Links between Prenatal Stress and Obstetrical Complications and Infant Behavior: A Twin Design

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved December 2011 by the
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ARIZONA STATE UNIVERSITY

December 2011

ABSTRACT

The main objective of this study was to use a genetically-informative design to examine the putative influences of maternal perceived prenatal stress, obstetrical complications, and gestational age on infant dysregulation, competence, and developmental maturity. Specifically, whether or not prenatal and obstetrical environmental conditions modified the heritability of infant outcomes was examined. A total of 291 mothers were interviewed when their twin infants were 12 months of age. Pregnancy and twin birth medical records were obtained to code obstetrical data. Utilizing behavioral genetic models, results indicated maternal perceived prenatal stress moderated genetic and environmental influences on developmental maturity whereas obstetrical complications moderated shared environmental influences on infant competence and nonshared environmental influences on developmental maturity. Gestational age moderated the heritability and nonshared environment of infant dysregulation, shared and nonshared environmental influences on competence, and nonshared environmental influences on developmental maturity. Taken together, prenatal and obstetric conditions were important nonlinear influences on infant outcomes. An evolutionary perspective may provide a framework for these findings, such that the prenatal environment programs the fetus to be adaptive to current environmental contexts. Specifically, prenatal stress governs gene expression through epigenetic processes. Findings highlight the utility of a genetically

informative design for elucidating the role of prenatal and obstetric conditions in the etiology of infant developmental outcomes.

To my parents Paul and Janine McDonald and my brother Jeff McDonald

You have always encouraged me to follow my dreams!

ACKNOWLEDGEMENTS

I wish to thank my committee for supporting this project. I would like to express my gratitude to Dr. Kathryn Lemery-Chalfant for being my mentor, listening to all of my ideas, directing my enthusiasm, and advising over the course of graduate school. I would also like to express my appreciation to Dr. William Fabricius, Dr. Linda Luecken, and Dr. Tracy Spinrad for their support throughout this invaluable process.

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Twin studies are informative designs that estimate the relative influences of nature and nurture on behavioral outcomes. Applying this method to prenatal studies will further enhance our knowledge of how prenatal risks relate to later developmental outcomes, controlling for genetic predispositions. To date, there is a sparse literature evaluating the relative influence of maternal prenatal risk, such as smoking or alcohol use during pregnancy, or perinatal environmental risks, including prematurity or low birth weight, to offspring developmental outcomes, controlling for genetic predispositions (Knopik et al., 2005; 2006; Koppen-Shomerus, Eley, Wolke, Gringas, & Plomin, 2000; Maughan, Taylor, Caspi, & Moffitt, 2004; Thapar et al., 2003; 2006; Wichers et al., 2002; Wilson, 1993). Thus far findings have illustrated that, in addition to prenatal environmental risk, it is important to consider genetic influences on child development and behavior. Evidence suggests strong influences of genetics for attention deficit- hyperactivity disorder (ADHD) and conduct problems in childhood. Importantly, maternal prenatal smoking and alcohol use remain significant predictors of ADHD in children, controlling for genetic influences on ADHD or problem behavior (Knopik et al., 2005; 2006; Maughan et al., 2004; Thapar et al., 2003). Moreover, research has documented significant gene-environment interactions; findings suggest interactions between genes and prematurity resulted in lower verbal and non-verbal communication in 24-month-old toddlers as well as interactions

between genes and low birth weight predicting cognitive development in young children (Koppen-Shomerus et al., 2000; Wilson, 1993). Other research exploring child behavior has shown negative interactions between genes and low birth weight; findings indicated the heritability of behavioral problems was lower when children were born with low birth weight whereas behavioral problems were highly heritable for children who were born with normal birth weights (Wichers et al., 2002).

The present study used a genetically-informative design to examine the putative influences of maternal prenatal perceived stress, obstetrical complications, and gestational age on infant dysregulation, competence, and developmental maturity. Specific goals of the study included estimating heritabilities on infant developmental outcomes and exploring whether prenatal perceived stress and obstetrical complications modified the heritability of infant behavior and developmental outcomes.

Prenatal and Obstetrical Risks and Developmental Outcomes

It is well-established that development can be impacted by a number of prenatal events (DiPietro et al., 2010). In fact, the importance of the prenatal environment for development and health has been scientifically studied from the early 1940's in humans, focusing primarily on prenatal nutritional deficiencies on pregnancy outcomes (Montague, 1962; Sontag, 1941). The fetal programming hypothesis states the environment in utero can influence fetal development during sensitive periods across pregnancy that lead to stable changes in offspring

development (Barker, 1995). To date, a number of researchers have investigated associations between the prenatal environment and infant cognitive outcomes; fewer studies have examined the association between prenatal risk and infant behavioral outcomes and temperament (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007; Davis, Glynn, Waffarn, & Sandman, 2011; Davis & Sandaman, 2010; Davis, Snidman, Wadhwa, Dunkel-Schetter, Glynn, & Sandman, 2004; Huizink, deMedina, Mulder, Visser, & Buitelaar, 2002; O'Connor, Heron, & Glover, 2002). Moreover, only a few studies have considered these associations in a genetically-sensitive design.

