a study used a topical Estrogen cream to successfully treat severe PPD and saw a reduction in self-reported depressive symptoms (Gregoire et al., 1996). Along with hormone changes, there are changes in the serotonergic system, which have been linked to mood disorders (Skalkidou et al., 2012). Disrupted circadian rhythm cycles could also be a cause and consequence of depression, specifically in the postnatal period, where sleep is generally lacking for most parents (Skalkidou et al., 2012). Changes have also been shown in the immune and thyroid systems to allow for the body to accept the fetus which could be linked to PPD through a host of biological pathways (Skalkidou et al., 2012). Although biological factors due to changes experienced during pregnancy would not explain enduring depressive symptoms into the preschool years, studies

have shown that women who experience a depressive episode or elevated levels of symptoms of depression in pregnancy and the postpartum period are more likely to experience future episodes of depression (Bradshaw et al., 2022).

In conjunction with the psychosocial risk factors and the biological vulnerabilities, there is also a factor that crosses the boundaries of both contributors: obstetrical complications. Obstetrical complications can create stress and anxiety for the mothers, which can also lead to negative delivery experiences which have been associated with increased depressive symptoms (Verreault et al., 2014). Furthermore, research has also shown that obstetrical complications are linked to lower brain serotonin synthesis in the offspring (Booij et al., 2012). Obstetrical complications in twin pregnancies include twin-to-twin transfusion, low birth weight, prematurity, fetal growth restriction, perinatal mortality, and an increased risk of miscarriage (Rao et al., 2004). The risks to the mother specifically during twin pregnancies include an increase in cardiovascular and hematological changes, as well as an increased risk for hyperemesis gravidarum and pre-eclampsia (Rao et al., 2004). Caesarean section rates are higher in certain countries when multiples are expected (Macfarlane et al., 2016). In certain cases, such as placenta previa or fetal distress delivery by caesarean section is crucial for both infant and maternal morbidity (Ylilehto et al., 2022). However, it has been shown in cases where a caesarean section was not medically necessary, that women expecting twins have far more positive experiences in delivery when done vaginally (Ylilehto et al., 2022).

Several of the factors that are affected by having a newborn are also key symptoms when screening for depression. It has been found that sleep deprivation exacerbates symptoms of depression in mothers (Okun et al., 2018). In the postnatal period, it can be increasingly difficult for parents to get the amount of sleep they were previously accustomed to, generally, the infancy

period is known for feeding the infant every few hours and keeping up with the demand is the main occupation of most mothers. Indeed, a recent study has found that in mothers experiencing sleep deprivation, the needs of their newborn child came before their (Hoegholt et al., 2023). It becomes increasingly difficult to disentangle how much of the adverse effects are due to potential depressive symptomatology or how much is due to just normal variation in this new journey. This would necessitate multiple assessments postpartum to study the evolution of depressive symptoms in mothers of twins.

Evolution of Depressive Symptoms

Previous research on the evolution of depressive symptoms in motherhood has focused primarily on mothers of singletons. The trajectories of depressive symptoms in mothers of singletons across studies have been relatively consistent. The majority of mothers will experience low stable levels of depressive symptoms, some will experience high levels of depressive symptoms that decrease with time, some that start with low levels that rise slowly over time, and some will experience high and persistent levels of depressive symptoms (Guyon-Harris et al., 2016; Park et al., 2018; Putnick et al., 2020; van der Waerden et al., 2015). Some researchers also examined the potential association that the trajectory course had on the children's behavioural and emotional outcomes in the future (Guyon-Harris et al., 2016; Park et al., 2018; van der Waerden et al., 2015). Consistent with the DoHAD framework, researchers posited that mothers who followed a trajectory of symptoms that were highly persistent or increasing symptom severity would be associated with behavioural outcomes in children in the future (Guyon-Harris et al., 2016; Park et al., 2018; van der Waerden et al., 2015). One study found that for mothers whose trajectory course resembled high persistent probability, their

children were more likely to display behavioural issues at five years of age in comparison to children of the same age with mothers who did not display symptoms of depression (van der Waerden et al., 2015). The effect was observed in conduct problems, hyperactivity/inattention, peer relationships, and prosocial behaviour all measured by the Strengths and Difficulties Questionnaire (van der Waerden et al., 2015). This observed association would suggest that maternal depressive symptoms, even those that are not severe enough to be diagnosed, may be linked to suboptimal outcomes in children's behaviour (van der Waerden et al., 2015). Additionally, another study found that in mothers who followed the trajectory course of increasing depressive symptoms, their children had higher levels of internalizing and externalizing behaviours at three years of age, and the children had poorer executive functioning at six years of age (Park et al., 2018). Conversely, another study did not observe an effect of trajectory course on behavioural and cognitive outcomes in toddlers (Guyon-Harris et al., 2016). Another study examined several perinatal predictors that could be associated with trajectory course, this study did not find that infertility, multiple births, pre-pregnancy BMI, gestational hypertension and infant sex affected trajectory course in this sample (Putnick et al., 2020). Although the sample size was large and these null findings are likely meaningful, it was suggested that the lack of a detailed depression measure and having a nonclinical sample may explain in part the lack of significant findings (Putnick et al., 2020).

Depressive Symptoms and Child Outcomes

It has been suggested that in some cases, maternal depression may be considered a risk factor for the development of depression in offspring in the future (Goodman et al., 2011). The heritability of depression has been observed in twin and adoption studies. Specifically for

behavioural outcomes, it has been suggested that elevated levels of depressive symptoms in mothers during critical periods were associated with higher scores on internalizing and externalizing problems in toddlers at five years of age (Farewell et al., 2021). A recent meta-analysis has shown that child functioning is negatively associated with the severity and chronicity of depressive symptoms in mothers (Sutherland et al., 2022). Some of the children from mothers who experience clinical or long-term depressive symptoms showed associations in some cases, with academic problems, cognitive functioning, and behavioural outcomes (Sutherland et al., 2022). However, associations do not necessarily equate to causation. It was also found that some children showed patterns of resilience, whereby being exposed to a certain amount of stress may have made them more apt and prepared to deal with the highs and lows that life can bring (Sutherland et al., 2022). The effect of depressive symptoms on cognitive development in children has been shown in another meta-analysis that examined symptoms of depression in general, not necessarily clinical symptoms (Liu et al., 2017). The study concluded that there can be a negative association between maternal depressive symptoms and children's cognitive outcomes (Liu et al., 2017). These results further emphasize a need for more regular and careful screening in women during pregnancy, the perinatal, and the postnatal periods.

The Present Thesis

The main objective of this dissertation is to better understand the mental health of mothers of twins, as well as explore behavioural and preliminary brain development outcomes in twins at preschool age. The current dissertation consists of three studies. The first study is a meta-analysis that includes 14 articles that were synthesized to quantify the differences between maternal depressive symptoms in mothers of twins in comparison to mothers of singletons. This

meta-analysis followed the PRISMA guidelines, and the inclusion and exclusion criteria were established *a priori*. We also specifically focused on depressive symptoms. We hypothesized that mothers of twins would experience more depressive symptoms than mothers of singletons. The second study of this dissertation is a latent growth analysis of the evolution of depressive symptoms in mothers of twins at three, six, and nine months postpartum. To conduct this study, we utilized archival data from a longitudinal cohort of monozygotic twins that have been followed closely since birth. Using Proc Traj in SAS we modelled the data and selected the trajectories that best fit our dataset. Subsequently, we tested to explore if the trajectory course would be associated with the twins' temperament at 12 months of age and behavioural outcomes at 24 months of age. We hypothesized that behavioural scores would be higher in twins whose mothers followed an increasingly severe depressive symptom trajectory. The third study is a case report paper that includes five families from the same longitudinal cohort of monozygotic twins. These five families agreed to participate in a neuroimaging study when the twins were four years of age to identify potential differences in brain development as a function of birth weight differences. The data presented in this study is descriptive and primarily aimed to characterize the twins' experiences and to demonstrate the feasibility of conducting MRI research in twins at preschool age, contributing to a larger project that will take place in the future.

Chapter 2: Depressive Symptoms in Mothers of Twins: A Meta-Analysis

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Submitted for Publication in the *Journal of Family Psychology*

Abstract

Although motherhood is widely considered to be a period of joy, the birth of a new child could come with various challenges, which may contribute to elevated levels of depression. Our objective was to quantify whether mothers of twins experience greater levels of depression symptoms than mothers of singletons. Included studies had to contain measures of depression for both mothers of twins and singletons. Only postnatal measures were included up to preschool age, and effect sizes had to be reported or calculated with the data provided. We compiled 14 studies (n=45-8069) that fit our search criteria and, using Meta-Essentials, we found that mothers of twins have slightly higher levels of depression symptoms than mothers of singletons (r = 0.08, p = .002). Findings are particularly relevant for mothers of twins who have other risk factors, such as a history of depressive episodes. Our meta-analysis highlights the importance of screening for common maternal mental health problems in the postpartum period among parents of twins.

Keywords: Postpartum depression, twins, singletons, meta-analysis, motherhood

Introduction

Although motherhood is often considered to be a period of joy and personal growth, it is also a vulnerable time for many women where some may experience mental health difficulties such as depression, stress, and anxiety (Arendell, 2000). However, the postpartum period is also associated with hormonal fluctuations, obstetrical complications, fatigue, body changes, and several other physiological changes. There could also be a host of psychosocial symptoms and stressors such as depression, anxiety, stress, the perception of lack of parental competence, financial pressures, lack of personal time, and changes in intimacy with a partner (Kehoe et al., 2016). The physiological changes combined with the psychosocial stressors could increase the risk of mothers experiencing mental health issues.

Most research on maternal mental health focuses on depression, anxiety, and perceived stress in association with outcomes in children (Graignic-Philippe et al., 2014; Lawless et al., 2014; Oyetunji & Chandra, 2020; Prino et al., 2016; Thompson & Henrich, 2022). Postpartum blues or maternity blues is a transitional psychological syndrome in the first ten days postpartum, with the peak being around the fifth day (Murata et al., 1998; Pearlstein et al., 2009). Symptoms such as mild depression, crying, unstable moods, anxiety and confusion are indices of postpartum blues (Reck et al., 2009). This transitional period does not demarcate itself well from precursory postpartum depression, but symptoms of postpartum blues cycle rapidly compared to postpartum depression (Murata et al., 1998). In the DSM-5, the specific diagnosis is a major depressive disorder with the specifier: peripartum onset which indicates that the depressive episode or symptoms occur during pregnancy or within four weeks postpartum (American Psychiatric Association, 2013). Postpartum depression occurs in approximately 10-15% of

mothers and should be screened for between two weeks and six months postdelivery, but the rate of screening among physicians is low (Goldin Evans et al., 2015; Goodman et al., 2011; Pearlstein et al., 2009). Prenatal depression, unemployment, socioeconomic status, poor social support, and age are all factors that predict elevated depressive symptoms and postpartum depression (Sampson et al., 2017; Verreault et al., 2014). Elevated symptoms of depression both prenatally and postnatally create a greater risk of developing clinically significant symptoms (Putnick et al., 2020; Verreault et al., 2014). Given that both prenatal and postpartum depression can be associated with subsequent child outcomes, it would be pertinent to screen and identify mothers who might be more at risk of developing postpartum depression.

There is some evidence that parents of multiples are particularly at risk for elevated levels of depressive symptoms and postpartum depression (van den Akker et al., 2016; Vilska & Unkila-Kallio, 2010). The typical challenges of motherhood are considered twice as heavy as those for twin parents than for singletons, and there could be double the financial pressure, even less personal time and time for intimacy (Kehoe et al., 2016; Wenze & Battle, 2018). In mothers of twins, the strongest predictor of postpartum depression is childcare stress (Damato, 2004). Further, prenatal stressors such as pregnancy-related stress (e.g., prematurity, and increased risk for obstetrical complications) are more common in twin births than in singleton births, which may further increase the risk for postpartum depression as prevalence is higher in parents who experience increased levels of stress during pregnancy (Dennis & Dowswell, 2013).

Globally, rates of twin births have been increasing. In North America, the twinning rate from 1980–1985 was 9.9, and from 2010–2015 it was 16.9, and as of 2020, in the United States, the twinning rate was 31.2 (*FastStats*, 2023; Monden et al., 2021). This rise is considered to be due to an increase in assisted reproductive techniques and bearing children at a later age

(Damato, 2005; Monden et al., 2021). Current research in twins focuses primarily on obstetrical complications for the fetuses associated with multiple births, such as twin-to-twin transfusion, low birth weight, prematurity, fetal growth restriction, perinatal mortality, and increased risk of miscarriage (Rao et al., 2004; Santana et al., 2018). There are additional risks to the mother during twin pregnancies, such as an increase in cardiovascular and hematological changes, as well as an increased risk for hyperemesis gravidarum and pre-eclampsia (Rao et al., 2004). However, despite an increasing number of studies showing a positive association between maternal mental health during singleton pregnancies and postpartum and child developmental outcomes, the maternal mental health of mothers of twins during pregnancy and postpartum is understudied (Graignic-Philippe et al., 2014; Oyetunji & Chandra, 2020). One systematic review included 27 studies that examined symptoms of depression, anxiety, and parenting stress in mothers of twins and singletons (Wenze et al., 2015). Assessments were taken at the earliest in the second trimester of pregnancy and up to 5 years of age. Among those studies, 20 articles compared mental health outcomes in parents of multiples and singletons (Wenze et al., 2015). It was found that parents of multiples experienced higher incidences of depression, anxiety and parenting stress compared to parents of singletons (Wenze et al., 2015). Another relevant meta-analysis, published in 2016, investigated whether or not mothers who conceived twins or singletons via assisted reproductive technologies (ART) were more at risk for depression than mothers who conceived twins or singletons via natural conception (van den Akker et al., 2016). This meta-analysis included six studies and found that there was no main effect of the type of conception. However, mothers of twins conceived via ART or natural conception were significantly more at risk than mothers of singletons conceived via ART or natural conception. (van den Akker et al., 2016). The results of the preceding meta-analysis coincide with findings

from a narrative review paper reporting that it is twin parenthood that puts parents more at risk for mental health problems rather than the type of conception, (Vilska & Unkila-Kallio, 2010). We are not aware of any meta-analysis that quantified differences between maternal depression postpartum in mothers of twins and singletons as the main goal. Studying whether or not twin parenthood is a risk factor for increased depressive symptoms regardless of type of conception is important and could highlight the need for additional resources for families expecting or having twins.

The current study aimed to systematically review the literature on postpartum depressive symptoms in mothers of twins and to quantify differences in depressive symptoms in mothers of twins and singletons using meta-analyses. For this study, articles that included at least a measure of depression in mothers of twins and mothers of singletons taken at least 6 weeks postpartum and up to preschool age were included. It was hypothesized that across studies, mothers of twins would have higher symptoms of depression than mothers of singletons. The overall aim of the present meta-analysis allowed for the retention of more articles than previous meta-analyses on this topic, which increased our sample size.

Method

Search Strategy

The current meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. The following two databases were searched: PubMed and PsycINFO. Google Scholar was used for acquiring articles from backward and forward referencing. Key terms were identified using previous relevant literature in the field and included: “maternal” OR “parental” AND “mental health” OR “depression” OR “anxiety” OR

"stress" AND "twins" OR "multiples". We included measures in mothers who used ART and natural conception, as previous research has suggested that there are no differences in levels of depression regardless of conception type (van den Akker et al., 2016; Vilska et al., 2009). The search was for English, French, and Portuguese articles. Articles were also published in peer-reviewed journals, and there was no restriction on the date of the studies. The initial search conducted in July 2021 yielded 439 articles. We included another 93 articles following an updated search conducted in September 2022.

Additional studies were retrieved by backward reference searching in previous meta-analyses or systematic review papers on the same topic (van den Akker et al., 2016). This search yielded another 14 studies not identified using keywords in previous searches, and Google Scholar was used to find these 14 articles.

Inclusion and Exclusion Criteria

Four criteria for inclusion were set up before conducting the searches. The first was that data from both mothers of twins and singletons were included in the article. Second, if the article used measures of distress in general, the operationalization of "distress" had to include depression. Third, the children (singleton or twins) had to have been born, and the measures of depression assessed six weeks after delivery when postpartum blues are less likely to be the cause (Pearlstein et al., 2009). Assessments taken during pregnancy were not included but assessments up to the preschool period were included. Finally, the articles had to have effect sizes reported or provide sufficient data to calculate the effect sizes. Our inclusion criteria would have been limited if only articles that had measures of depression, stress, and anxiety inclusively were retained. By focusing specifically on depression and not requiring measures of stress and anxiety, more articles were included in the current meta-analysis than the most recent meta-

analysis on this topic to our knowledge (van den Akker et al., 2016). Additionally, since both ART and naturally conceived twins were included in the search criteria this further increased the number of articles retained. The articles were initially reviewed based on the title and abstract by the first author (SS). Articles that were duplicates (n =166), not empirical (qualitative studies, reviews, and meta-analyses; n = 9), and were not relevant to the research question (n = 191) were excluded from the next step. The PRISMA flow diagram for new systematic reviews or meta-analyses was used throughout the screening process (Figure 1).

Quality Assessment

The National Heart, Lung, and Blood Institute created a Quality Assessment Tool for the Observational Cohort and Cross-Sectional Studies (*Study Quality Assessment Tools | NHLBI, NIH*, 2021), which we used to evaluate the quality of the articles from the search return. Two independent reviewers assessed the quality of the articles (SS, RD). Overall, there was moderate agreement (Rigby, 2000; Tang et al., 2015) between the two reviewers (κ = .534). Discrepancies were centred on suitable measures used to assess depression. A third reviewer (LB) mediated any discrepancy in quality assessment. We retained 14 studies of the 51 reviewed for data extraction.

Data Extraction

The data from the included articles were extracted and inputted into a spreadsheet to perform the required statistics. We used the effect size analysis method to weigh the individual results from the studies. The software chosen for this meta-analysis was Meta-Essentials (Suurmond et al., 2017). We used two effect size converters: Practical Meta-Analysis Effect Size Calculator and Psychometrica, to extract the correlation r from the studies. The programs were used depending on the type of data provided in each article. Effect sizes were calculated or retrieved regarding depressive symptoms in mothers of both singletons and twins.

Data Analysis

The random effects model was employed over the fixed effects model to obtain the combined effect size. The random effects model was preferred over fixed because in a random effects model, the results can be generalized outside of the population of the meta-analysis whereas in a fixed effects model, it should not be generalized (Borenstein et al., 2010). To examine the differences in symptoms between parents of twins and parents of singletons, we used the raw means and standard deviations reported in the papers to calculate the effect sizes. A conversion tool was then used to convert the scores to Cohen's *r*. Cochran's Q and the I^2 statistic were employed to assess heterogeneity across the articles (Cochran, 1954). Publication bias was assessed using a funnel plot and by interpreting Egger regression values (Egger et al., 1997).

Results

Fourteen studies were included (Table 1). The total sample size across studies ranged from 45 to 8,069, with most studies coming from North America. The age range of mothers across the 14 studies was between 19 and 45 years (See Table 1). The symptoms of depression were all based on well-known and validated self-report questionnaires. The depression assessments were taken when the twins were six weeks old up to 12 years old. The most commonly used measures included the Center for Epidemiologic Studies of Depression (CES-D: $n = 4$) or the Edinburgh Depression scale ($n = 4$) when assessing only depression and the general health questionnaire ($n = 3$) when assessing depression, except for one study that studied general mental health (Treyvaud et al., 2016).

Results are summarized in Figure 2. Heterogeneity was nonsignificant ($Q = 27.26$ & $I^2 = 52\%$), requiring no further inquiry. The association of differences in depression scores between parents of twins and singletons was small, but significant ($r = 0.08$, $p = .001$). We examined

publication bias using a funnel plot and an evaluation of Egger's test. For this meta-analysis, the funnel plot appears symmetric (Figure 3), and Egger's test was nonsignificant (t = -0.73, p = .48). These results suggest that publication bias did not impact the results of this meta-analysis in a significant way.

Discussion

Despite the emerging evidence of twin parenthood being a risk factor for mental health issues, psychological well-being in parents of twins remains understudied (Prino et al., 2016). The current meta-analysis showed that across studies, mothers of twins have higher symptoms of depression than mothers of singletons. Given that higher symptoms of depression can be associated with difficulties in bonding during critical periods of a child's development, this can negatively impact behavioural outcomes in children (Crugnola et al., 2020; Sampson et al., 2017). Our results highlight the importance of screening for mental health difficulties in the postpartum period and beyond, with a particular focus on mothers of twins.

Compared to the most recent meta-analyses (Van den Akker et al. 2016), we were able to include 75% more articles (n=14 vs. n=6). Although the obtained overall effect size in the present study across studies was small, our meta-analysis indicated that mothers of twins have, on average, higher depression symptoms than mothers of singletons. Results are in line with previous work (Van den Akker et al. 2016) and are of clinical relevance, since previous studies in singletons have shown that mothers who have high levels of postpartum depressive symptoms are more at risk for subsequent or sustained symptoms, potentially reaching clinical levels (Putnick et al., 2020). In mothers of twins, a study also found that, despite half of their sample supporting the need for mental health treatment, only 10% were offered treatment (Wenze &

Battle, 2018). All in all, our results highlight the need for more awareness regarding postpartum depression during pregnancy and onwards in individuals pregnant with multiples.

Higher symptoms of depression can be due to several psychosocial factors, such as twin parenthood putting enormous pressure on marital relations (Thompson & Henrich, 2022). Additionally, the financial pressure associated with having two babies is substantial. Sleep quality is also significantly worse postpartum, and poorer sleep quality is associated with more severe symptoms of depression and anxiety (Wenze & Battle, 2018). In sum, the psychosocial effects of motherhood may be experienced to a larger extent in mothers of twins, thereby suggesting that risk and protective factors should be screened for in the early postpartum period.

A fundamental aspect of motherhood is attachment to the new infant. Attachment to twins is considered more challenging than attachment to singletons due to delivery method, neonatal intensive care unit stay, infant temperament, and maternal age (Damato, 2004). Mothers who are experiencing depressive symptoms may have more difficulty with bonding due to the depression affecting their perception of their child (Lawless et al., 2014). Mothers of twins may also experience challenges when interacting with their newborns in comparison to mothers of singletons (Ionio et al., 2022). Mothers of twins may experience high parenting stress and state anxiety by trying to interact with both babies equivalently (Crugnola et al., 2020).

In conjunction with psychosocial risk factors, there also exists a host of biological factors that can be associated with postpartum depression due to the physiological changes experienced in pregnancy. During pregnancy with singletons and twins, several hormones are produced at an increased level, the subsequent drop after labour can be a biological factor that contributes to depressive symptoms (Skalkidou et al., 2012). Indeed, a study used a topical Estradiol cream to successfully treat severe postpartum depression (Gregoire et al., 1996).

Disrupted circadian rhythm cycles could also be a cause and consequence of depressive symptoms, specifically in the postnatal period, where sleep is generally lacking for most parents (Skalkidou et al., 2012). Although biological factors due to changes experienced during pregnancy would not explain enduring depressive symptoms into the preschool years, studies have shown that women who experience a depressive episode in pregnancy and the postpartum period are more likely to demonstrate future episodes of depression (Bradshaw et al., 2022).

The findings of the present study may be particularly relevant for women who have experienced a previous depressive episode before or during pregnancy (Silverman et al., 2017). One of the largest longitudinal population-based studies has found a 20-fold increased risk for an episode of postpartum depression if there is a history of depression (Silverman et al., 2017). Other risk factors identified in mothers of singletons include younger maternal age and preterm delivery (Silverman et al., 2017). History of depression, maternal age, and preterm birth are risk factors for mothers in general, expecting twins may put mothers of twins even further at risk. This would highlight the need for careful perinatal screening up to a year afterward, particularly if mothers experienced more difficult pregnancies, the twins were preterm, mothers had earlier episodes of major depressive disorders, were younger, and lacked social support. The worst possible outcome in untreated postpartum depression is maternal suicide, therefore treatment during the perinatal period is vital to reduce this tragic outcome (Cook et al., 2023).

The present meta-analysis has some limitations. The paucity of research indicates that although a problem is evident, mothers of multiples remain understudied. Thus, this limited the possibility to study the effect in a larger number of studies, and to include studies with more diverse and larger samples of parents of twins. Although advantageous that we examined only depressive symptoms to retain more studies, it was also a limitation due to possible

comorbidities and associations between depression and anxiety symptoms for example. Further, there is little to no research on the extent to which twin parenthood may also increase the risk for mental health difficulties in fathers. Fathers are relevant to consider since there is research highlighting the relevance of paternal depression in child development; additionally, marital quality is a risk factor for paternal depression given that maternal depression is the highest risk factor for paternal depression (Shorey et al., 2018; Thompson & Henrich, 2022; Wenze & Battle, 2018). Therefore, future research would benefit from studying not only mothers but fathers as well. Another limitation was that it was not possible to account for whether the mothers had other children at home or if this was their first time becoming parents. Future research is needed to examine the moderating role of the presence of other children and social support. Likewise, it could also be relevant for future research to account for the age of parents when the twins are born a young age is also a factor that can increase depressive symptoms in mothers (Silverman et al., 2017). Lastly, 85% of the included studies were cross-sectional. Longitudinal studies will help to study depressive symptoms over time and investigate the possible impacts of depressive symptoms on child outcomes.

In summary, the present meta-analysis found that mothers of twins report higher levels of depressive symptoms than mothers of singletons. Our findings contribute to the literature on maternal mental health and highlight the importance of mental health screening and preventative intervention in mothers of multiples as part of routine prenatal and postpartum care.

Table 1. Characteristics of Articles Included in the Meta-Analysis

Author Name	N *	Assessment Time Point	Maternal age (years)	Maternal depression measure	Effect size (r)**
Anderson et al., 2014	206	6-12 years	43-44	Adult Self-Report Anxious Depressed Scale	0.04
Choi et al., 2009	8069	9 months	18-35	CES-D	0.06
Ellison et al., 2005	249	1-4 years	35	CES-D	0.15
Glazebrook et al., 2004	260	1 year	29-34	General Health Questionnaire	0.16
Golombok et al., 2007	45	3 years	35	Edinburgh Depression Scale	0.18
Noy et al., 2014	274	2 years	32	Mental Health Inventory	0.04
Olivennes et al., 2005	688	2-5 years	36	Edinburgh Depression Scale	0.09
Riva Crugnola et al., 2020	112	3 months	34-35	Edinburgh Depression Scale	0.17
Roca-de Bes et al., 2009	123	6 months – 4 years	--	CES-D	0.01
Roca-de Bes et al., 2011	636	6 months – 4 years	35-38	CES-D	0.09
Sheard et al., 2007	147	6 weeks	33-34	Edinburgh Depression Scale	-0.12
Taubman-Ben-Ari et al., 2010	147	1 year	19-45	Mental Health Inventory	-0.02
Treyvaud et al., 2016	143	2 years	30	General Health Questionnaire	-0.09
Vilska et al., 2009	579	1 year	25-35	General Health Questionnaire	0.2

*Sample size includes mothers of twins and singletons

** Small ES: r =0.10, Medium ES: r =0.20, and Large ES: r =0.30 (Gignac & Szodorai, 2016).

Figure 1. PRISMA Flow Chart of Articles Retained in Meta-Analysis

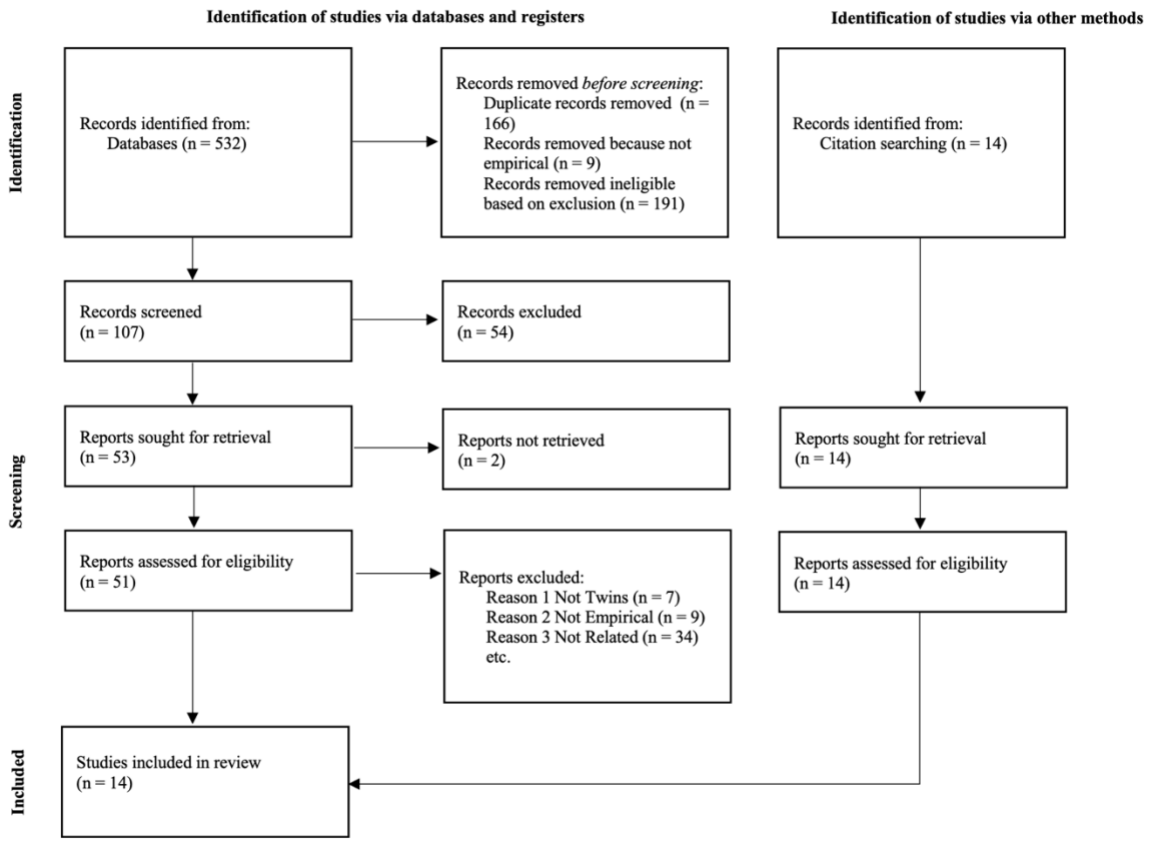


Figure 2. Forest Plot of Effect Sizes of Studies Included in the Meta-Analysis

Figure 2. Forest plot of effect sizes of studies included in the meta-analysis.

#	Study name	Correlation	CI Lower limit	CI Upper limit
1	Anderson et al., 2014	0.04	-0.10	0.18
2	Ellison et al., 2005	0.15	0.02	0.27
3	Glazebrook et al., 2004	0.16	0.03	0.27
4	Golombok et al., 2007	0.18	-0.13	0.46
5	Noy et al., 2014	0.04	-0.08	0.16
6	Olivennes et al., 2005	0.09	0.01	0.16
7	Riva Crugnola et al., 2020	0.17	-0.02	0.35
8	Roca-de Bes, et al., 2009	0.01	-0.17	0.19
9	Roca-de Bes, et al., 2011	0.09	0.01	0.16
10	Sheard et al., 2007	-0.12	-0.28	0.04
11	Taubman-Ben-Ari et al., 2010	-0.02	-0.18	0.15
12	Treyvaud et al., 2016	-0.09	-0.25	0.07
13	Vilska et al., 2009	0.20	0.12	0.28
14	Choi et al., 2009	0.06	0.04	0.08

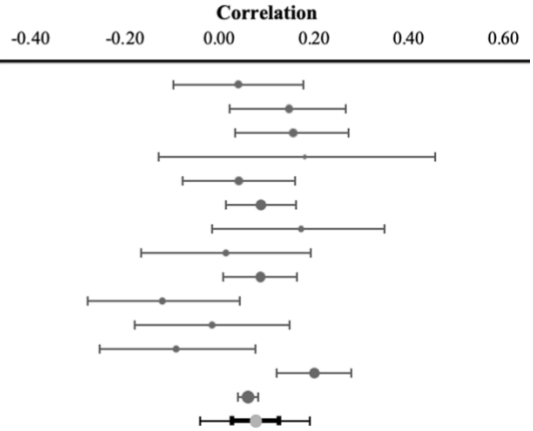
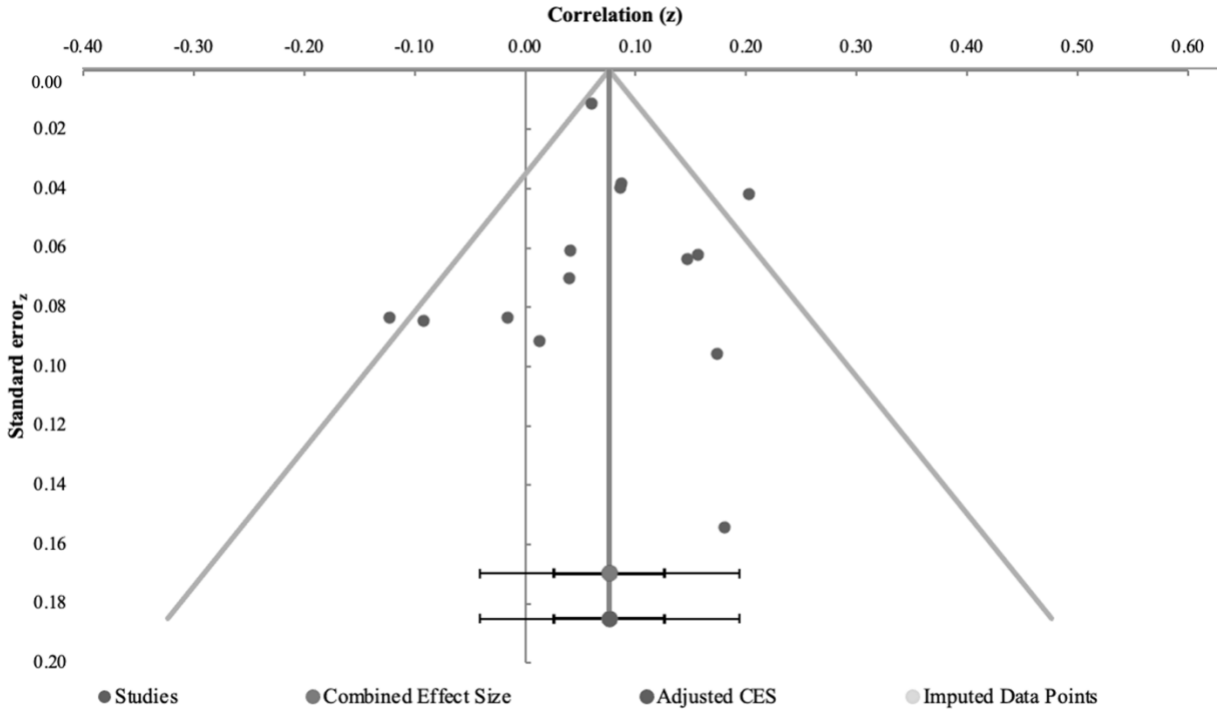


Figure 3. Funnel Plot of Publication Bias of Studied included in the Meta-Analysis

Figure 3. Funnel plot of publication bias of studies included in the meta-analysis.



Chapter 3: Trajectories of Maternal Depression Symptoms: A Longitudinal Study in Mothers of Twins and their Children

Manuscript in preparation

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Abstract

Postpartum depression is one of the most common mental health disorders experienced by women after having a child. Previous studies have shown that mothers of twins are at greater risk than mothers of singletons. However, most studies in parents of twins are cross-sectional and the evolution of maternal depressive symptoms in the first year after giving birth to twins has not been studied. This study used data from a longitudinal cohort of monozygotic twins which recruited 104 families and took depression assessments using the Center for Epidemiologic Studies in Depression (CES-D) at 3, 6 and 9 months postpartum. We also assessed temperament and behaviour in the twins at 12 and 24 months of age to study whether maternal depression trajectories could predict child outcomes. To model the evolution of depressive symptoms at three time points we used Proc Traj in SAS. We identified three trajectories of depressive symptoms in mothers of twins over the first nine months postpartum: low and stable (66.1%), decreasing (17.2%), and increasing (16.7%). Children whose mothers were on the increasing depressive symptoms trajectory tended to have higher levels of emotional difficulties (r=.226) and shyness (r=.239) (both p=.058). The findings of the present study highlight the importance of repeated screening for depression symptoms at various time points in at least the first year after giving birth to twins.

Keywords: Postpartum depression, twins, evolution, trajectories, DOHaD, behaviour

Introduction

Postpartum depression (PPD), defined by depressive episodes that occur within the postpartum period, is one of the most common mental health disorders experienced by women after having a child. Symptoms of PPD include sleep and appetite problems, loss of energy, feelings of worthlessness or guilt, lack of concentration, and suicidal thoughts (American Psychiatric Association, 2013; Pearlstein et al., 2009). Prevalence rates of PPD are estimated at 12 to 16.5% (Stewart & Simone, 2016). Approximately 20% of women will have depressive symptoms past the first year postpartum, 12% after two years postpartum, and 40% of women will relapse in future pregnancies or the future for causes unrelated to pregnancy (Stewart & Simone, 2016). Considering that maternal depression is a risk factor for future episodes of depression and may affect a child's emotional and cognitive development (Thompson & Henrich, 2022; Weissman, 2018), early screening and prevention of maternal depression are important.

In addition to cross-sectional studies focusing on clinical samples, various longitudinal studies conducted in community samples of mothers of singletons have described the evolution of depressive symptoms during pregnancy and after childbirth using trajectory modelling. For example, in one study that modelled the evolution of depressive symptoms from pregnancy to 60 months postpartum, five trajectories were identified: no symptoms, persistent intermediate depressive symptom levels, persistently high levels of depressive symptoms, high symptoms only in pregnancy, and high symptoms only in preschool period (van der Waerden et al., 2015). Subsequent regression analyses showed that maternal trajectories of depressive symptoms that were persistently high or material depressive symptoms occurring only in the preschool period predicted children’s emotional and behavioural difficulties at five years of age (van der Waerden et al., 2015). However, findings on how maternal depression trajectories predict child behaviours

have not consistently been replicated. For example, in another large longitudinal study, maternal depressive symptom course did not predict child outcomes (Guyon-Harris et al., 2016).

While the cause of maternal depressive symptoms is multifactorial, several psychosocial risk factors for high levels of depressive symptoms have been identified in the literature, including social support, marital difficulties, financial difficulties, and stress during and after pregnancy (Kehoe et al., 2016). Biologically, there are also hormonal fluctuations that occur after childbirth and women do differentiate based on their sensitivity levels to these hormonal fluctuations (Pearlstein et al., 2009). In addition to psychosocial and biological risk factors, a relatively understudied risk factor for maternal depression is the type of birth. Specifically, various studies have shown that parents of multiples experienced higher incidences of depression compared to parents of singletons (Wenze et al., 2015). In one meta-analysis, it was found that twin parenthood was associated with higher depression symptoms, irrespective of whether the children were conceived via natural conception or via assisted reproductive technologies (van den Akker et al., 2016). In a recent meta-analysis involving 14 studies, we further confirmed that mothers of twins have higher symptoms of depression compared to mothers of singletons (Smith et al., under review).

While the specific mechanisms of how raising twins could increase depression are unknown, one possible explanation is that raising twins may increase the postnatal parental psychosocial burden. For example, sleep deprivation, exhaustion, lack of personal time, stress, and financial difficulties – all shown to be risk factors for depression – may be more pronounced in parents of twins than in parents of singletons (Kehoe et al., 2016), while avenues to receive additional resources or benefits are often limited. Furthermore, studying the well-being of parents of twins is relevant, considering that in Western countries, the rate of twin births has

doubled (Martin & Osterman, 2019) (Wenze & Battle, 2018). Furthermore, most studies on depressive symptoms in mothers of twins during the postpartum period are cross-sectional, measuring symptoms at a single point in time. Whether or not depression levels remain stable or change over time throughout the postpartum period is unknown. Modelling the evolution of depressive symptoms over time in mothers of twins would allow us to better understand how often and at what time points screening for depressive symptoms in the postpartum period in mothers of twins would be important.

Previous research has also found that maternal depressive symptoms in infancy were associated with an increased likelihood of the children being diagnosed with depression themselves in adolescence (Murray et al., 2011). The proposed mechanism for this was insecure attachment in infancy and low ego resilience in childhood and adolescence (Murray et al., 2011). However, some studies also suggest that it is the duration and the severity of the symptoms that may be associated with unfavourable behavioural outcomes for children in the future (Sutherland et al., 2022). Thus, the importance of understanding and clarifying the evolution of depressive symptoms is two-fold, to inform intervention when mothers may be more vulnerable and to elucidate when exposure to depressive symptoms may be associated with behavioural outcomes in children in the future.

The primary aim of the present study was to characterize the evolution of maternal depressive symptoms over the first 9 months postpartum among mothers with twins. We also explored whether trajectories of maternal depressive symptoms between 3 and 9 months postpartum predict internalizing and externalizing symptoms in children at 12 and 24 months of age. We used data from the Projet des Ressemblances entre Jumeaux Monozygotes (PDIMOZY)

study, a longitudinal cohort of monozygotic twins recruited from and regularly followed since birth.

Methods

The study has been approved by the Human Research Ethics Committee at Centre Hospitalier Saine-Justine and the University Human Research Ethics Committee of Concordia University. All parents provide written consent and assent for their children before study participation.

Participants:

The present study used data from the PDIMOZY longitudinal cohort recruited in Quebec. There were 104 families of identical twins recruited between 2015 and 2019 from hospitals in Montreal, Quebec City and Sherbrooke. Twin families were recruited if the twins were born with no serious complications (e.g., twin-twin transfusion, serious medical complications resulting in extended NICU care, and medical conditions from birth such as heart abnormalities). Data were collected when the twins were 3, 6, 9, 12, and 24 months. A telephone interview was conducted with the mother when the twins were 3, 6 and 9 months old and included measures of family functioning, social support, and maternal depression. We also evaluated temperament outcomes when the twins were 12 months of age and behavioural outcomes when the twins were 24 months of age.