The harmful effects of some obstetrical complications, including maternal smoking, alcohol, and drug use during pregnancy on neural, cognitive, and behavioral development in infancy and early childhood has been well documented (Chasnoff et al., 1998; Mayes, Bornstein, Chawarska, Haynes, & Granger, 1996; Mayes, Grillion, Granger, & Schottenfield, 1998; Orlebeke, Knol, & Verhulst, 1999; Pollack, Lantz, & Frohana, 2000). Maternal smoking during pregnancy can result in complications during pregnancy, including placental abruption and miscarriage, or at birth, such as increased risk of still birth, prematurity, and low birth weight (Orlebeke et al., 1999; Pollack et al., 2000). There is strong evidence for the association between maternal prenatal smoking and psychological and behavioral problems in children, including attention problems, hyperactivity, and conduct problems, and lower academic achievement in adolescence, controlling for maternal age, household income, maternal education, maternal employment,

family psychiatric history and prenatal care (D'Onofrio et al., 2010; Linnet et al., 2003). In addition, an extensive literature indicates maternal alcohol use during pregnancy may result in a variety of outcomes for exposed infants, including increased risk of still birth (Kesmodel, Wisborg, Olsen, Henrickson, & Secher, 2002), congenital malformations, Central Nervous System (CNS) dysfunction and impaired mental functioning, such as fetal alcohol syndrome (FAS), delayed learning, memory problems, poor concentration and coordination, symptoms of ADHD, and poor social skills in toddlers and young children (Aroson, Hagber, & Gillber, 1997; Coles, Platzman, Smith, James, & Falek, 1992). Lastly, less research has focused on potential harmful effects of maternal drug use during pregnancy or prenatal poly-drug use (i.e., amphetamines, barbiturates, cocaine, marijuana, methampthamine, opiates), yet studies that have explored these links have shown impaired neural, cognitive, and behavioral development in infancy and childhood (Mayes et al., 1996; 1998; Weiss, John-Seed, & Harris-Muchell, 2007) and risk of psychiatric problems in childhood and adolescence (Chasnoff et al., 1998).

Maternal prenatal psychological stress and anxiety have been less studied. Growing evidence from research in the animal and human literatures suggests maternal prenatal stress can affect offspring physiological and behavioral development (see review by Gutteling, de Weerth & Buitelaar, 2005; Weinstock, 1997). Stress during human pregnancy poses a risk for pre-term delivery (<37 weeks gestation), lower birth weight (<2500 grams), smaller head circumference,

conotruncal heart defects, and lower Apgar scores (reflecting the newborn's heart rate, respiration, color, muscle tone, and reflexes) (Crandon, 1979; Dunkel-Schetter,1998; Lobel, Dunkel-Schetter, & Scrimshaw, 1992; Lou, Hanson, Nordentoft, Pryds, Jensen, & Nim, 1994; Paarlberg, Passchier, Dekker, & Ven Geign, 1995). Other research has illustrated maternal prenatal stress is associated with newborn irregularity of biological functions, poor soothability shortly after birth, more irritability (crying, fussiness), and more distress to novelty (Van den Bergh, 1990). Maternal prenatal stress and anxiety are also associated with lower mental and psychomotor development (Bergman et al., 2007; Brouwers, van Barr, & Pop, 2001; Buitelaar, Huizink, Mulder, Robles de Medina, & Visser, 2003; Davis & Sandman, 2010; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), difficult temperament (Austin, Pavlovic, Leader, Saint, & Parker, 2005; Gutteling, de Weerth, Willemsen-Swinkels, Huizink, Mulder, Visser et al., 2005), negative behavioral reactivity, controlling for postnatal maternal depression (Davis, Glynn, Waffarn, & Sandman, 2011; Davis, Snidman, Wadhwa, Glynn, Dunkel-Schetter, & Sandman, 2004) and inattention (Buitelaar et al., 2003) in infancy and toddlerhood. Furthermore, maternal anxiety during pregnancy is associated with risk for symptoms of ADHD, externalizing problems (aggression, acting out behavior), and internalizing problems (anxiety, emotional inhibition) in young children with effects persisting into late childhood (Gutteling et al., 2005; O'Connor et al., 2002; Van den Bergh & Marcoen, 2004). Importantly, effect sizes from these well-controlled studies are of equal

magnitude to the effect of smoking during pregnancy on birth weight and head circumference, an index of brain development (see review Lou et al., 1994).