Measures:

The Center for Epidemiologic Studies Depression Scales (CES-D; (Radloff, 1977) was used to evaluate depressive symptoms post-partum at 3, 6 and 9 months. Participants were asked to indicate how many times during the week they related to the statements provided. Items included “I was bothered by things that usually don’t bother me” and “I felt depressed”. The

scale was evaluated on a Likert scale using "0= rarely or none of the time (less than 1 day), 1= some or a little of the time (1-2 days), 2= occasionally or a moderate amount of time (3-4 days) and 3= most or all of the time (5-7 days)". The CES-D is scored on a scale of 0-60 with a higher score indicating more symptomology of depression, a score of 16 or more is indicative of a risk factor for clinical depression (Lewinsohn et al., 1997). For this study, total scores on the CES-D were used to model the trajectories at 3 different time points: 3, 6 and 9 months postpartum (Cronbach's $\alpha = .759$).

The National Longitudinal Survey of Children and Youth examines child development and well-being. We assessed behaviour (See Appendix A) and temperament (See Appendix B) outcomes using the same questions as those from this longitudinal study (Comeau et al., 2020; Government of Canada, 2009). At 12 months of age, we assessed the temperament of the twins (Cronbach's $\alpha = .709$). The behavioural assessments were hyperactivity, inattention, hyperactive-inattention, emotional troubles, anxiety, separation anxiety, global physical aggression, pure physical aggression, prosocial behaviour, opposition, shyness, and depression (Cronbach's $\alpha = .877$). Behavioural measures were all taken at 24 months of age.

Analysis:

Latent class growth analysis was used to model the different groups of CES-D trajectories in mothers of twins. This was conducted using the Proc Traj program of SAS (version 9.4). The Proc Traj procedure provides an estimate of the percentage of individuals from the sample following each trajectory and the probability of the individuals belonging to the different trajectories. To identify the fit of the model, the Bayesian Information Criterion (BIC) was used; the closer the BIC is to 0 would indicate a better fit for the model. To find the

trajectory groups that fit the sample the best (see Table 2), several models were tested using a different number of groups (2, 3 and 4) and trajectory shapes (constant and linear).

CES-D total scores at the 3 timepoints which were coded in months (3 (T1), 6 (T2) and 9 (T3) months) were modelled in our sample. Across the time points, there were missing data for CES-D at 3 months (n=28), 6 months (n=22), and 9 months (n=14). Missing data were dealt with using the maximum likelihood method of estimation, which is standard in latent class growth analysis (Nagin, 1999). Missing data at any time point were verified using an ANOVA to examine if there was a relation between missing data and weeks of gestation and birth weight discordance. Overall, we found there was no significant difference in weeks of gestation and missing data in CES-D scores (F= 1.51, p=.222). We also found a significant difference in birth weight differences and missing data in CES-D (F= 2.87, p= .093).

Results

Sample Description:

Our sample consisted of 104 families, half of the sample was of Canadian and European origin (n=74; 71%), the majority of the twins were naturally conceived (n=90, 86.5%), and 54 pairs were female pairs (51%). The twins were born between 32 and 38 weeks *(*M= 35.2, SD=1.55). The twins had birth weights that ranged from 640g to 3180g (M = 2289.6, SD = 424.4).

Trajectories of symptoms of depression:

To find the model with the best fit for depressive symptoms in our overall sample, we tested several trajectories (see Table 2). Overall, four different trajectory shapes were tested, and although a four-group model with a constant linear shape had a lower BIC, visual assessment and associations with previous literature led us to conclude that the three-group linear model was the

best fit for our data. The three-trajectory group model was defined by a linear equation (p <0.01). There was a group with stable low symptoms of depression (66.1%), a group with decreasing symptoms (17.2%), and a group with increasing symptoms (16.7%; see Figure 4). The mean posterior probability for group one was 0.92, group two was 0.80, and group three was 0.93 which represents a good model fit (Nagin, 1999). Figure 1 showed that for group one the levels of depressive symptoms across time remained stable and low, for group two the levels of depressive symptoms started high at three months postpartum and decreased by nine months postpartum whereas, for group three depressive symptoms started low at three months postpartum and steadily increased until nine months postpartum.

Group assignment and children's behavioural outcomes:

We performed multiple regression analyses on the temperament data from when the twins were 12 months old and the behavioural data from when the twins were 24 months of age. Group assignment (one, two or three) were the predictors and the outcomes tested were several subscales (See Appendix A: hyperactivity, inattention, hyperactive-inattention, emotional trouble, anxiety, physical global aggression, physical pure aggression, prosocial behaviour, opposition, and shyness) from a questionnaire that assesses temperament and behaviour.

Our results indicated that there was no significant association between group assignment and temperament at 12 months and all behavioural outcomes tested at 24 months (hyperactivity, inattention, hyperactive-inattention, emotional trouble, anxiety, physical global aggression, physical pure aggression, prosocial behaviour, opposition, and shyness). Since there were no significant differences in scores on questionnaires within twin pairs (see Table 3), we took the average of the mean scores on the behavioural outcomes to examine the potential association with group trajectory. We noted that children whose mothers were on the increasing depressive

symptom trajectory tended to have higher scores on emotional trouble (β = -.704, *p*=.058, *r*=.226) and shyness subscales (β = -1.237, *p*=.058, *r*=.239; see Table 4).

Discussion

The present study investigated the evolution of depressive symptoms in mothers of twins in the first nine months postpartum, it was found that most of our sample maintained low and stable symptoms of depression over time (66.1%). For the remainder of the sample, some mothers began with high symptoms of depression that decreased with time (17.2%) or started with low symptoms of depression that increased over time (16.7%). At three months postpartum with twins, we would expect to see variation in depressive symptom levels. As previous literature has shown, the infancy period (the first three months postpartum) can be a time of stress for parents of singletons, and even more so for parents of twins (Prino et al., 2016). The same psychosocial and biological factors that may affect all women after having a child come into play here and the burden can be increased for parents of twins, especially if they are first-time parents (Kehoe et al., 2016; Prino et al., 2016). The infancy period is a time that is well known for frequent feedings, lack of personal time, lack of sleep, infant colic, and more (Kehoe et al., 2016; Prino et al., 2016; Twomey et al., 2012).

Our findings showed that the group with the highest levels of depressive symptoms at 9 months, affecting 1 out of 6 women, had a pattern of severity that was not stable but gradually *increased* over time, although unrelated to child outcomes at 12 and 24 months. Total scores on the CES-D at 9 months in this group were above 16, suggesting an increased risk of developing clinical depression (Lewinsohn et al., 1997). The importance of depression screening postpartum has been well-documented in the literature (Gjerdingen & Yawn, 2007). We and others have shown that mothers of twins are more at risk for depression than mothers of singletons (Van den

Akker et al., 2016; Wenze et al., 2015; Smith et al., under review). However, depressive symptoms as shown in the increasing trajectory, do not always present immediately postpartum. In certain individuals (e.g., 16.7% of the sample), depressive symptoms present later which would suggest that screening for depressive symptoms in mothers of twins should be done repeatedly rather than in the first six weeks postpartum. Additionally, this trajectory would suggest that offering resources may be more pertinent in later periods to mitigate depressive symptoms in those particularly at risk, as we show that depressive symptoms can be higher in later periods.

Our findings also showed that 1 out of 6 women were affected by depressive symptoms in the first three months postpartum (e.g., 17.2% of our sample). This trajectory would suggest that there is a potential risk of increased depressive symptoms in the first three months postpartum, which converges with the above point that pre-screening should be done repeatedly in the first year postpartum. The severity of the symptoms descended over time likely due to several factors. For example, protective factors such as partner and familial support, financial status, or availability of childcare. The third trajectory which comprised most of our sample (66.1%) shows a low and stable evolution of depressive symptoms. This trajectory suggests that not all women who are expecting and giving birth to twins will experience the same psychosocial outcomes as others. The current study adds to the literature on the well-being of parents of multiples by showing the importance of depression screening in mothers of twins repeatedly at various time points in time during the first year after giving birth, rather than at a single point in time. These trajectories also suggest that should intervention be considered, timely intervention will depend on when additional resources (e.g., financial aid or psychosocial support) are necessary which may be different for different individuals.

We did not find any significant association between temperament and behavioural outcomes in the twins and the group trajectory assignment. Results from some studies in singletons reported that maternal depressive symptom trajectories are predictive of behavioural outcomes, although the preceding finding has not been replicated consistently (Guyon-Harris et al., 2016). One possible explanation for the lack of association in the current study may relate to our study methodology. In the present study, behavioural outcomes in the twins were assessed by one parent rather than by an external observer. Parents of twins tend to compare them similarly, which in turn can bias their assessments of their children (Scourfield et al., 2004; Vitaro et al., 2009). Alternatively, it is possible that the measures may not have been sensitive enough to assess the risk for internalizing or externalizing symptoms at 24 months of age, as they are young and varied behaviour is typical of this age group due to developing executive functions (Hughes et al., 2020). We plan to continue to follow up with the cohort, which allows us to study risk for behavioural problems at the school age and onward. Additionally, the findings of near-significant associations between maternal depressive symptom trajectories between 3-9 months of age and children's emotional trouble and shyness at 24 months of age require further study. A possible interesting avenue of research in this context is further examining attachment style and interaction style between mother and child as possible mediating factors (Reck et al., 2016). Higher depressive symptoms may create barriers to bonding between mothers and their infants, and for mothers of twins, there is an increased pressure to bond equally with both babies (Crugnola et al., 2020), which in turn may particularly affect the development of internalizing problems in children. When depressive symptoms may be associated with bonding, children may experience more mood disorders and a more difficult temperament in the future (Slomian et al.,

2019) Future studies in larger samples should combine measures of depressive symptoms, child behaviour and bonding to test potential underlying mechanisms further.

Strength and Limitations

The current study is to our knowledge the first examining longitudinally the evolution of depressive symptoms in mothers of twins. The measure of temperament was taken at 12 months of age which allowed us to have an assessment of behavioural outcomes late in the first year, as well as measures of behaviour taken at approximately 24 months which allowed us to examine toddlerhood. The cohort remains active, allowing us to take follow-up behavioural measurements in the future. Finally, the sample is homogenous with the twins all considered relatively healthy, and there were no major health complications in the mothers at the time of giving birth.

This study has the following limitations. The sample size is relatively small and there was attrition at various time points which further extenuated the sample size issue. While having a homogenous sample in terms of zygosity and being relatively healthy, future studies are needed to examine to what extent findings can be generalized to samples with clinical populations and families with dizygotic twins. Additionally, given the episodic nature of depression including more time points could have been beneficial to examine the variation in changes in symptoms of depression in mothers of twins. Assessments every three months may not be sufficient to capture the variability that is typically seen in depression. Future research should also consider taking measurements in fathers, as maternal depression is a strong predictor of paternal depression (Shorey et al., 2018) and having one parent not experiencing depressive symptoms is a protective factor for the risk in child outcomes in the future (Martin et al., 2022).

Conclusion

Overall, we modelled the evolution of depressive symptoms in mothers of twins and found three trajectories. Within these three trajectories, we found that most mothers of twins experienced stable low symptoms of depression. One out of six mothers began with high symptoms at 3 months that lowered by 9 months and one out of six mothers started with low symptoms at 3 months that increased by 9 months. Along with findings that mothers of twins are particularly at risk for depression, results from the present study highlight the importance of repeated screening for depression symptoms at various time points in at least the first year after giving birth. Mothers on the trajectory of increasing symptoms over time may be at a higher risk for developing clinical symptoms of depression and may benefit the most from early preventive interventions.

Table 2. Model fit for depression symptom trajectories.

Model	Number of trajectories	Order (0= constant, 1 = linear, 2 = quadratic)	BIC
Identify number of groups:			
1	**3**	**1 1 1**	**-837.70**
2	4	1 1 1 1	-837.43
3	2	1 1	-841.88
Identify shape of trajectory			
4	3	0 1 1	-837.72
5	3	1 0 1	-838.42
6	4	0 1 0 1	-841.26
7	4	0 1 1 1	-836.71

Figure 4. CES-D trajectories at 3, 6 and 9 months postpartum (dashed lines are estimates, solid observed)

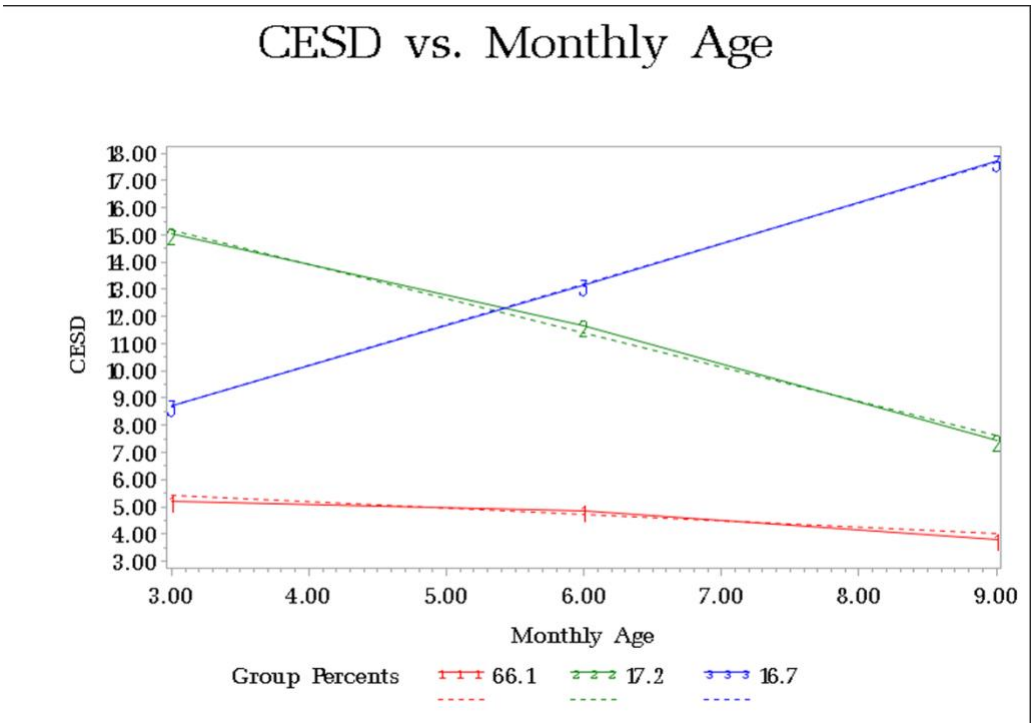

Table 3. Paired Samples T-Test of Within Twin Pair Differences on Behavioural Outcomes

Variable	Mean	Standard Deviation	t	df	Two-sided p
Hyperactivity	.214	2.41	.808	82	.422
Inattention	.359	2.46	1.31	79	.196
Hyperactive/Inattention	.240	2.21	.990	82	.325
Emotional Troubles	.261	1.71	1.39	82	.167
Anxiety	.062	2.38	.233	80	.816
Separation Anxiety	-.141	2.19	-.585	82	.560
Global Physical Aggression	-.155	1.84	-.765	82	.446
Pure Physical Aggression	.024	1.97	.112	82	.911
Prosocial Behaviour	.085	2.58	.298	80	.767
Opposition	.277	2.12	1.19	82	.238
Shyness	-.369	2.46	-1.37	82	.175
Depression	.163	1.94	.764	82	.447
Temperament	-.026	1.51	-.162	87	.872

Table 4. Behavioural variables: emotional trouble and shyness regressed on the group from trajectories.

Predictor	Unstandardized ß	SE	t	p	r	r^2	df
Behavioural emotional trouble							
Group 2 (Decreasing)	-.454	.401	-1.132	.261	.226	.051	(2,80)
Group 3 (Increasing)	-.704	.366	-1.921	.058	.226	.051	(2,80)
Behavioural shyness							
Group 2 (Decreasing)	-1.007	.705	-1.430	.157	.239	.057	(2,80)
Group 3 (Increasing)	-1.237	.644	-1.920	.058	.239	.057	(2,80)

Chapter 3: Supplemental Material

Appendix A: Behavioural Assessment Questionnaire

Web interview at 24 months (BEH). Study of resemblance between monozygotic twins (PDIMOZY)

Using the answers "Never or not true, Sometimes or somewhat true, Often or very true, I don't know or Refusal", please indicate how often Twin 1 or Twin 2 :

BEH-Q8B. Can't sit still, is restless or hyperactive?

BEH-Q8D. Will try to help someone who has been hurt?

BEH-Q8D1. Is shy with children he/she does not know?

BEH-Q8E1. Is defiant?

BEH-Q8EA. Kicks others?

BEH-Q8F. Seems unhappy or sad?

BEH-Q8GA. Gets into fights?

BEH- Q8I1. Is easily distracted, has trouble sticking to any activity?

BEH – Q8JA. Takes away things from others when they won't give it to him/her?

BEH-Q8J1. Doesn't seem to feel guilty after misbehaving?

BEH-Q8K. Is not as happy as other children?

BEH-8K1. Readily approaches children he/she does not know?

BEH-Q8N. Fidgets?

BEH-Q8NA. Pushes others to get what he/she wants?

BEH-Q8P. Can't concentrate, can't pay attention for long?

BEH-Q8Q. Is too fearful or anxious?

BEH-Q8R1. Punishment doesn't change his/her behaviour?

BEH-Q8R2. Takes a long time getting used to being with children he/she does not know?

BEH-Q8S. Is impulsive, acts without thinking?

BEH-Q8T1. Has temper tantrums or hot temper?

BEH-Q8U. Offers to help other children (Friend, brother or sister) who are having difficulty with a task?

BEH-Q8UI. Is shy with adults he/she does not know?

BEH-Q8UA. Threatens to hit others?

BEH-Q8V. Is worried?

BEH-Q8W. Has difficulty waiting for his/her turn in games?

BEH-Q8XC. When somebody accidentally hurts him/her (such as by bumping into him/her), he/she gets angry and tries to injure the other?

BEH-Q8Z1. Has angry moods?

BEH-Q8Z1A. Physically attacks others?

BEH-Q8BB. Confronts a child (friend, brother or sister) who is crying or upset?

BEH-Q8CC. Cries a lot?

BEH-Q8CCA. Is cruel to others?

BEH-Q8DD1. Clings to adults or is too dependent?

BEH-Q8EE. Gives up easily?

BEH-Q8EEA. Punches others?

BEH-Q8HH1. Cannot settle down to do anything for more than a few moments?

BEH-Q8HHC. Bullies others?

BEH-Q8LL1. Constantly seeks help?

BEH-Q8MM. Is nervous, high-strung, or tense?

BEH-Q8NNA. Bites others?

BEH-Q8PP1. Doesn't want to sleep alone?

BEH-Q8QQ. Is inattentive?

BEH-Q8QQ1. Readily approaches adults he/she does not know?

BEH-Q8RR. Has trouble enjoying him/herself?

BEH-Q8SS. Helps other children (friends, brother or sister) who are feeling sick?

BEH-Q8SS1. Takes a long time getting used to being with adults he/she does not know?

BEH-Q8TT1. Gets very upset when separated from parents?

BEH-Q8UU1. Helps those who do not do as well as he/she does?

BEH-Q8UUC. Hits (in the sense of slapping) others?

Appendix B: Temperament Assessment Questionnaire

Web interview at 12 months (TMP). Study of resemblance between monozygotic twins (PDIMOZY).

The following questions are about how Twin 1 behaves. Please answer them for him/her in comparison to others. "About average" means how you think the typical child would be scored.

TMP-Q1. How easy or difficult is it for you to calm or soothe twin 1 when he/she is upset?
- Very easy
- 2
- 3
- About average
- 5
- 6
- Difficult
- I don't know
- Refusal

TMP-Q3. How easy or difficult is it for you to predict when he/she will become hungry?
- Very easy
- 2
- 3
- About average
- 5
- 6
- Difficult
- I don't know
- Refusal

TMP-Q5. How many times per day, on average, does twin 1 get fussy and irritable – for either short or long periods of time?
- Never
- 1-2 times per day
- 3-4 times per day
- 5-6 times per day
- 7-8 times per day
- 10-14 times per day
- 15 times per day or more
- I don't know
- Refusal

TMP-Q6. How much does he/she cry and fuss in general?
- Very little; much less than the average baby/child
- 2
- 3
- Average amount; about as much as the average
- 5
- 6
- A lot; Much more than the average baby/child
- I don't know
- Refusal

TMP-Q7. How easily does he/she get upset?
- Very hard to upset (by things that upset most babies/children)
- 2
- 3
- About average
- 5
- 6
- Very easily upset (by things that wouldn't bother most babies/children)
- I don't know
- Refusal

TMP-Q8. When he\she gets upset (e.g., before feeding, during diapering, etc.) how vigourously or loudly does he/she cry and fuss?
- Very mild intensity or loudness
- 2
- 3
- About average
- 5
- 6
- Very loud intensity or loudness
- I don't know
- Refusal

TMP-Q19. On the average, how much attention does he/she require, other than for caregiving (feeding, bathing, diaper changes, etc.)?
- Very little
- 2
- 3
- Average amount
- 5
- 6
- A lot; much more than the average baby/child
- I don't know
- Refusal

TMP-Q20. When left alone, he/she plays well by him/herself?
- Almost always
- 2
- 3
- About half of the time
- 5
- 6
- Almost never – Won't play by self
- I don't know
- Refusal

TMP-Q33. Please rate the overall degree of difficulty your twin would present for the average parent.
- Very easy
- 2
- 3
- Ordinary, some problems
- 5
- 6
- Highly difficult to deal with
- I don't know
- Refusal

Chapter 4: Studying early environmental contributions on early brain development; a series of case reports on the MRI study participation experiences of monozygotic twins at the preschool age.

In preparation

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Abstract

The early environment comprising both the *in-utero* environment and the early life are critical periods in child development. A proxy for the quality *in-utero* environment is birth weight, whereby a lower birth weight would be an index of a suboptimal *in-utero* environment. Low birth weight has been associated with differences in behavioural, cognitive and brain outcomes in children in the future. A twin design allows researchers to control for the effect that genetics and shared environment may play in the association between birth weight and behavioural, cognitive, and brain outcomes in the future. In the present case report, a monozygotic (MZ) discordant twin design was employed in a longitudinal cohort of twins. Four-year-old twins were recruited to participate in a neuroimaging study. The twins were followed closely since birth, allowing for multiple waves of data collection to occur. Five families participated in a neuroimaging study following a play approach familiarization protocol. Preliminary results include an assessment of family functioning using the HOME questionnaire, as well as temperament and behavioural outcomes at 12 and 24 months. During the imaging session, the CBCL was used to collect behavioural data at four years of age by one parent. The present case report observes subtle differences within twin pairs in behavioural outcomes. Overall, the present case report suggests that neuroimaging in a pediatric population for non-medical purposes is feasible.

Keywords: birth weight, twins, discordant method, neuroimaging, behaviour, DOHaD

Introduction

Developmental Origins of Health and Disease (DOHaD) Framework

The DOHaD framework postulates that exposure to adversity in the prenatal and early postnatal periods can alter physiological aspects which increases the risk for disease (Gluckman et al., 2008; Van Den Bergh, 2011). Initially, the original hypothesis regarding DOHaD specified the critical period to be the fetal period, however, it has now expanded to include the earliest generation of cell division that occurs in pregnancy to the entire postnatal period, up to and including adolescence (Suzuki, 2018). Earlier versions of this hypothesis were specifically designed regarding physical health fields (e.g., cardiovascular trouble and metabolic issues; Suzuki, 2018). However, along with an expansion of the defining period where the effects of DOHaD can be observed, the framework has also been expanded to the mental health field (Gluckman et al., 2008; Suzuki, 2018; Van Den Bergh, 2011).

A term used synonymously with DOHaD is fetal programming, which occurs when early physiological alterations occur *in-utero* during gestation (Lieshout & Krzeczkowski, 2016; Suzuki, 2018). The prenatal environment has been linked to mental health and cognitive development outcomes in the future; whereby an unfavourable prenatal environment would increase the risk for future mental health issues and cognitive difficulties (Lieshout & Krzeczkowski, 2016). One of the most common negative prenatal exposures is stress *in-utero*, defined as psychosocial, cultural and environmental stressors that women experience during pregnancy (Coussons-Read, 2013) A study that examined the mental health trajectories of children exposed to several potentially negative prenatal exposures (e.g., low socioeconomic status and high levels of family stress) demonstrated that they were more likely to experience

high levels of behavioural problems and have more problematic trajectories whereby their mental health would continue to decline into adolescence (Tearne et al., 2015).

To objectively measure prenatal environments several birth size parameters can be evaluated as a proxy for the quality of the *in-utero* environment: birth weight (BW), head circumference, ponderal index, and length at birth. All of these parameters have been associated with future cognitive ability in children (Krishna et al., 2019). The most common proxy and widely used in the literature is birth weight (Martinussen et al., 2005; Skranes et al., 2013). Low birth weight has been associated with malnutrition, maternal depression and stress, exposure to certain toxins, and most significantly social disadvantage (Lewis et al., 2014; Luby et al., 2023). However, an important challenge that studies examining the effect of environment on brain development experience is discriminating the possible effect of the environment and genetics (Chiarella et al., 2015). Individuals exposed to a suboptimal *in-utero* environment may not only differ environmentally but also genetically from other individuals who did not experience the same level of *in-utero* adversity (Teh et al., 2014).

In-utero Environment and Birth Weight Discordance

To differentiate genetic from environmental contributions a monozygotic twin design can be employed since they are the ideal and naturally occurring sample that allows researchers to control for genetics and shared environment. Monozygotic (MZ) twins are assumed to share nearly all of their genetic sequence. Furthermore, early in life in most cases they also have overall similar shared environments. Therefore, when utilizing monozygotic twin designs researchers can attempt to measure the effect of the non-shared environment on an association (Chiarella et al., 2015; Kato et al., 2005). As a widely used and accepted proxy for *in-utero*

environmental exposures, we can examine birth weight discordance within monozygotic twin pairs (Salafia et al., 2008), where the twin with the lower birth weight would indicate a less optimal *in-utero* environment compared to their higher birth weight cotwin. Birth weight discordance within a twin pair can at times predict discordance in behavioural and cognitive outcomes in the future (Chiarella et al., 2015).

Birth Weight Discordance Association with Behavioural, Cognitive and Brain Development

Behavioural and cognitive outcomes may be associated with adversity *in-utero* or in early life. Birth weight discordance has been associated with differences in behaviour within a twin pair. For example, in a population-based study that assessed symptoms of attention deficit/hyperactivity disorder (ADHD) at two time points, one when the twins were eight- to nine years old and again when they were 13 to 14. The lower birth weight twin scored higher on average at both assessments on symptoms of ADHD compared to their cotwin with a higher birthweight (Hultman et al., 2007). Other research, focusing on the association between BW discordance in MZ twins and cognitive outcomes, showed that the lower birth weight twin performed less well on measures of intelligence testing than their higher birth weight cotwin (Newcombe et al., 2007; Ross et al., 2012). These differences in behaviour and cognition appear to endure from childhood into adulthood (Van Os et al., 2001) and have also been found when the twins' birth weight is considered to be within a normal range (Newcombe et al., 2007; Ross et al., 2012; Van Os et al., 2001).

Concerning brain development, previous research has found that in monozygotic twin pairs between the ages of three and thirty, birth weight discordances predicted differences in cortical surface area (Raznahan et al., 2012). Specifically, the twin with the lower birth weight

had a smaller cortical volume compared to their higher birth weight cotwin (Raznahan et al., 2012). In research conducted in our lab, we have found that birth weight discordance is associated with differences in brain development in adolescent monozygotic twin pairs, where the lower birth weight twin has a smaller overall brain volume than their cotwin (Casey et al., 2017). At the regional level, the lower birth weight twin had lower total gray and white matter volumes in the superior frontal cortex and the thalamus compared to the higher birth weight twin (Levesque et al., 2015). More recently, we observed differences in limbic network functional connectivity in MZ adolescent twins. Specifically, we found that the lower birth weight twin had less connectivity in the right hippocampus and amygdala than their high birth weight cotwin (Hayward et al., 2020).

To summarize, in some cases negative *in-utero* experiences may be associated with problematic behavioural and cognitive outcomes within twin pairs, as assessed via lower birth weight. These differences in developmental outcomes have been expanded to include neurodevelopment, whereby brain development in a lower birth weight twin can at times differ from the higher birth weight cotwin. Little is known about the association between *in-utero* environment and brain development at the preschool age. Studying the preceding association at this age is important because the brain is in a critical period of development, Furthermore, the role of early life on the neural outcome is studied closely before other environmental changes occur. Although brain development can be studied in various ways, Magnetic Resonance Imaging (MRI) is of most interest, since (unlike EEG) this technique allows for studying brain structure, function, and connectivity between brain regions. However, there are several challenges when doing MRI research in children. Namely, it is difficult for children under the age of six to follow instructions and remain immobile without sedation (Barkovich et al., 2019).

Older infants and toddlers are at an age where they are more aware of their surroundings and have greater reactions to noises (Barkovich et al., 2019). Obtaining high-quality scans with limited head movement is thus essential. The latter can be even more challenging in the case of a twin design where within-pair differences are the primary outcome (i.e., requiring high-quality scans in both twin members to have valid data for subsequent analyses). Thus, when conducting MRI research in twins, having a protocol in place that minimizes discomfort is particularly essential.

Present Study

The overall aim of the present study is to present the first pilot data of five pairs of MZ twins that participated in an MRI study. The twins are part of a cohort of MZ twins that are regularly being followed since birth. As such, before the children participated in the current brain-imaging study, data on the family environment at 1, 12 and 24 months after birth using the home observation of the environment (HOME) interview was collected, as well as data on the twin's temperament and behaviour. There are also several waves of data collection on postpartum maternal mental health, perception of twin behaviours, nutrition, accidents, and illnesses.

In this case report we describe MRI results from the scans that were taken at 4 years of age. We also describe our experiences with the familiarization protocol to acclimatize the twins to what will happen and conduct the scan in the twins without sedation (Thieba et al., 2018). We also present data on twins' experiences with the MRI and results of the Child Behaviour Checklist (CBCL) that parents completed for each twin during the MRI session.

The current study was a pilot for a larger project funded by the Canadian Institutes of Health Research, examining the role of *in-utero* environment in early brain development and associated epigenetic processes (PI: Booij). For the larger study, recruitment was paused due to the global COVID-19 pandemic and subsequent renovations of the MRI facility where the imaging takes place. The pilot data described below informs the continuation of the larger study, for which data collection is expected to resume in early fall 2023.

Methods

The study has been approved by the Human Research Ethics Committee at Centre Hospitalier Sainte-Justine (Ste Justine Hospital) and the University Human Research Ethics Committee of Concordia University. Before participating in the study, parents are sent a detailed consent form outlining the project, what their participation will entail, the confidentiality of their data, their rights to withdraw at any point as well as the compensation agreement. All parents provided written consent and assent for their children before study participation.

Participants

The Projet des Ressemblances entre Jumeaux Monozygotes (PDIMOZY) cohort was recruited from hospitals in Montreal; Centre Hospitalier Universitéé (CHU) Sainte Justine (47 pairs), CHU Montreal (CHUM; 11 pairs), McGill University Health Centre (MUHC; 15 pairs) as well as in Sherbrooke at the CHU Sherbrooke (4 pairs) and in Quebec City at the CHU Quebec (CHUQ; 27 pairs). Recruitment of more MZ twin pairs is currently ongoing. The current attrition rate in this cohort is 3% where 3 families have withdrawn from the cohort making the current retention rate 97%. The PDIMOZY cohort is comprised of 104 monozygotic twin pairs where 51

pairs are male, and 53 pairs are female. The twins were born between 32 and 38 weeks of gestation, where 80 were born before 37 weeks and 24 were born between 37 and 38 weeks. This cohort had various birth weight discordances present; we categorized them as high (20% discordant or more) and low (10% discordant or less). In the low discordant group, there were 58 twin pairs and in the high group, there were 46 twin pairs, nearly an even split. The twins in the dataset are categorized based on birth order, where twin one was the firstborn and twin two was the second. Birth weight for twin one was between 1375-3180g, and for twin two birth weight was between 640-3040g. Most of the sample were conceived naturally (*n*=90, 86.5%) and were of Canadian European descent (*n*=74, 71%).

The Quebec Newborn Monozygotic Twin Study (QNTS) cohort recruited participants across Quebec, where all MZ twin births beginning in April 1995 until December 1998 were reported to the registry. All families were contacted, and appointments were scheduled when the twins were between 59 and 61 weeks old (Boivin, et al., 2005). The first wave had a sample size of 662 families. Brain imaging data was taken when the twins were 15 years of age, where 52 MZ twins participated (Casey et al., 2017). The imaging data from the QNTS cohort as described by Casey et al. (2017) were used for comparisons between outcomes in the four-year-old twins and the adolescent twins.

Data Collection

The twins have been followed closely since birth, with the first waves of data collection occurring at 1 month of age. Specifically, at 1, 12 and 24 months of age, the HOME interview was conducted with the families by a nurse who visited the family in their home environment. At 3-, 6-, and 9-months data on illness and accidents, nutrition, family environment, maternal

mental health, and parenting/parental cognitions was also collected. Temperament was assessed in the twins at 12 months of age and behaviour at 24 months of age. There was also data collection on maternal mental health and parental hassles at approximately 36 months of age. At 4 years old, we commenced recruitment for the MRI study where we had five families that participated. For these families, we have MRI data for four of them, CBCL for all five of them, as well as data on the familiarization protocol and general enjoyment of the study.

Case Reports on Five Families

Families in this cohort are identified by ID number, for this paper, we will be referring to them as Family 1, 2, 3, 4, and 5. Within these five families, family 2 did intend to participate in the study, however, the twins were too scared on the day of the scan and therefore did not complete the MRI portion of the study. These families were considered eligible after a phone interview was conducted and they passed the eligibility criteria (see Appendix A). Before participation, each family was emailed the consent form to read over, if they had any questions, they were strongly encouraged to contact the researcher (n = 1). They were also sent a brochure (Appendix B) to commence familiarization with the MRI in the twins. Along with the brochure, we attached an MP3 file with MRI sounds to have the twins listen to for about a week before the MRI to begin acclimating them to the sounds of the machine.

Familiarization Protocol

Upon arrival at the hospital, the trained researchers brought the family into the MRI waiting room where the familiarization protocol continued. The protocol begins by telling the children a story about Youpala the turtle and his friends that are in a submarine trying to find

their friend (See Appendix C for images). The images in the story are of the Sainte Justine Hospital, while reading the story the researchers point at places in the building that the children might have recognized from their arrival to make them more comfortable. While reading the story, the researchers used some props including a wood-play version of an MRI and some turtle puppets (See Appendix C). While reading the story the children held the puppets and put the turtle into the MRI as it happens in the story. During the story, the children are again exposed to the sound sequence of the MRI, because Youpala the turtle will be hearing the MRI as well. The researcher put the headphones onto the children and played an MP3 of the MRI sounds again to habituate the children further. The researcher also pointed out the hospital robe Youpala wears when he enters the submarine as this is the same robe the children will be wearing when they go into the MRI. The story, listening to the MRI, and playing with the wood MRI lasted a total of 10 minutes.

Once the story was completed, we allowed the children to play with the puppets and the toy MRI at their leisure to habituate the children to the imaging process (5 minutes). The children then selected a film of their choice to watch during the MRI, where they had access to a screen as well as headphones to further block out any noises. To assess how the familiarization worked, the children were asked to rate their experience and the story using the scale found in Appendix D. The protocol has been used in past research and is effective in habituating children to the MRI as well as time efficient (Thieba et al., 2018).

While one twin was in the MRI, the other research assistant was with the parent and the other twin. During that time, the research assistant was playing with the other twin and helping them feel more comfortable while the parent completed the CBCL for each twin. When it was time to switch, the research assistants attempted to do this as swiftly as possible. Once the MRI

was done, and the child was back in the waiting room, the child was asked how the scan went and asked to rate their experience (2 minutes) (Appendix E). Once this was completed the children were given a gift for participating and were allowed to play freely. When the last twin was done, they also got to rate their experience and were given a gift as well. In total, the familiarization protocol took 17-20 minutes.

Measures

Home Observation Measurement of the Environment (HOME).

The HOME questionnaire (Bradley & Caldwell, 1977) was created to assess how the child's immediate environment is associated with their cognitive development. It also addresses a discrepancy between socioeconomic status and child development, as well as offers unique information to create intervention measures (Black et al., 2007), if necessary, while also identifying the areas of immediate need (Valadi et al., 2020).

The infant-toddler HOME was used to study the family environment. Trained nurses went to the families' home and administered the HOME when the twins were 1, 12 and 24 months of age. The measure has 45 items and 6 subscales that are observed by trained nurses. Items 1 through 11 comprise the subscale of emotional and verbal responsivity of the primary caregiver; this subscale assesses the interaction and quality of the interaction between the primary caregiver and the child in question by observation of the nurse. The second subscale comprising items 12 through 19 assesses the avoidance of restriction and punishment which the nurse observes how the adults choose to discipline their children. Items 20 through 25 comprise the organization of the physical and temporal environment. This subscale specifically includes observations on how the child's time is devoted outside of the family and home, as well as how

their personal space appears. Items 26 through 34 include the provision of appropriate play materials, observing the presence or absence of toys and if the toys are age-appropriate or educational. The subscale of parental involvement with the child includes items 35 through 40 of the scale which observes how the parent interacts physically with the child. Finally, the last subscale observes the opportunities for variety in daily stimulation. This subscale includes observations on whether the child's routine has been designed to allow for alternate social interaction rather than just with the mother.

All items are responded to with a yes or no answer option. The items are then scored. There are no specific cut-off points established; however, a summary sheet (Bradley et al., 2003) indicates that scores falling in the lowest quarter present a home environment that is of risk to the child. In this preliminary set of case reports, we have results from the HOME subscale of communication and implication at 1, 12 and 24 months.

Behavioural and Temperament Assessments

The National Longitudinal Survey of Children and Youth is a long-term study in Canada that examines child development and well-being at multiple time points throughout their life. Temperament at 12 months (Appendix H) and behaviour outcomes at 24 months (Appendix G) were assessed using the same questions as those from this longitudinal study (Comeau et al., 2020; Government of Canada, 2009). Outcomes were separated as in Forget-Dubois et al.; calculating the mean of all the 3-point Likert scale items of physical aggression, hyperactivity/impulsivity, and opposition (2007). Examples of items included in the physical aggression subscale are: How often does your child bite? Do they kick others? Or do they physically attack others? (See Appendix G for full measure). Examples of items in the

hyperactivity/impulsivity subscale are: can your child sit still? Do they fidget a lot? Are they impulsive? Finally, in the opposition subscale, some included items are does your child feel guilty after misbehaving? Does punishment change their behaviour? Is your child defiant?

Parental Cognitions and Conduct Towards the Infant Scale (PACOTIS)

The Parental Cognitions and Conduct Towards the Infant Scale (Boivin et al., 2005) was used to assess aspects of parental behaviour with their twins at approximately 3, 6, and 9 months postpartum. This questionnaire has 28 items with four main scales: parental self-efficacy, perceived parental impact, parental hostile-reactive behaviours, and parental overprotection. The measure was completed by one parent. Each item in this questionnaire is assessed on a 0-10 Likert scale. This scale has a Cronbach's α between .68 - .82 in our overall sample of 104 MZ twins (Boivin et al., 2005; Forget-Dubois et al., 2007).

Child Behaviour Checklist (CBCL)

The Child Behaviour Checklist (Achenbach, 1999) was used to assess child behaviours as well as language development. The questionnaire took between 10 and 15 minutes to complete. The form was completed by the primary caregiver present on the day of the MRI.

The questionnaire includes 100 items asking about behaviour in the child within the last two months. The items are scored with a 0 (not true as far as you know) a 1 (somewhat or sometimes true) or a 2 (very true or often true). The scoring of the items is divided into eight subscales, Emotionally Reactive (α= .73), Anxious and Depressed (α = .66), Somatic Complaints (α = .80), Withdrawn (α = .75), Sleep Problems (α = .78), Attention Problems (α = .68), Aggressive Behaviour (α = .92), and Other Problems. The first four subscales identify

internalizing problems, sleep problems are a category on their own, and the last three identify externalizing problems. Therefore, the scores for internalizing and externalizing behaviours can be computed separately, as well as the total problems behaviour score (α = .95) can also be computed. When scoring the internalizing (α = .89), and externalizing disorders (α = .92), researchers and clinicians can use the raw score to obtain a T score. The T score is comparable across the literature for different questionnaires as well. Specific cut-offs are established here, where a T score of 60 to 63 is considered on the edge of clinical significance. A T score of 63 or higher is considered in the clinical range. However, the CBCL alone cannot be the only tool to determine the need for clinical intervention. The range of scores for total problematic behaviours is between 0 and 200.

McMaster Family Assessment Device

The McMaster Family Assessment Device (Epstein et al., 1983) assessed family functioning based on seven dimensions: Problem Solving, Communication, Roles, Affective Responsiveness, Affective Involvement, Behaviour Control and General Functioning. For our study, only the General Functioning dimension was used. This dimension has one item from Problem-Solving, four from Communication, two from Roles, one from Affective Responses, three from Affective Involvement, and one from Behavioural Control. The scale is rated between 1 and 4 indicating agreement or disagreement with the 12 statements. All odd items are reverse scored. Once items are reverse scored, the 12 items can be added together to make a total. This dimension has a Cronbach's α = .92, the highest of all dimensions.

MRI

The MRI obtained images of both brain structure and white matter integrity. This was done using a GE 3T scanner at CHU Sainte Justine.