In parallel with this work in humans, there has been an extensive body of well-controlled, experimental animal studies that provide support for a causal relation between the harmful effects of prenatal stress, nicotine, alcohol, and drug exposure on fetal development, with long lasting effects on early brain and psychological development. A consistent finding in the rodent and non-human primate work is that exposure to smoking, alcohol, or drugs prenatally causes long-lasting effects on learning, attention, regulatory behaviors, neuromotor behavior and performance, social behaviors, and poor adaptive ability in novel situations, such as increased anxiety (Ajarment & Ahmad, 1998; Driscoll, Stresissguth, & Riley, 1990; Erikksson, Ankarberg, & Fredriksson, 2000; Schneider, Roughton, Koelher, & Lubach, 1999; Schneider, Moore, Kramer, Roberts, & DeJesus, 2002; Weinstock, 2001). Epigenetic mechanisms are thought to underlie the long-lasting effects by affecting an individual's genome permanently following exposure environmental factors (Szyf, Weaver, Champagne, Diorio, & Meany, 2005).

Many of the effects of maternal prenatal stress on regulation of the hypothalamic-pituitary- adrenal axis (HPA axis) and resulting neuroendrocrine responses, motor development, behavioral adaptation, and attention and learning (Schneider, Roughton, Koehler, & Lubach, 1999; Weinstock, 2001) remain through adolescence (Clark & Schneider, 1997; Schneider et al., 1999), and

adulthood (Weinstock, 2001). Many of these same outcomes can be brought on by maternal administration of natural or synthetic corticosteroids during pregnancy, implicating HPA axis activity as a mediator of these effects (Benesova & Pavlik, 1989; Matthews 2002; Schneider et al., 1999; Weinstock, 2001). All together, compelling evidence from experimental animal studies has yielded strong support for a causal role for long-term effects of maternal prenatal stress or exposure to nicotine smoke, alcohol, or drugs on the development of the fetal brain and behavior in offspring (Weinstock, 2001).

In research with humans, confounding prenatal, perinatal, and postnatal risks may underlie the link between prenatal risk and adverse developmental outcomes in infants and toddlers, and increased behavioral problems and psychopathology in children. Moreover, it is possible that maternal smoking, alcohol use, or drug use during pregnancy may be markers of psychological or behavioral problems in the mother that are also inherited by the child, and that inherited risk may cause behavioral problems in children. An important consideration is the coexistence of uncontrolled covariates that may also affect development, including genes and traits of the mother that can be inherited. Although current research exploring prenatal risks and developmental outcomes has put forth important associations between the prenatal environmental and development, it is critical to pursue the relative contribution of genetic and environment influences as previous literature suggests maternal and infant

behavior may be linked for both genetic and environmental reasons (Agrawal et al., 2008). Genetically informative designs can differentiate types of influences.

The development of the brain is established early in life though a series of interactions between genetic influences and environmental factors (Fox, Levitt, & Nelson, 2010). The twin method applied in the current study takes advantage of the differing levels of genetic relatedness between Monozygotic (MZ) twin pairs who are genetically identical, and Dizygotic (DZ) twin pairs who are, on average, 50% genetically similar. By comparing the within-pair correlations for MZ and DZ twins, estimates for the contribution of additive genes, shared environment, and unique environment to the variance of a trait or behavior of interest may be obtained. A twin method exploring links between prenatal risk and developmental outcomes is highly informative and can address whether associations between prenatal risks and development occur independently of a genetic association.

Obstetrical Complications and Developmental Outcomes

Events occurring during pregnancy and childbirth have been related to the newborn's condition as well as development at later ages. Prematurity and low birth weights are two obstetrical complications that have received a great deal of research attention. Previous studies have shown consistent findings suggesting low birth weight and prematurity place the infant at risk for various health problems. Children born with low birth weights often experience more learning difficulties and emotional problems than children born within the range of normal weight (Saigal, 2000). However, the long-term consequence of low birth weight

largely depends on the environment. Children born with low birth weights and who were raised in stimulating home-environments that facilitate cognitive and emotional development are more physically and psychologically healthy as compared to children who were raised in less stable or economically disadvantaged families (Taylor et al., 2000).