The first scans were anatomical which lasted on average 8 minutes and included whole brain anatomical T1-weighted gradient echo (FSPGR BRAVO, TE=3.16ms, 190 axial slices, FOV = 240mm, voxel size=0.8mm^3) and multi-slice T2-weighted fast spin echo (TR=3200ms, TE=499ms, 120 sagittal slices, FOV=256mm, voxel size=1mm^3).

To obtain images on the white matter integrity Diffusion Tensor Imaging (DTI) was done and lasted 10 minutes. The DTI included 32 diffusions encoding directions, 2mm isotropic voxel size, 63 slices, b=1000 s/mm^2, TE=98ms, TR=12s and parallel reconstruction. Images were controlled for slice-based signal-to-noise and motion artifacts. Also included are resting phase sequences (4 minutes) after the anatomical and DTI scans.

Preliminary Results

Characterization of Case Report Families

In the five families that participated in the neuroimaging study (See Table 5), there were 4 pairs of male twins and 1 pair of female twins. The twins had birth weight discordances that ranged between 10g – 960g. The twins were all born from 33 to 37 weeks of gestation. Overall, all the twins had excellent health at the 9-month-old assessment. Maternal depressive symptoms reported in Table 5 are based on our results from Study 2. In this sample, two families had mothers who followed the low and stable course of depressive symptoms, one followed the decreasing trajectory (higher symptoms at three months and lower at nine months) and finally,

two followed the increasing trajectory (lower symptoms at three months and higher at nine months). McMaster Family Assessment device indicated a stable home environment, as well, as HOME communication and implication scores in these five families, indicate that the home environment was above average (See Table 5). The results for the PACOTIS by family and twin are summarized in Table 6. Overall, on the PACOTIS we do not see any noticeable differences within twin pairs. Higher scores on the parental overprotection subscale are considered negative.

Behaviour and Temperament

Temperament assessments were taken when the twins were 12 months old, and behaviour assessments were taken at the target age of 24 months old. Overall, there is minimal variability in temperament and behavioural outcomes within twin pairs. There is some variability between twin pairs, in the five families that participated. The individual questionnaire scores per twin are reported in Table 7.

Familiarization Protocol

Overall, of the five families, four took part in the MRI. In the four pairs that did, all the participants indicated that they very much enjoyed the MRI (M=4.5, SD=0.76). The participants also indicated that they found the MRI a little to a lot noisy (M=2.87, SD=1.64), but 75% of the participants responded that they would like to go in the MRI again. The participants were also asked if they were excited for the MRI to end, they were a little and a lot excited for it to end (M=3, SD=1.6). The participants enjoyed being able to watch a movie during the MRI (M=4.6, SD=0.5) and 87.5% indicated they could see it well and 100% indicated they could hear the movie well. The participants also enjoyed the story about Youpala the turtle for the

familiarization protocol (*M*=4.4, *SD*=1.41). The individual score for the twins is available in Table 8.

MRI Results:

Figure 5. Anatomical Brain Images for Family 1: Twin 1 and Twin 2

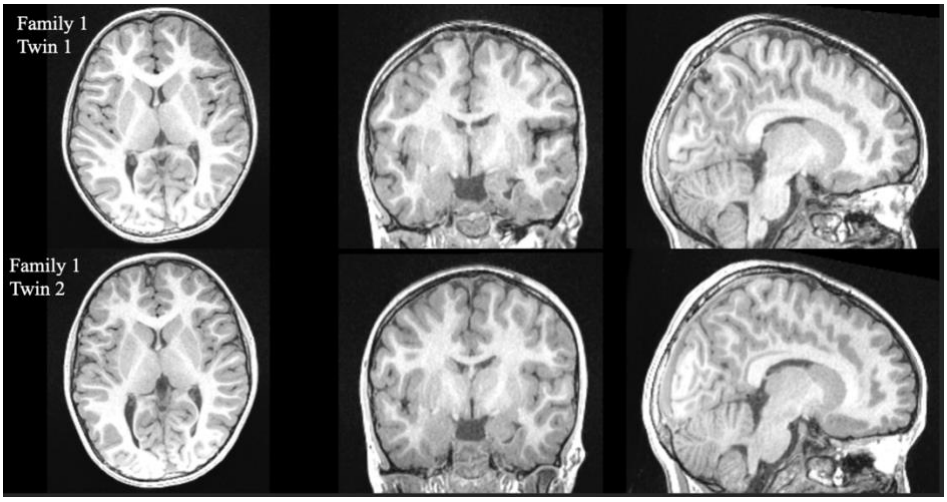


Figure 6. Anatomical Brain Images for Family 3: Twin 1 and Twin 2

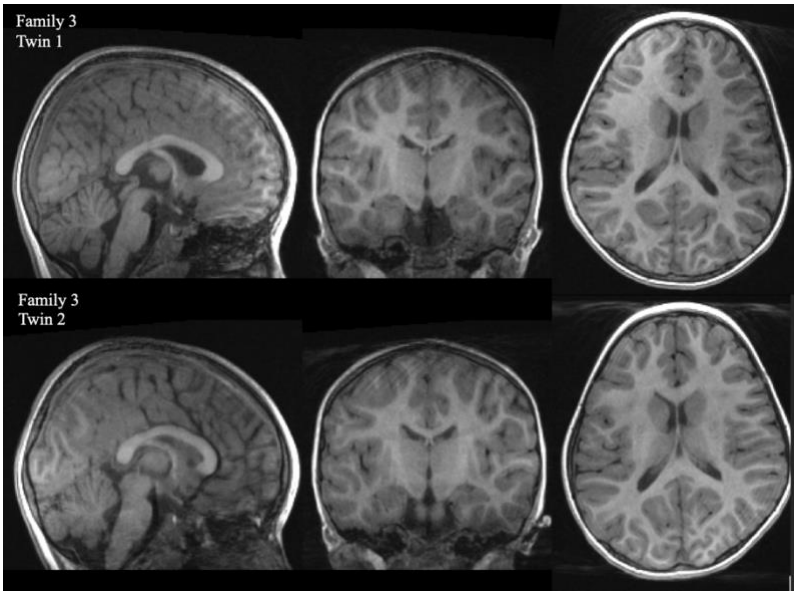


Figure 7. Anatomical Brain Images for Family 4: Twin 1 and Twin

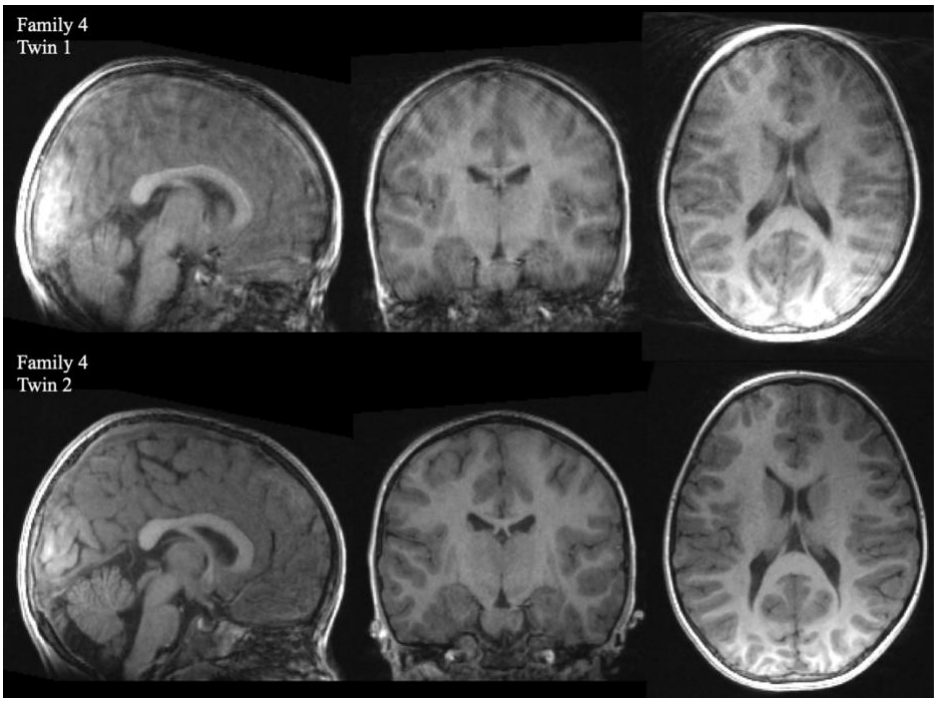
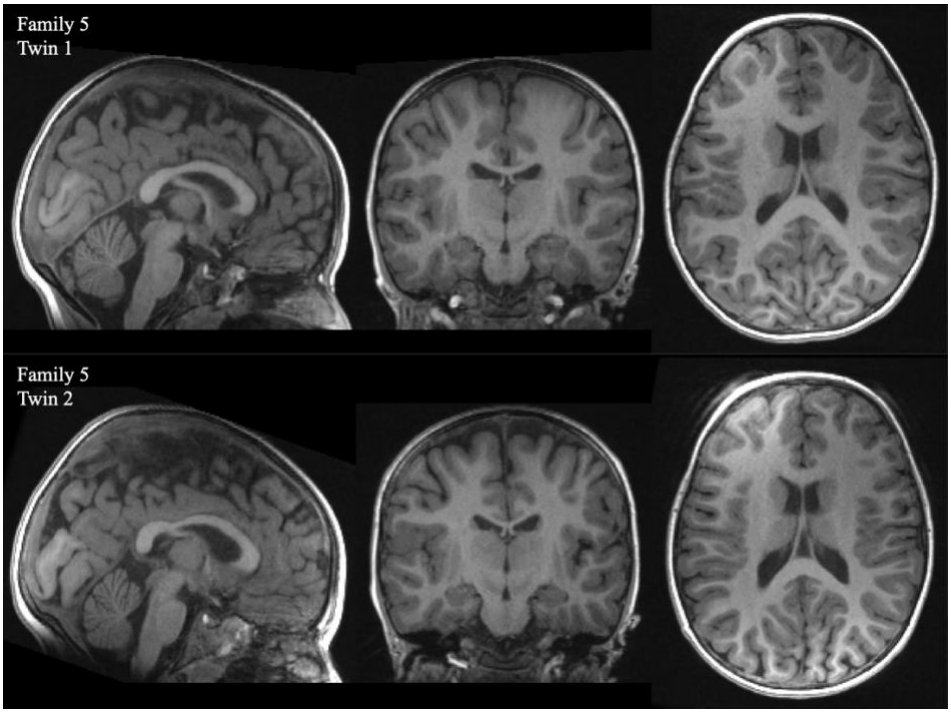
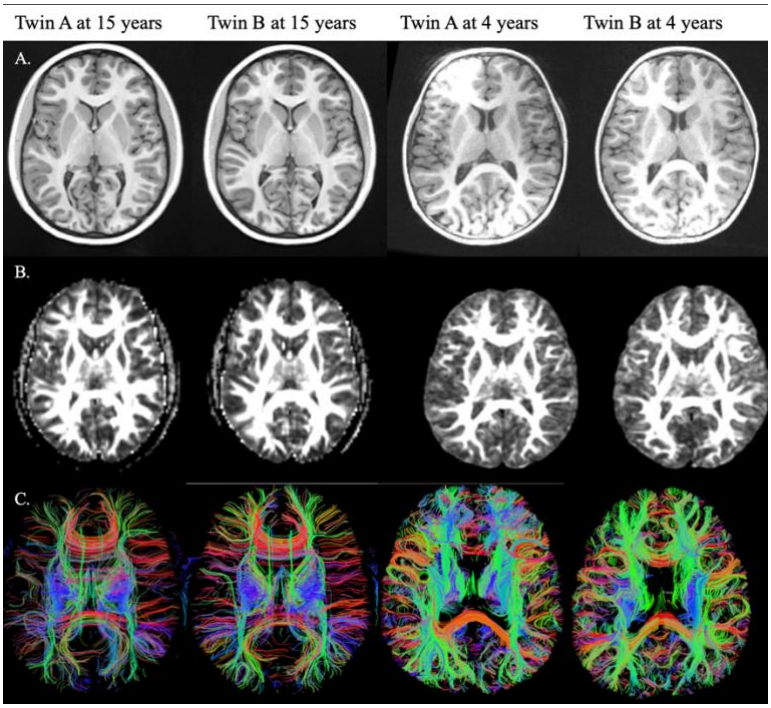


Figure 8. Anatomical Brain Images for Family 5: Twin 1 and Twin 2



Quality metrics indicate high contrast, sensitivity, low blurring, low noise, and absence of artifacts. Given that there were not enough twin pairs to make formal statistical comparisons, the results here are suggested upon visual inspection. Overall, and in line with the brain development literature, the four-year-old twins appear to have less distinction between grey and white matter, and some differences can be seen visually in brain areas within twin pairs. In the future, comparisons will be made within twin pairs and between twin pairs at four years of age and fifteen years of age. The data from the MZ twin pairs at fifteen years of age were collected from a previous study conducted in our lab and were also taken using a 3T scanner (Levesque et al., 2015). In Figure 9, some preliminary comparisons between twin pairs at four years of age and fifteen years are shown.

Figure 9. Comparison between twins four years of age and fifteen years of age.



Note: A. Brain structure T1-Anatomic, B. Fractional Anisotropy, C. Fibre Tracking.

Comparisons of brain structure suggest that when twins are fifteen years of age there is a better distinction between white and grey matter, whereas as expected at four years since the

brain is in development, we do not see such distinction. Fractional anisotropy indicates the degree of flow of water in the direction of white matter pathways, in these images there are no distinct differences in the brain at fifteen years of age and four years of age. Finally, there is fibre tracking, which is where most differences between twin pairs can be observed. Fibre tracking monitors the probabilistic traces of white matter in the brain. Overall, it can be observed that in the fifteen-year-old twins there are far fewer connections but much longer, whereas in the four-year-old twins, we can see many connections, but they are shorter. The fibre tracking conforms to what would be expected as differences between the fifteen-year-old twins and the four-year-old twins as at fifteen years old, neuronal pruning would have occurred.

Child Behaviour Checklist Results

Family 1

Overall CBCL profiles of each twin can be found in Appendix F. For one twin, scores on internalizing behaviours were raw score 17 and T score 63, externalizing behaviours was raw score 26 and T score 65, for a total of raw score 63 and T score 65. As can be seen in the graph (Twin 1=higher BW twin), scored in the clinical range for withdrawn behaviours and in the borderline clinical range for aggressive behaviour. For the other twin, they had a raw score of 15 and a T score of 61 for internalizing behaviours, followed by a raw score of 23 and a T score of 62 for externalizing behaviours. These summed for a total raw score of 58 and T score 63. As

can be seen in the graph (Twin 2= lower BW twin) this twin scores in the normal range on all evaluated profiles.

Family 2

On the CBCL, one twin had a raw score of 20 T score of 65 on internalizing behaviours, a raw score of 7 and a T score of 43 on externalizing behaviour, for a total raw score of 44 and a T score of 57. As can be seen in the graph (Twin 2= higher BW twin), this twin scored in the borderline clinical range for emotionally reactive. They also scored in the clinical range for anxious/depressed and sleep problems. Following this, the other twin had an internalizing behaviour raw score of 9 and T score of 53, and an externalizing behaviour score of 16 and a T score of 55 which, gives a total raw score of 39 and T score of 54. From this graph (Twin 1=lower BW twin) we can see that this twin scored in the normal range on all profiles.

Family 3

On the CBCL, both twins were within the normal range on all the dimensions (see Appendix F). Overall, one twin had a total raw score of 4 and T score of 43 on internalizing behaviours, they also had a raw score of 3 and a T score of 37 on the externalizing behaviour component. This added up to a total score of 17 and T score of 41. For the second twin on internalizing behaviours, they had a raw score of 6 and a T score of 41, on externalizing behaviours they had a raw score of 10.5 and a T score of 48. Twin two had a total raw score of

31.5 and a T score of 55. For the second twin, the mother indicated that they had much greater sleeping difficulties (Twin 1).

Family 4

On the CBCL, one twin had a total internalizing behaviour raw score of 22 and a T score of 67, on externalizing behaviours this twin had a total raw score of 30 and a T score of 69. This made a total raw score of 77 and a T score of 69. This twin (Twin 1=lower BW twin) also scored in the clinical range for emotionally reactive, sleep problems, and aggressive behaviour. On all other dimensions, this twin was within the normal range. The second twin had a total raw score of 23 and a T score of 68 for internalizing behaviours, for externalizing they had a total raw score of 36 and a T score of 77 which added up to a total raw score of 86 and a T score of 72 for this twin. This twin (Twin 2= higher BW twin) scored in the clinical range for emotionally reactive, somatic complaints, attention problems, and aggressive behaviours. On the rest of the dimensions, they scored within the normal range.

Family 5

On the CBCL, one twin had a total raw score of 4 and a T score of 45 on the internalizing portion, on the externalizing behaviours, they had a total raw score of 7 and a T score of 43. This created a total raw score of 18 and a T score of 42. This twin (Twin 1 = higher BW twin) scored within the normal range on all the dimensions of the CBCL. For the second twin, they had an internalizing behaviour raw score of 6 and a T score of 47, they also had an externalizing raw score of 8 and a T score of 44. This added up to a total raw score of 22 and a T score of 45. This

twin (Twin 2=lower BW twin) scored within the normal range on all dimensions, with the highest score being for the withdrawn dimension.

Overall, the CBCL within twin pairs revealed some variability in each of the twins' behavioural outcomes, as per the parent who was present at the imaging session. Although analyses were not conducted to see if there were differences in higher or lower birth weight twins, by visually inspecting the scores there is some variability in higher and lower birth weight twins.

Discussion

The data presented here is descriptive in nature, therefore no conclusions or statistical associations can be derived from what is presented above. This study presented cases of the five families that have thus far participated in this wave of data collection in the PDIMOZY cohort. This cohort is unique in that it is solely monozygotic twins that have been followed very closely since birth, which allowed researchers to take multiple waves of assessments to identify patterns and evolutions. It is ongoing and recruitment of new MZ twin pairs is continuing. The goal of the above data is to demonstrate the feasibility of conducting brain imaging on twins in childhood. These data are important for the larger project on the role of the in-utero environment in brain development studied in a longitudinal MZ twin design. Additionally, we also have saliva samples collected at one, twelve, and twenty-four months of age to study the mediating effect that epigenetic processes might play in this association between in utero environment and brain development. Upon completion, this study will be unique in nature as brain imagining in young children is lacking and the mediating effect of epigenetic processes in the association between birth weight and behavioural outcomes is understudied in young children.

Overall, the pilot data described here suggested that the MRI scans were well-received and that conducting MRIs at the preschool age using a twin design is feasible. It was noted that the twins generally enjoyed the MRI, and that the familiarization protocol prepared the twins well for the MRI. By capitalizing on a playing procedure, we were able to attain and retain their attention, as well as make sure the experience was rewarding for the twins. Although one family was unable to complete the MRI, the parent indicated that the children were not used to laying still and would also not enjoy that they could not enter the room with them. The parents could not enter the room with the twins because they had a newborn child with them as well and did not want to leave the newborn, who could not enter.

While the sample size was too small to do statistical analyses, we compared the data qualitatively with those obtained in an adolescent twin cohort. Based on visual inspection, we noted that in the adolescent twins, the fibre tracts are much longer but there are fewer. The preceding is consistent with the brain development literature. Specifically, at four years of age, neuronal pruning has not yet occurred, this can be observed in the short but many fibre tracts (Stiles & Jernigan, 2010). Moreover, the distinction between white and grey matter is also consistent with a developed brain in the fifteen-year-old twins, whereas in the four-year-old twins, this development is not yet complete.

Behaviourally, we noted that the family is high functioning (as shown by the HOME scores and McMaster Family Environment), and the twins were all in excellent health. We can also observe that based on scores on the CBCL, there were subtle differences within twin pairs in behaviour.

Strengths and Limitations

Due to the COVID-19 pandemic, recruitment was stopped to comply with the governmental laws of health and safety. Currently, the scanner of the main site is being upgraded causing further delays. Due to the preceding delays, the twins can no longer be scanned at the preschool age. MRI data collection will resume this fall. Although the twins are now of school age and subsequent testing will thus occur later than planned, as MRI research in MZ twins of school age is scarce, the study remains of interest.

A strength of this study relates to the uniqueness of this cohort, with multiple waves of data collection and extensive measures of child and maternal behaviour, family environment and epigenetics. Another strength of this study is that this study is being done in a non-clinical MZ twin sample, which was further shown by the results on the CBCL. Although testing a nonclinical sample limits generalizability, it increases homogeneity.

Conclusion

The preliminary results presented here suggest that scanning four-year-old children is feasible without sedation and for research purposes, with the proper protocol in place.
The cases presented here demonstrated the feasibility and informed the further continuation of the study for which data collection is expected to resume this fall.

Table 5. Characterization of families

	Sex of twins	BW Discordance	Weeks of gestation	McMaster Scores	HOME Communication	HOME Implication	Depressive symptom trajectory
Family 1	M	340g	33	3m: 4.62 6m: -- 9m: 4.87	1m: -- 12m: 53 24m: 46	1m: -- 12m: 30 24m: 12	1
Family 2	M	655g	33	3m: 3.59 6m: 3.85 9m: 5.38	1m: -- 12m: 49 24m: 45	1m: -- 12m: 26 24m: 13	1
Family 3	F	10g	37	3m: 4.10 6m: 3.33 9m: 3.59	1m: 50 12m: 53 24m: --	1m: 19 12m: 29 24m: --	3
Family 4	M	480g	36	3m: 4.62 6m: 5.13 9m: 4.87	1m: -- 12m: -- 24m: --	1m: -- 12m: -- 24m: --	3
Family 5	M	960g	35	3m: 5.90 6m: 5.64 9m: 5.90	1m: -- 12m: -- 24m: --	1m: -- 12m: -- 24m: --	2

Table 6. PACOTIS Scores

	Family 1			Family 2			Family 3			Family 4			Family 5		
	3M	6M	9M	3M	6M	9M	3M	6M	9M	3M	6M	9M	3M	6M	9M
Parental Efficiency Twin 1	--	5.00	7.00	8.75	9.00	10.00	9.75	8.25	8.25	4.50	6.25	7.00	9.00	9.50	9.00
Parental Efficiency Twin 2	--	5.00	6.75	8.75	9.00	10.00	9.75	8.25	8.25	4.50	6.25	7.00	9.00	9.50	9.00
Parental Impact Twin 1	--	4.80	6.40	0.20	0.00	0.00	.60	.20	.40	.40	1.00	1.20	3.40	.40	2.00
Parental Impact Twin 2	--	4.80	6.20	0.20	0.00	0.00	.60	.20	.40	.40	1.00	1.20	3.40	.40	2.00
Parental Coercion Twin 1	--	5.00	4.00	.33	0.00	.33	.67	1.67	4.00	1.00	.33	1.00	2.00	1.67	3.33
Parental Coercion Twin 2	--	5.00	5.33	.33	0.00	.33	.67	1.67	4.00	1.00	.33	1.00	2.00	1.67	3.33
Parental Affection Twin 1	--	8.60	9.60	9.60	9.60	10.00	10.00	10.00	10.00	7.00	6.60	7.80	8.40	4.40	9.40
Parental Affection Twin 2	--	8.60	9.60	9.60	9.60	10.00	10.00	10.00	10.00	7.00	6.60	8.00	8.40	4.40	9.40
Parental Overprotection Twin 1	--	4.50	5.00	7.75	5.50	6.00	3.50	8.00	8.00	3.00	4.00	4.25	4.75	2.75	1.50
Parental Overprotection Twin 2	--	4.50	5.00	7.75	5.50	6.00	3.50	8.00	8.00	3.00	4.00	4.25	4.75	2.75	1.50
Parental Perception of child qualities Twin 1	--	6.75	8.25	8.50	8.75	10.00	10.00	8.75	8.50	6.75	7.00	8.75	4.50	6.50	5.50
Parental Perception of child qualities Twin 2	--	6.75	8.00	8.75	8.75	10.00	10.00	8.75	8.50	6.25	7.25	8.75	4.50	7.00	5.50

Table 7. Scores on behavioural variables and temperament.

	Family 1		Family 2		Family 3		Family 4		Family 5	
	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2
Hyperactivity	8.00	7.00	6.00	7.00	6.00	8.00	3.00	1.00	3.00	5.00
Inattention	6.25	7.50	5.00	6.25	5.00	3.75	3.75	.00	.00	1.22
Hyperactive/inattention	7.22	7.22	5.56	6.67	5.56	6.11	3.33	.56	1.67	3.33
Emotional Trouble	5.00	3.33	5.00	.00	1.67	.00	.00	.00	.00	.00
Anxiety	3.75	6.25	7.50	2.50	5.00	1.25	3.75	1.25	1.25	1.25
Separation Anxiety	7.50	8.33	7.50	3.75	6.25	2.50	5.00	3.75	5.00	5.00
Global Aggression	4.50	4.09	.91	2.73	5.45	5.00	1.36	.91	2.27	4.55
Pure Aggression	5.00	4.17	.83	2.50	5.83	4.17	.83	.00	2.50	3.33
Prosocial Behaviour	3.75	1.00	2.00	3.00	8.75	5.00	7.00	8.00	9.00	9.00
Opposition	9.00	7.00	5.00	4.00	5.00	8.00	3.00	4.00	1.00	5.00
Shyness	3.33	4.17	5.00	6.67	5.00	3.33	5.00	5.00	4.17	5.00
Depression	4.00	4.00	7.00	2.00	3.00	.00	2.00	.00	1.00	.00
Temperament	4.05	5.48	5.00	1.67	3.81	2.38	3.81	3.33	4.52	5.00

Table 8. Familiarization scores

	Family 1		Family 3		Family 4		Family 5	
	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2
Did you like it?	5	5	5	5	5	4	4	3
Was it noisy?	3	5	1	1	5	4	2	2
Would you do it again?	No	Yes	Yes	No	Yes	Yes	Yes	Yes
Were you excited for it to end?	5	5	3	3	2	4	1	1
Did you like the movie?	5	5	5	4	5	4	5	4
Did you see the movie well?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Did you hear the movie well?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did you like the story about Youpala?	5	5	5	5	5	1	4	5

Chapter 4: Supplemental Material

Appendix A: Eligibility Criteria

Questionnaire phone interview

1) Does your child take any medication? NO YES

[IF YES] Which ones? _____

2) Does your child currently have health problems? NO YES

[IF YES] Which ones? _____

3) Did your child have health problems in the past? NO YES

[IF YES] Which ones? _____

4) Is your child seeing a psychiatrist or other therapist and/or experiencing mental health problems? NO YES

[IF YES] For what reason does your child see this person? Does your child see this person regularly? _____

5) Does your child have metals in his/her body (dental implants, prosthesis, braces, implants, aneurism clip)? NO YES

[IF YES] Which ones? _____

6) Did your child undergo surgery? NO YES

[IF YES] For what? _____

Questions	Response for exclusion	Response of the participant
1)		
2)		
3)		
4)		
5)		
6)		

Appendix B: Brochure

What Will Happen During The Visit?

Before proceeding with the scan, we will ask you to fill out a questionnaire to make sure that the scan can be performed safely. We have planned a brief session of activities with your child (15 minutes) to help your child get familiarized with the environment. We will also ask your child and the accompanying parent to wear pyjamas and to remove any metal object that they might have on them (jewellery, belt, glasses, etc.).

We will then ask your child to lie down on the bed and remain still. The bed will slowly slide through the tunnel. Once the bed is in the tunnel, we will start scanning for approximately 30 to 45 minutes. MRIs are painless, but they do make a lot of noise. Your child will be able to wear earplugs. Your child can also watch a movie during the scan to pass the time.

Contact Information:

Research Center of Sainte-Justine University Hospital 3175 Chemin de la Côte-Sainte-Catherine, Montréal H3T 1C4

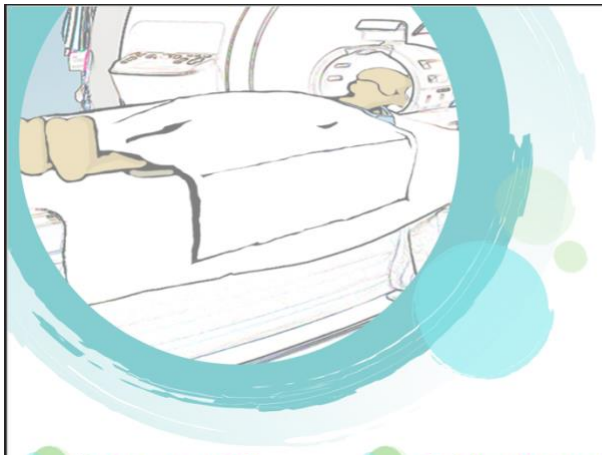
514-345-4931 ext. 4041

Jumeaux.HSJ@gmail.com
linda.booij@umontreal.ca

We look forward to meeting you!

Magnetic Resonance Imaging

Information for parents and children



What Is An MRI?

A Magnetic Resonance Imaging scan or MRI, is a medical imaging technology that allows us to safely acquire brain images. The MRI machine looks like a tunnel with a bed that slides inside.

You'll even get a picture at the end!!!

Children's Questions

1. Can one of my parents stay with me?
Yes! After filling out the safety questionnaire, your parent can stay with you with the technicians' authorization. Your parent can enter the room and rest their hand on your leg.

2. Why can't I move during the scan?
The MRI works like a giant camera. So it is important that you do not move so that the pictures are not blurry.

3. Why can't I wear any metal objects?
The MRI is also like a big magnet. If you wear metal objects they will be pulled towards the machine.

4. What will I be doing during the scan?
You will be able to watch a movie of your choice or simply rest.

Parent's Questions

1. Could the MRI be painful or dangerous ?
Absolutely not, MRIs are harmless, but they are noisy.

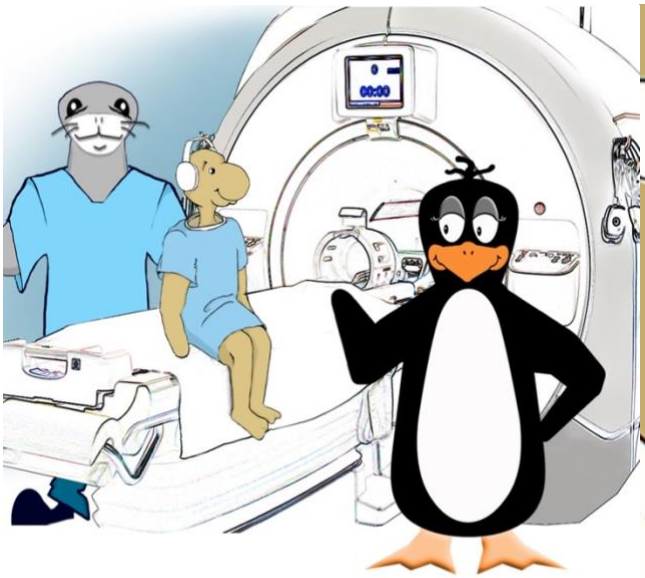
2. How long does the scan last ?
Anywhere from 30 to 45 minutes, preceded by a few activities for 15 minutes.

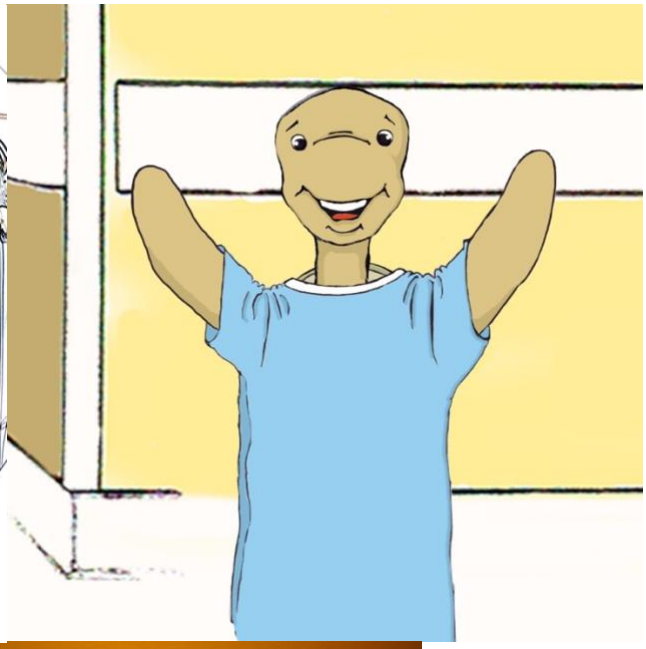
3. How can I prepare my child ?
Be informed, do not hesitate to ask the research team questions. Explain to your child that we will be taking pictures of his or her brain. Reassure your child by telling him/her that it will be painless and that he/she can watch a movie or even sleep. Children are usually very curious to see and learn about the MRI machine. We will also spend time with your child before the scan to familiarize them with the machine and procedure. They will be able to look at pictures, play with a small replica of the machine, and listen to the noises the MRI makes. We will answer all of your child's questions.

4. Will my child be restrained ?
To ensure the success of the scan, your child should not move during the scan. For this reason, we will stabilise his/her head with soft pads on the bed before starting.

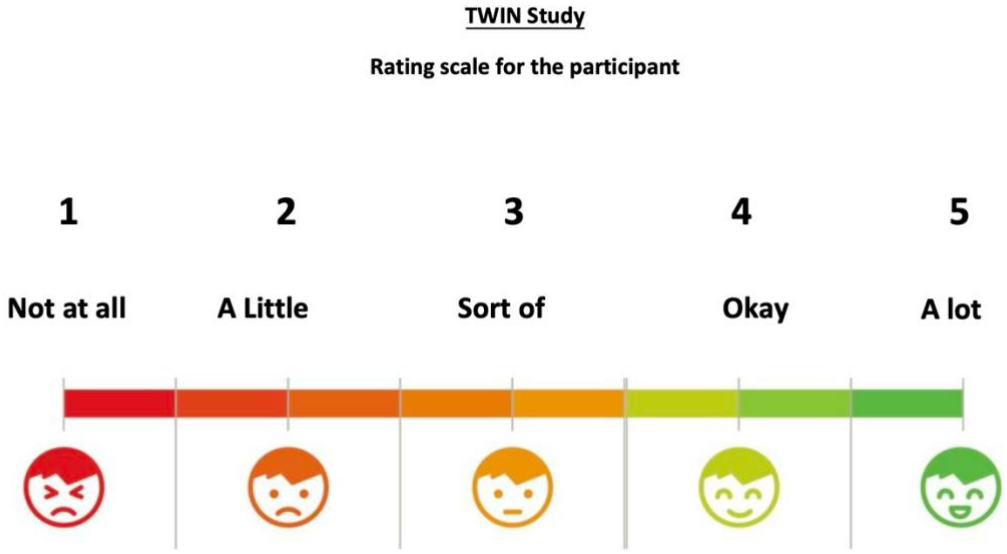
5. Where will the scan take place ?
The research coordinators of the study will meet you at the main entrance of Sainte-Justine Hospital. She will accompany you to the radiology department.

Appendix C: Photos from the story of Youpala the turtle and the MRI in the lab





Appendix D: Scale used for twins to rate experience in MRI.



Appendix E: Questionnaire used to ask twins about MRI.

Participant code : _____

INFANT OPINION ON THE STUDY

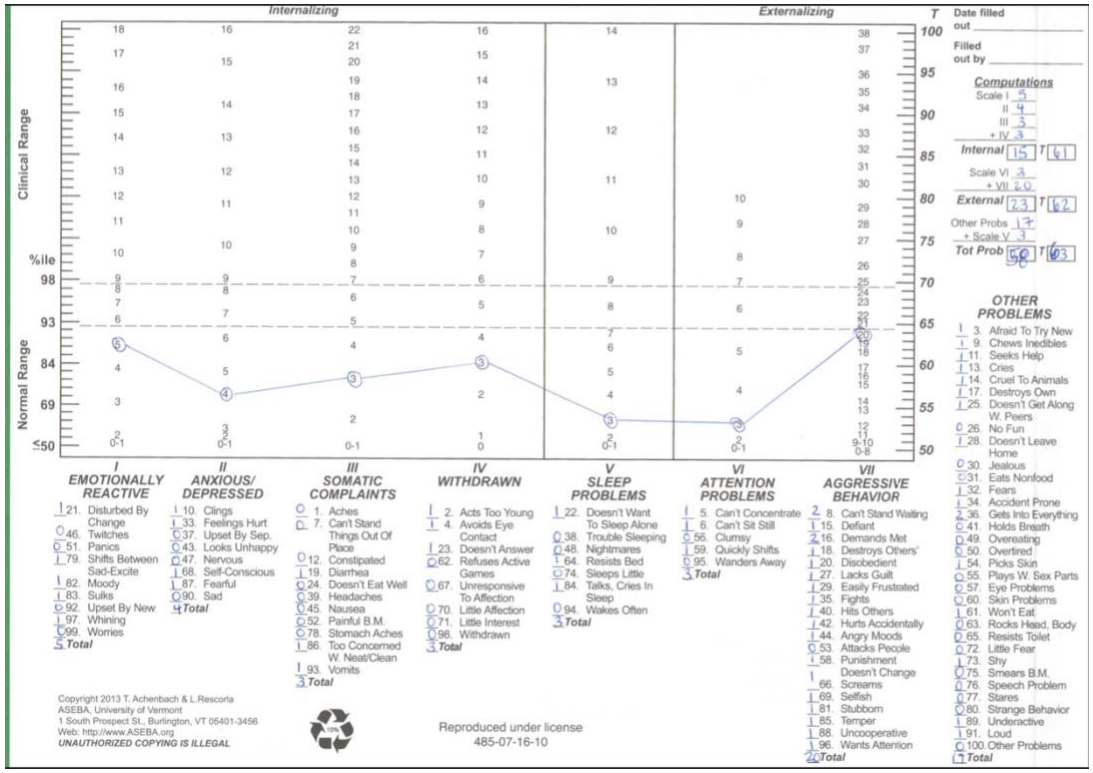
Complete with child after the MRI

Not at all	A little	Between a little and a lot	A lot	Very much
1	2	3	4	5

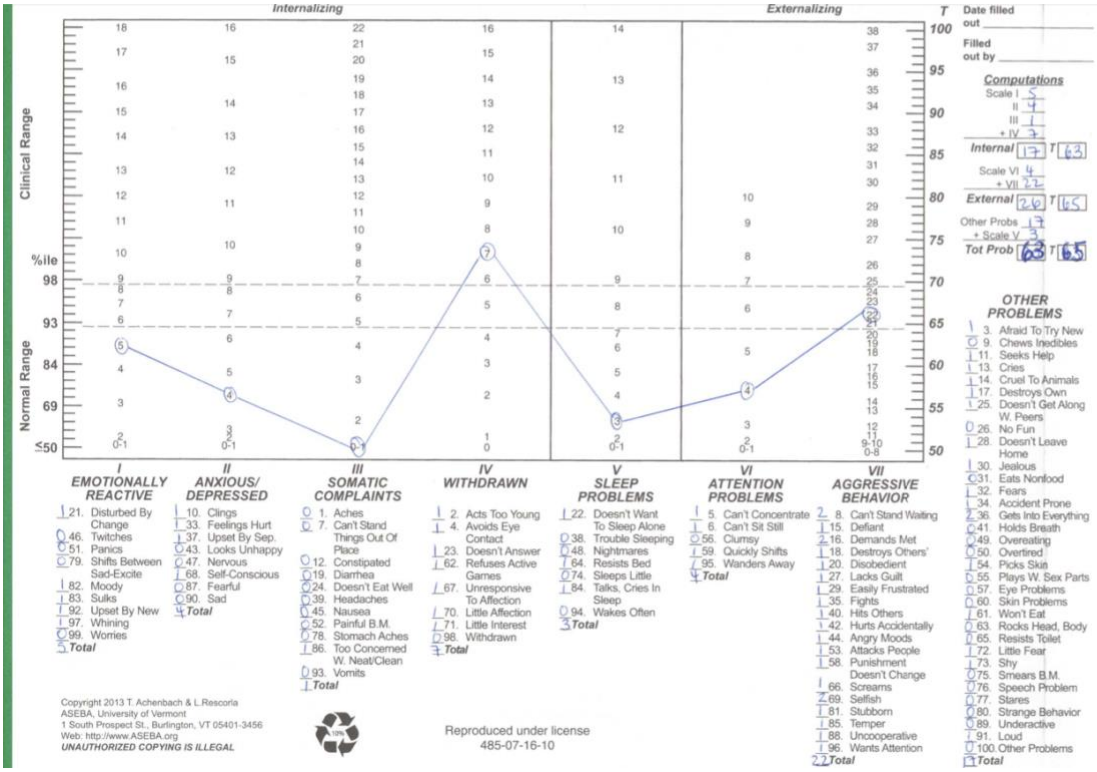
1. Did you like that ?
   1 2 3 4 5
2. Was it noisy ?
   1 2 3 4 5
3. Would you like to do it again?
   No Yes
4. Did you want it to end? Were you bored?
   1 2 3 4 5
5. Did you like the video? (Video chosen : _____)
   1 2 3 4 5
6. Were you seeing well the video?
   No Yes
7. Were you hearing well the video?
   No Yes
8. Did you like the book about Youpla?
   1 2 3 4 5

Appendix F: CBCL scores per twin:

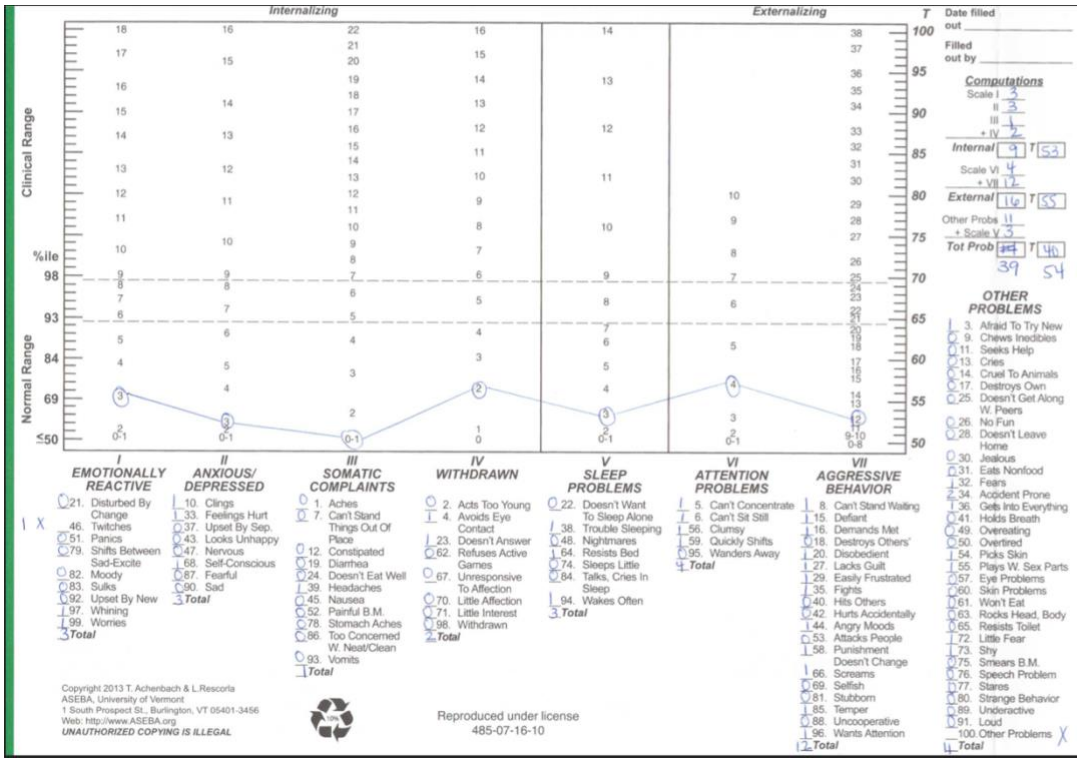
Family 1: Twin 1



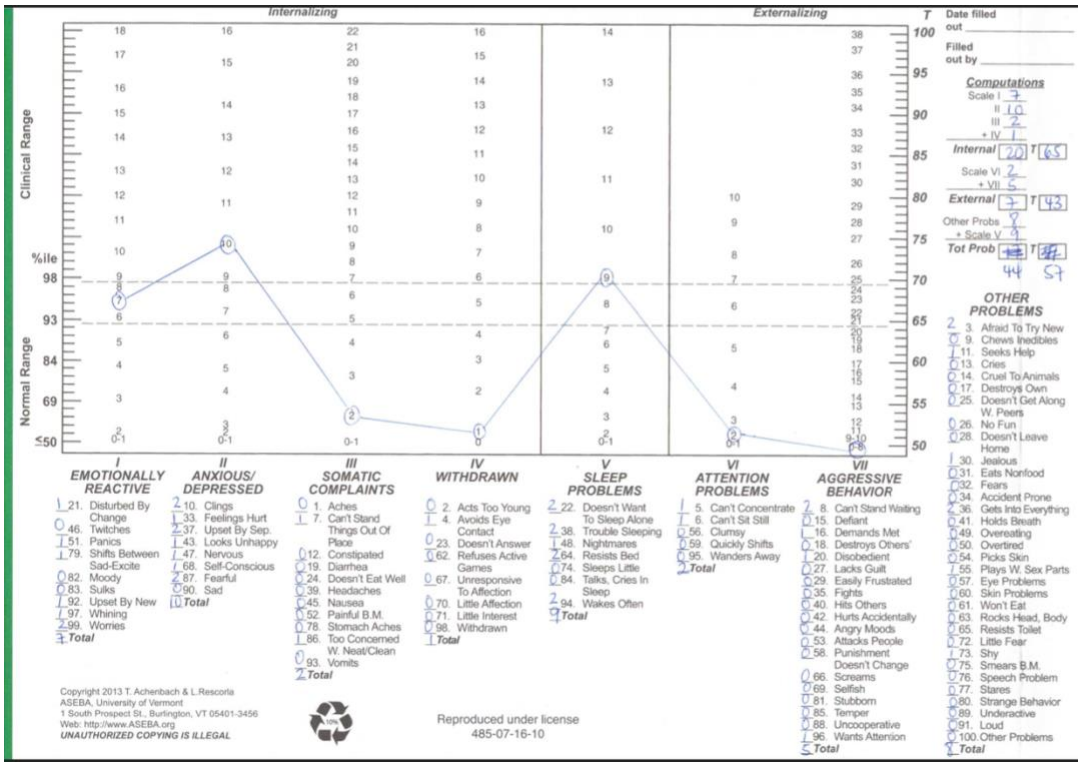
Family 1: Twin 2



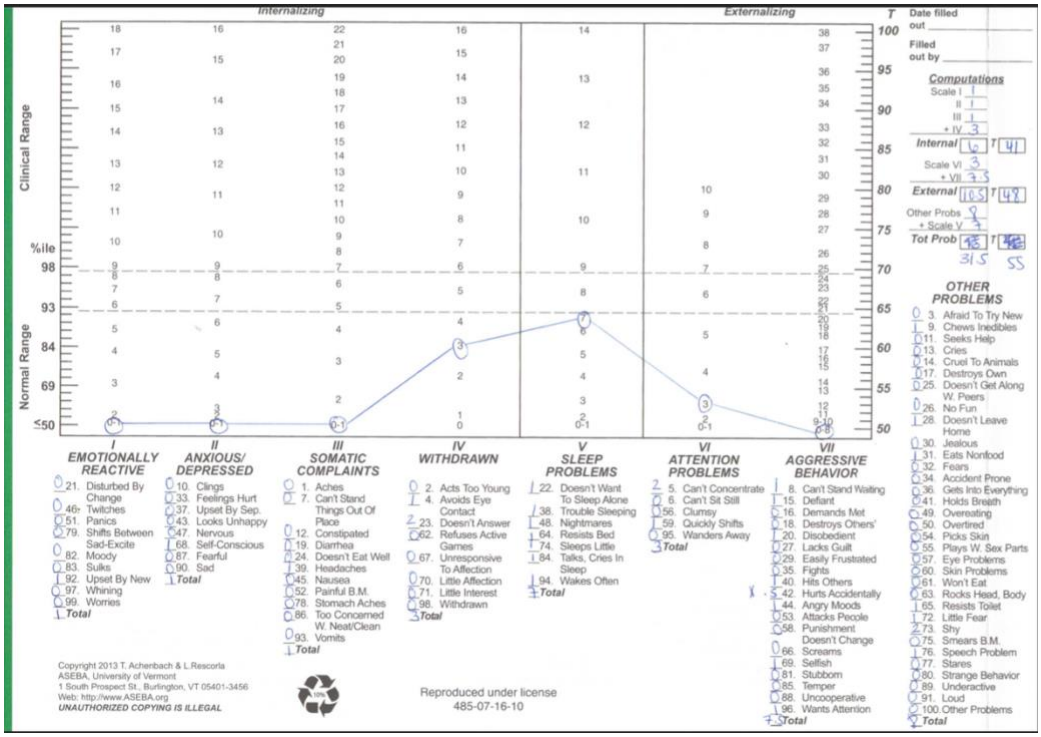
Family 2: Twin 1



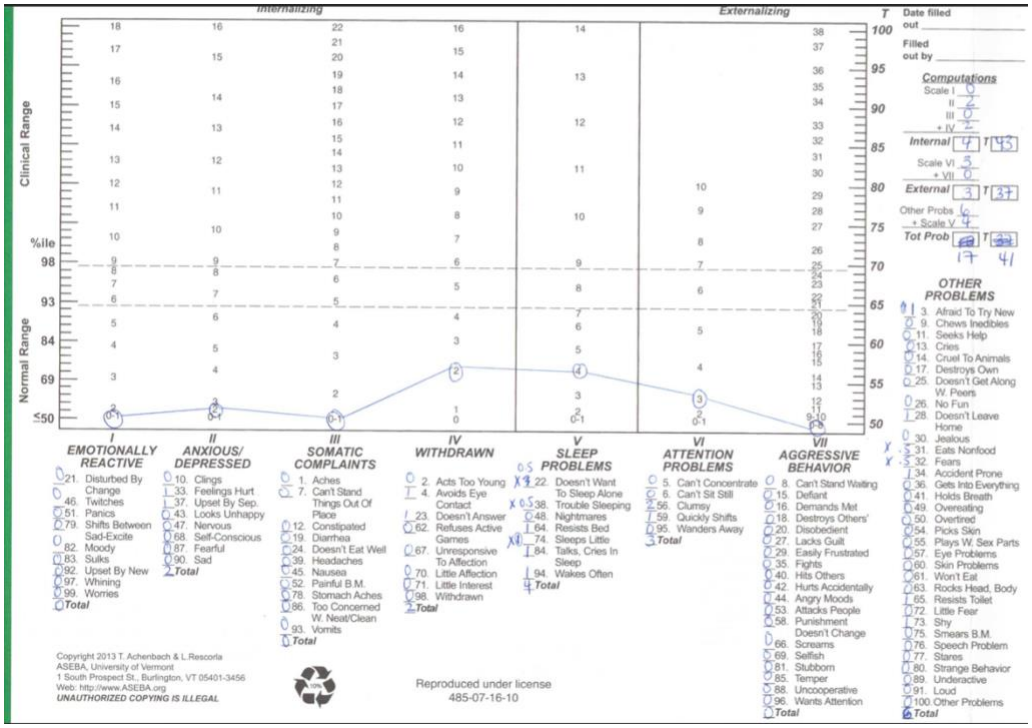
Family 2: Twin 2



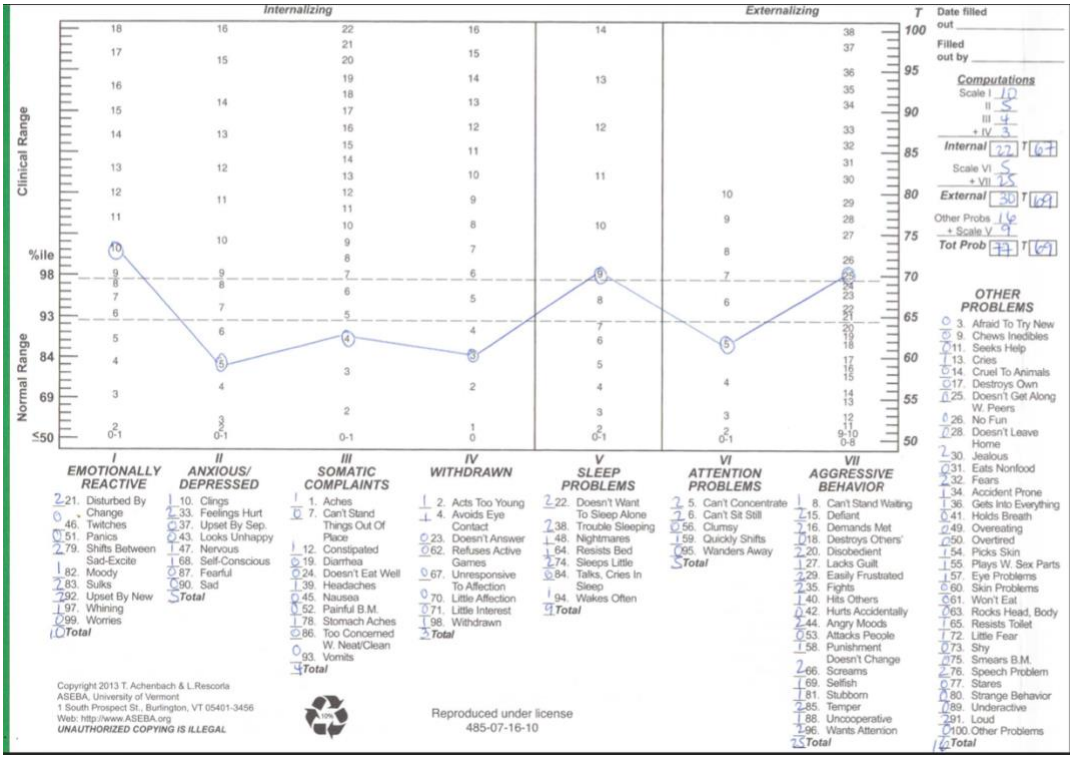
Family 3: Twin 1



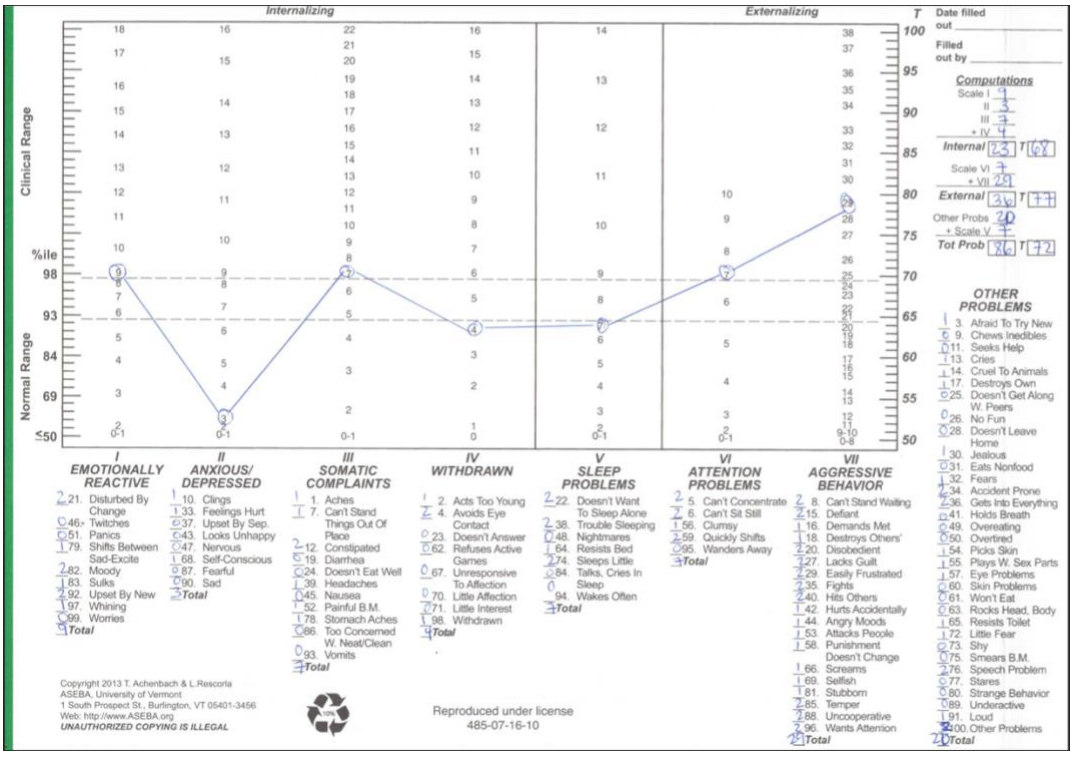
Family 3: Twin 2



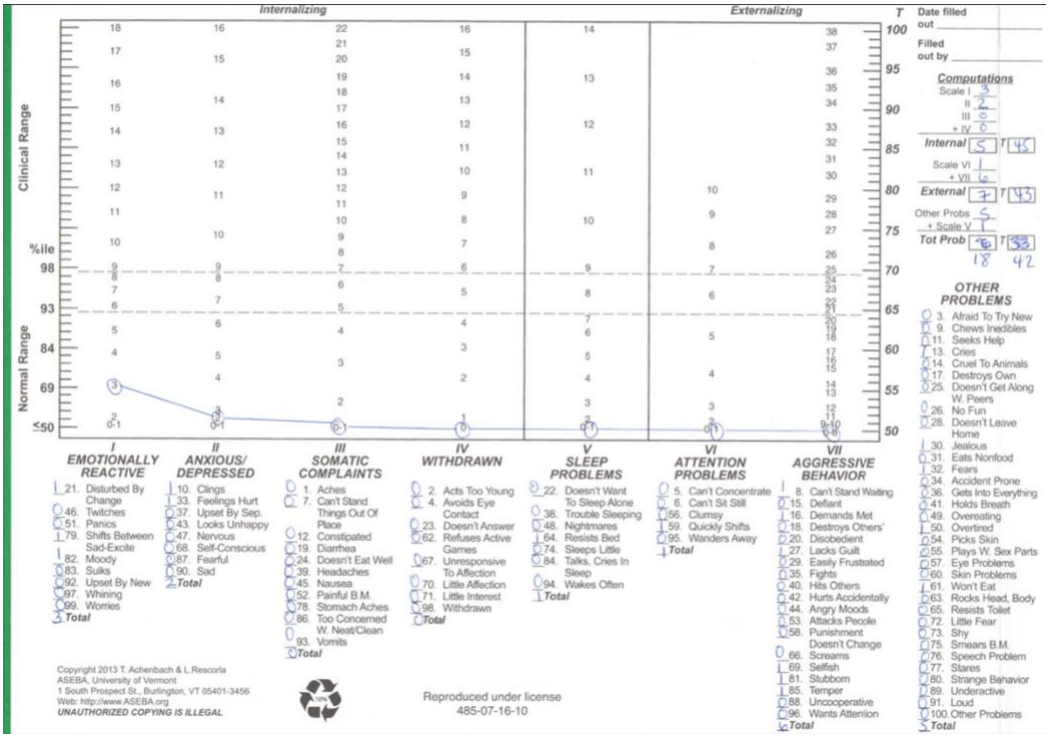
Family 4: Twin 1



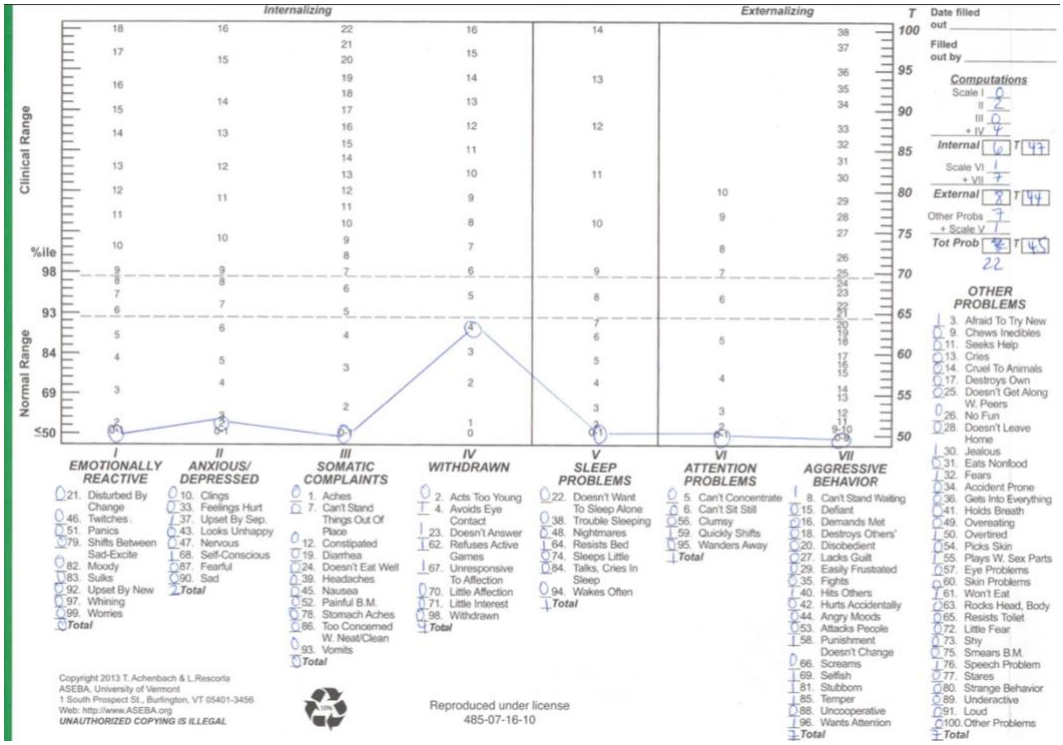
Family 4: Twin 2



Family 5: Twin 1



Family 5: Twin 2



Appendix G: Behavioural Assessment Questionnaire

Web interview at 24 months (BEH). Study of resemblance between monozygotic twins (PDIMOZY)

Using the answers "Never or not true, Sometimes or somewhat true, Often or very true, I don't know or Refusal", please indicate how often Twin 1 or Twin 2 :

BEH-Q8B. Can't sit still, is restless or hyperactive?

BEH-Q8D. Will try to help someone who has been hurt?

BEH-Q8D1. Is shy with children he/she does not know?

BEH-Q8E1. Is defiant?

BEH-Q8EA. Kicks others?

BEH-Q8F. Seems unhappy or sad?

BEH-Q8GA. Gets into fights?

BEH- Q8I1. Is easily distracted, has trouble sticking to any activity?

BEH – Q8JA. Takes away things from others when they won't give it to him/her?

BEH-Q8J1. Doesn't seem to feel guilty after misbehaving?

BEH-Q8K. Is not as happy as other children?

BEH-8K1. Readily approaches children he/she does not know?

BEH-Q8N. Fidgets?

BEH-Q8NA. Pushes others to get what he/she wants?

BEH-Q8P. Can't concentrate, can't pay attention for long?

BEH-Q8Q. Is too fearful or anxious?

BEH-Q8R1. Punishment doesn't change his/her behaviour?

BEH-Q8R2. Takes a long time getting used to being with children he/she does not know?

BEH-Q8S. Is impulsive, acts without thinking?

BEH-Q8T1. Has temper tantrums or hot temper?

BEH-Q8U. Offers to help other children (Friend, brother or sister) who are having difficulty with a task?

BEH-Q8UI. Is shy with adults he/she does not know?

BEH-Q8UA. Threatens to hit others?

BEH-Q8V. Is worried?

BEH-Q8W. Has difficulty waiting for his/her turn in games?

BEH-Q8XC. When somebody accidentally hurts him/her (such as by bumping into him/her), he/she gets angry and tries to injure the other?

BEH-Q8Z1. Has angry moods?

BEH-Q8Z1A. Physically attacks others?

BEH-Q8BB. Confronts a child (friend, brother or sister) who is crying or upset?

BEH-Q8CC. Cries a lot?

BEH-Q8CCA. Is cruel to others?

BEH-Q8DD1. Clings to adults or is too dependent?

BEH-Q8EE. Gives up easily?

BEH-Q8EEA. Punches others?

BEH-Q8HH1. Cannot settle down to do anything for more than a few moments?

BEH-Q8HHC. Bullies others?

BEH-Q8LL1. Constantly seeks help?

BEH-Q8MM. Is nervous, high-strung, or tense?

BEH-Q8NNA. Bites others?

BEH-Q8PP1. Doesn't want to sleep alone?

BEH-Q8QQ. Is inattentive?

BEH-Q8QQ1. Readily approaches adults he/she does not know?

BEH-Q8RR. Has trouble enjoying him/herself?

BEH-Q8SS. Helps other children (friends, brother or sister) who are feeling sick?

BEH-Q8SS1. Takes a long time getting used to being with adults he/she does not know?

BEH-Q8TT1. Gets very upset when separated from parents?

BEH-Q8UU1. Helps those who do not do as well as he/she does?

BEH-Q8UUC. Hits (in the sense of slapping) others?

Appendix H: Temperament Assessment Questionnaire

Web interview at 12 months (TMP). Study of resemblance between monozygotic twins (PDIMOZY).

The following questions are about how Twin 1 behaves. Please answer them for him/her in comparison to others. "About average" means how you think the typical child would be scored.

TMP-Q1. How easy or difficult is it for you to calm or soothe twin 1 when he/she is upset?
- Very easy
- 2
- 3
- About average
- 5
- 6
- Difficult
- I don't know
- Refusal

TMP-Q3. How easy or difficult is it for you to predict when he/she will become hungry?
- Very easy
- 2
- 3
- About average
- 5
- 6
- Difficult
- I don't know
- Refusal

TMP-Q5. How many times per day, on average, does twin 1 get fussy and irritable – for either short or long periods of time?
- Never
- 1-2 times per day
- 3-4 times per day
- 5-6 times per day
- 7-8 times per day
- 10-14 times per day
- 15 times per day or more
- I don't know
- Refusal

TMP-Q6. How much does he/she cry and fuss in general?
- Very little; much less than the average baby/child
- 2
- 3
- Average amount; about as much as the average
- 5
- 6
- A lot; Much more than the average baby/child
- I don't know
- Refusal

TMP-Q7. How easily does he/she get upset?
- Very hard to upset (by things that upset most babies/children)
- 2
- 3
- About average
- 5
- 6
- Very easily upset (by things that wouldn't bother most babies/children)
- I don't know
- Refusal

TMP-Q8. When he\she gets upset (e.g., before feeding, during diapering, etc.) how vigourously or loudly does he/she cry and fuss?
- Very mild intensity or loudness
- 2
- 3
- About average
- 5
- 6
- Very loud intensity or loudness
- I don't know
- Refusal

TMP-Q19. On the average, how much attention does he/she require, other than for caregiving (feeding, bathing, diaper changes, etc.)?
- Very little
- 2
- 3
- Average amount
- 5
- 6
- A lot; much more than the average baby/child
- I don't know
- Refusal

TMP-Q20. When left alone, he/she plays well by him/herself?
- Almost always
- 2
- 3
- About half of the time
- 5
- 6
- Almost never – Won't play by self
- I don't know
- Refusal

TMP-Q33. Please rate the overall degree of difficulty your twin would present for the average parent.
- Very easy
- 2
- 3
- Ordinary, some problems
- 5
- 6
- Highly difficult to deal with
- I don't know
- Refusal

Chapter 5: Discussion

The present dissertation consists of three studies, designed to further our understanding of maternal mental health in mothers of twins; as well as to elucidate the association between the early environment, brain, and behaviour development. Utilizing a longitudinal cohort of genetically identical twins, these studies contribute to the paucity of research on the mental health of mothers of twins, and preliminary associations to brain development in preschool-age twins.

Overall Aim of the Studies

The first study was a meta-analysis designed to examine whether there are differences in depressive symptoms between mothers of twins and singletons. PRISMA guidelines were followed, and the inclusion criteria were set *a priori*. In comparison to other meta-analyses on this topic, the main objective was to examine depression in mothers of twins, regardless of method of conception and without a focus on other variables of mental health (i.e., anxiety or stress). Overall, there was a small but significant difference between depressive symptoms in mothers of twins and mothers of singletons, whereby mothers of twins experience more elevated levels of depressive symptoms ($r = 0.08$, $p = .002$). The results of our meta-analysis corroborate with another meta-analysis, which also found that twin parenthood in general may be a risk factor for elevated depressive symptoms (van den Akker et al., 2016). However, compared to the most recent meta-analyses (van den Akker et al., 2016), we were able to include 75% more articles (n=14 vs. n=6).

The second study was designed in association with the findings from Study 1. Given that twin parenthood was suggested to be indicative of elevated depressive symptoms, the aim was to characterize depressive symptoms in mothers of monozygotic (MZ) twins over time, during the

first year after giving birth. That is because we noted that 85% of the included studies in the meta-analyses were cross-sectional, and thus the evolution of symptoms postpartum is largely known. A longitudinal cohort of MZ twins and their parents (called the Projet des Ressemblances entre Jumeaux Monozygotes cohort) was utilized to model the evolution of depressive symptoms in mothers of twins in the first nine months postpartum. We also explored whether the course of depressive symptoms predicts temperament outcomes in the twins at 12 months of age and behavioural outcomes in the twins at 24 months of age. Latent class analysis findings revealed that a 3-group constant model was the best fit. Mothers of twins followed the course of one of three trajectories: low and stable depressive symptoms at three-, six- and nine-month assessment (66.1%), decreasing symptoms where higher symptoms were observed at three months postpartum and steadily declined at the six- and nine-month assessment (17.2%), and finally increasing, where symptoms were low at the three-month assessment but steadily increased at the six- and nine-month assessment (16.7%). Regression analyses revealed that there was no significant association between the course of maternal depressive symptoms and toddler temperament and behaviour outcomes at 12 and 24 months. There was a trend towards elevated emotional trouble and shyness in twins' outcomes from mothers experiencing increasing depressive symptoms (p=.058). This is the first study to our knowledge that specifically examined mothers of MZ twins' evolution of depressive symptoms in the first nine months postpartum.

The third study was a series of case reports on five families that took part in a neuroimaging study in preschool-aged twins. The series of case reports aimed to demonstrate the feasibility of conducting an MRI on MZ twins at the preschool age and describe the twins' experiences as well as behavioural data in the twins that participated at four years of age.

Utilizing the same longitudinal cohort of MZ twins as study two, families interested in participating in an MRI study were recruited. Overall, five families participated. To conduct a neuroimaging study in four-year-old twins, a play approach was utilized to render the children familiar with what to expect in an MRI: how it works, the noises it makes, and the contribution they would be making to science. Overall, in this study, the familiarization protocol was effective in habituating the children to the MRI and adequately preparing them for what would happen. The MRI data we obtained were of good quality, and the twins overall indicated that they were satisfied with the MRI experience. Further, visual inspection of the brain images in adolescent twins compared to the preschool-aged twins confirmed that at four years of age, the brain is still in a critical period of development as there appears lack of distinction between white and grey matter. As well, the four-year-old twins have many, but shorter fibre tracts compared to the fewer but longer observed in the adolescent twins, which would suggest that neuronal pruning has not yet occurred. CBCL results also indicated that there are subtle behavioural differences within twin pairs and that the twins had a high-functioning family life, as indexed by the HOME assessment.

The case report series will inform the larger study for which data collection is expected to resume in fall 2023, now that COVID restrictions are lifted and (new) scanning facilities at the primary research site will be operational. The larger study aims to evaluate whether birth weight differences within twin pairs, which indexes a less optimal *in-utero* environment, could be associated with brain development differences when the brain is in a critical period of development, and to test for underlying epigenetic mechanisms accounting for the preceding associations.

Integrative Association across Studies

An integral theme that is presented across each study is the importance of the early environment, which includes both the *in-utero* environment and the postnatal period. Adversity in early life has been associated with negative behavioural and cognitive outcomes in children in the future (Raznahan et al., 2012; Van Os et al., 2001). The mechanism that may be behind the association between early life adversity and negative behavioural and cognitive outcomes is differences in brain development. However, it is also important to note that adversity does not affect all children the same way, some children who have experienced adversity in early life are resilient and thrive regardless (Cicchetti & Rogosch, 1996). Specifically, there can be biological, social, and psychological risk factors and protective factors that explain the disparities in outcomes in children.

In study one, the systematic review of the literature revealed that experiencing depressive symptoms is not only in the first year postpartum but also when children are of school age. This would suggest that the evolution of depressive symptoms in the first year is of interest to researchers as it could help identify potentially vulnerable mothers for early intervention. Study two was conceptualized to address this gap, as there have been several studies conducted on the evolution of depressive symptoms in mothers of singletons. In mothers of twins, most studies are cross-sectional focusing on clinical samples. There is a paucity of research examining the longitudinal evolution of depressive symptoms in mothers of twins from a community sample. Except for one study that included mothers of multiples in their larger sample but a small sample (Putnick et al., 2020). In modelling the evolution of depressive symptoms over the first nine months postpartum, there was a proportion of the sample of identical twins' mothers (16.7 %) that may be more vulnerable to experiencing clinically significant depressive symptoms in the

future. As maternal well-being is a contributing factor in early life experiences, it is pertinent to establish timely intervention in mothers who may be more vulnerable. In its larger scope, study three was designed to assess the association between behavioural outcomes, brain development, and early life adversity holistically by examining biological, psychological, and social factors that may be behind the association. The pilot study presented in study 3 showed that neuroimaging in a pediatric population of twins is feasible and preliminary behaviour data suggests subtle differences within twin pairs.

Previous research has found an association between the evolution of maternal depressive symptoms and childhood outcomes at five years of age in singleton designs (van der Waerden et al., 2015), but as study two suggests, there was only a trend present toward behavioural subscales of emotional troubles and shyness. As was discussed in study one, mothers of twins are at higher risk of displaying elevated symptoms of depression compared to mothers of singletons. As study two showed, in a small proportion of mothers in our sample some experienced enduring and increasingly severe levels of depressive symptoms, which would suggest a higher risk for reaching clinical threshold levels of depressive symptoms (16.7%). Not finding a significant association between behavioural outcomes and maternal depressive symptoms in study two could be due to a limitation in twin designs, using one individual to report on the MZ twins' behaviours (Vitaro et al., 2009). Although, another singleton design that regressed maternal depressive symptoms and child behavioural outcomes at two years old, did not find a significant difference (Guyon-Harris et al., 2016). In study three, in the five families that participated, two of the mothers were identified to be following the course of increasing depressive symptoms when the twins were nine months of age. An avenue of research that may be of interest to study is whether the association between developmental trajectories and behavioural outcomes is

mediated by brain processes. Our results could also be interpreted as further evidence for the multifinality principle in developmental psychology, whereby a certain adverse event should not be presumed to be associated with the same psychopathological issue as another (Cicchetti & Rogosch, 1996).

Although poor maternal mental health has been associated with unfavourable behavioural and cognitive outcomes in children in the future, there remains a paucity of research and limited resources available (Cook et al., 2023; Wenze & Battle, 2018). Studies one and two contribute to the limited research on mothers of twins. Research on families of twins remains generally focused on the twins due to their unique ability to allow researchers to control for genetic and environmental factors without experimental manipulation (Vitaro et al., 2009). Study one suggests that mothers of twins are at higher risk for experiencing clinically significant levels of depression, which highlights the need for additional resources for mothers of twins. Yet, pre-screening for mothers in general is not done consistently, nor are adequate resources offered (Cook et al., 2023; Lamere & Golova, 2022; Wenze & Battle, 2018). Study two highlights that 1 out of 6 mothers of twins are particularly vulnerable to elevated symptoms of depression. Depression symptoms in mothers of twins do not always necessarily display in the early postpartum period, as is demonstrated in the increasing trajectory, the symptoms can be low immediately postpartum but increase with time. This would not only signify like study one that early pre-screening is important, but repeatedly screening up to two years after would be crucial. As has been suggested, 12% of women can experience depressive symptoms in the first two years postpartum (Stewart & Simone, 2016). Moreover, the results from study two would also suggest that is critical to provide resources to mothers of twins more than just immediately postpartum. As symptoms at nine months were highest for the most vulnerable this would

suggest that offering psychosocial, financial, and possibly childcare support later in the postpartum period would also be beneficial to mitigate depression symptoms in mothers of twins that are particularly at risk. Increases in adverse behavioural outcomes in children include higher incidences of anxiety and depression and disruptions in how children regulate and cope with their emotions, which increases the odds of the children having negative cognitive biases in the future (Thompson & Henrich, 2022). Not all children are affected the same, which suggests that perhaps these adverse behavioural outcomes are related to the severity and chronicity of maternal depressive symptoms (Sutherland et al., 2022).

Theoretical and Practical Implications

The findings from the present dissertation suggest that monitoring during pregnancy would be an effective means of targeting mothers at higher risk of developing increased depressive symptoms. Mothers who experience more difficult pregnancies, or who experience more stress during pregnancy are at an increased likelihood of experiencing depressive symptoms postpartum. In addition to the trials, ordeals, and delights that twin motherhood provides, mothers of twins may also be at increased risk for depressive symptoms due to twin pregnancies being more difficult and resulting in higher incidences of preterm birth and subsequent neonatal intensive care being necessary (Fogel, 2017). As study two would suggest, proper monitoring during pregnancy may help identify a proportion of women who may be more vulnerable in the early postpartum period. This was shown by the evolution of depressive symptoms that were higher at three months postpartum but reduced by nine months, which could suggest more anxiety in pregnancy. Mothers found that medical professionals did not adequately prepare them for the disparities in emotional experiences, and when to seek professional help (Fogel, 2017). This lack of information would also suggest that an early intervention in the

prevention of maternal distress would be education. However, it may be more pertinent to include this education during pregnancy, as it has been suggested that educating mothers about depression and anxiety at discharge from the hospital was not effective in the reduction of depressive symptoms in mothers (McCarter-Spaulding & Shea, 2016). During pregnancy, new parents are encouraged to take part in courses and seminars about what to expect when in the postnatal period, and how to manage a newborn. These classes provide mothers with confidence and have shown an association with decreased depressive symptoms postpartum (Shimpuku et al., 2022). However, when having multiples, pregnancy and postpartum are similar and in so many ways, very different. To bridge the gap between what is common and what is different when expecting multiples, new classes were developed and implemented (Kuhnly et al., 2015). For example, offering more suggestions for how to breastfeed two babies, safe ways to bottle feed, a tour of the neonatal intensive care unit, and more details of the realities that having two newborn children will bring (Kuhnly et al., 2015). Nonetheless, the classes specific to multiples are still not widely accessible. As study one suggests, mothers of twins are more at risk than mothers of singletons, it would be pertinent to normalize discussing what mothers may experience postpartum, what can be considered average disparities in emotional well-being, and what would no longer be considered as average (Cook et al., 2023). In our current society, the importance of mental health care and well-being are at the forefront. Extending this normalization of taking wellness days, and prioritizing the self, not just physiologically but also psychologically to mothers postpartum would be greatly beneficial.

In concordance with our evolving society, the use of telehealth has been suggested for reducing symptoms of depression postpartum in mothers. Telehealth has been suggested to be an effective mode of intervention to address several barriers that some women face after having

children. Specifically, some women internalize the role and responsibility to be the perfect mother deeply which makes them less likely to seek intervention as doing so, would seem like an admission to experiencing difficulty (Gelabert et al., 2012). An additional benefit of telehealth is convenience, it is far less troublesome for mothers to take part in a treatment program from home than by attending face-to-face psychotherapy (Zhao et al., 2021). As the literature review in study one suggests, mothers of twins are already overextended and have very little time, this new means of intervention could be critical in targeting mothers of multiples. Another intervention that could be effective as a means for targeting mothers who may be vulnerable to depressive symptoms in pregnancy could be the Toi, Moi et Bébé adapted for usage in Quebec from Mothers and Babies (Côté et al., 2023). This program is a distance intervention that follows mothers during pregnancy through phone meetings to help new moms manage the stress of pregnancy and new motherhood, as well as relaxation techniques and communication to properly co-parent (Côté et al., 2023).

The implications of early and effective intervention in the reduction of depressive symptoms may have an association with reducing negative behavioural and cognitive outcomes in some children. As has been suggested across the literature, adversity in early life during critical periods of neurodevelopment may create long-lasting changes in brain development, measuring the extent of this association is the overarching goal of study three (Nelson & Gabard-Durnam, 2020). Therefore, developing early interventions targeting maternal mental health postpartum would increase protective factors for children. Specifically mothers of twins who as suggested by studies one and two are more at risk for developing depressive symptoms and for experiencing them long term.

Future Directions and Limitations

Despite the strengths of the current dissertation, there are ways in which studies are limited. Despite that MZ twins share almost all their genes, some biological contributors can be associated with the outcome between early life adversity, and behavioural, cognitive, and brain outcomes in children in the future. A future direction suggested in study three is the possible contribution of epigenetic processes, whereby genes are modified due to *in-utero* or early-life environmental factors. Additionally, the potentially mediating effect of epigenetics could also explain resiliency in certain children (Kentner et al., 2019). Given that the twins recruited in study three were followed closely from birth, and saliva was collected at one, twelve, and twenty-four months of age, in the larger upcoming study, the potential role of epigenetic processes will be examined. Another limitation is concerning behavioural assessments reported specifically by the mother and the reports for twins one and two were taken at the same time. In the future, when asking parents to assess their twin's behaviour they will be asked to evaluate twins one and two with a one-week time gap separating the assessments. This has already been implemented in the new families recruited to this cohort, to reduce the potential bias in responding (Vitaro et al., 2009).

This dissertation was limited to only studying depression in mothers of multiples. Future research should also study differences in anxiety between mothers of twins and singletons. Additionally, a variable that could also be of interest is loneliness. It has been found that new motherhood is isolating, and even more so for mothers of multiples, therefore including loneliness as a potential moderator could be of interest when studying the evolution of depressive symptoms over time. Furthermore, future research may benefit in studying the mediating role that having other children at home may be associated with depressive symptoms following a twin

birth. Specifically, is it new mothers of twins who are more at risk or potentially over-extended mothers who already have children at home? The directionality of this effect should also be studied, specifically, does having other children at home better prepare mothers for the birth of twins as they can be confident in their skills as a parent? Or does having other children at home exacerbate other psychosocial risk factors for depressive symptoms?

Future studies should examine how prenatal education classes specific to pregnancy with multiples may help educate mothers of multiples on the unique stressors and risks that are associated with the pregnancy of multiples. However, courses should not remain heavy on the negative as this can create unnecessary stress and anxiety in the mothers. Interventions in the early postpartum period should be commonplace and widely accessible to women, and specifically to mothers of multiples who are more at risk. Future research should explore specifically the effectiveness of telehealth intervention in mothers of multiples to study whether the same reduction in depressive symptoms is present. Social support has been suggested as a protective factor in new mothers. Future research could also include an examination of how brain processes may mediate the association between depression trajectories in mothers of twins and behavioural outcomes in the twins in the future. Study three suggests that neuroimaging in a pediatric population for non-medical purposes is feasible.

Conclusion

Overall, this dissertation contributes to the paucity of research examining the mental health of mothers of multiples. Studies one and two highlight the need for additional resources provided to mothers of twins as they are at increased risk for developing depression. Additionally, offering these resources strictly in the first three months postpartum may not be enough. As study two suggests, at the nine-month assessment particularly vulnerable mothers of

twins showed the highest levels of depressive symptoms, suggesting that offering resources in the later postpartum period may be critical for mitigating depression symptoms in those more particularly vulnerable. Additionally, the thesis also showed the feasibility of doing brain imaging research conducted in preschool-aged twins. We were able to successfully image four twin pairs, with minimal movement and obtained high-quality images. This would suggest that brain imaging using a play approach technique is feasible for non-medical purposes in young children.

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