Childbirth factors, such as mode of delivery, length of labor, and signs of fetal stress, have been found to relate to the newborn's condition after birth. Newborns born by elective cesarean section have higher rates of respiratory morbidity, possibly because labor and delivery produce a surge of catecholamines in the fetus which are important for lung functioning postnatally (Van den Bergh, Van Elburg, van Geijn, & Fetter, 2001). The mode of delivery and the neonate's condition after birth also has been related to the infant's stress reactivity (Taylor, Fisk, & Glover, 2000) and subsequent behavior (Keenan, Grace, & Gunthorpe, 2003). Infants born from emergency Cesarean-section (c-section) deliveries, which refers to the removal of the infant from the uterus through a surgical incision on the mother's abdomen, have less adrenocortical reactivity to stress (Lewis & Thomas, 1990), and showed the longest durations of crying bouts at the age of three months as compared to infants from those of elective c-sections (Keller, Lohaus, Volker, Capenberg, & Chasiotis, 1998).

Multiple gestation pregnancies and deliveries are more challenging than a single pregnancy and delivery. Twins gain weight at about the same rate as single born infants until the seventh week of pregnancy. Due to the nutritional and

placental challenges in sharing a common uterine environment, labor and delivery of more than one infant frequently results in early rupture of the membranes. Subsequently twins tend to be born early (about 35 weeks gestation) with lower birth weights (on average about five pounds) (Demissie, Ananth, Martin, Hanley, MacDorman, & Rhoads, 2002). Multiple gestation pregnancies are at an increased risk of maternal hypertension, diabetes, excessive bleeding in labor and delivery, and need for tocolysis, a drug treatment for preterm labor that results in suppressing uterine contractions and inhibition of the birth process (Luke & Brown, 2007). Medical treatment for preterm labor also involves the administration of corticosteroids (magnesium sulfate, betamethasone, and betamimetics) to improve fetal maturation and enhance fetal CNS functioning by means of stimulating the HPA-axis (Davis et al., 2004; Welberg, Seckl, & Holmes, 2000). Fetal suppression of the HPA-axis, on the other hand, is linked to reduced responsivity to environmental stressors whereby the infants' stress response system is affected, specifically the ability to regulate stress response and adapt to change (Davis et al., 2004; Coe & Lubach, 2005). Tocolysis increases the risk of intraventrical hemorrhage, infant mortality, and seizures; however, in combination with corticosteroids these risks are substantially decreased. Labor and delivery medications can cross the placenta, and in heavy doses, influence infant behavior postnatally. Infants of heavily medicated mothers were inattentive, more lethargic and irritable when aroused, more difficult to feed, smiled and cuddled less during the first few months of life (Davis et al., 2004;

deWeerth & Buitelaar, 2005). Common obstetrical complications among twin pregnancies include prematurity, intrauterine growth retardation, twin to twin transfusion, cardio-respiratory depression, and respiratory distress syndrome (Zuppa, 2003) all of which are likely to influence development beyond the neonatal period. Thus, studying developmental risk and outcomes in infant twins is especially important.

Stressful pregnancies as determined by mode of delivery (vaginal versus c-section) and delivery complications (duration of labor, fetal heart function, and 5-min Apgar scores) predict more crying and fussing as well as more difficulties in regulating infants' behavior, especially in stressful situations (i.e., inoculation) at the ages of six weeks and two months (deWeerth & Buitelaar, 2007). Importantly, fetal heart rate and Apgar scores, rather than mode of delivery, predicted infants' behavior, suggesting mild childbirth complications may serve as better predictors of early infant behavior than mode of delivery. Delivery mode is regarded as a global characterization of labor and delivery that may be less closely associated with the infant's condition after birth but not necessarily linked to later behavior. In contrast, complications specific to labor and delivery, such as long durations of labor or fetal heart rate functioning that indicate more physical distress during the process childbirth have been linked to poor physical health and behavior problems (de Weerth & Buitelaar, 2007). Similarly, four adversities during labor including duration of stage two labor (dilation stage that begins once cervix is completely open and lasts until the infant is born),

administration of syntocinon to accelerate labor, use of analgesia, and abnormal fetal heart rate were related to more crying and colic in 12-week-old infants (James-Roberts & Conroy, 2005). Moreover, in the same study, researchers found maternal prenatal anxiety, maternal alcohol consumption, and prenatal smoking were also predictive of infant crying and fussiness, but unrelated to infant colic (James-Robert & Conroy, 2005). Obstetrical complications and neonatal condition at birth also have been linked to delays in language development; specifically low Apgar scores and infant's total number of days in the NICU was significantly associated with delayed word production in toddlers (Marschik et al., 2007).

Further, a wide range of obstetrical and neonatal complications have been related to ADHD, conduct disorder (CD), and oppositional defiant disorder (ODD) in seven-and eight- year-old twins with more symptoms of ADHD and conduct behaviors in female children (Wagner, Schmidt, Lemery-Chalfant, Leavitt, & Goldsmith, 2009). Other research with adults has indicated obstetrical complications increase risk for schizophrenia, personality disorders, antisocial behavior, and mood disorders, including bi-polar disorder (e.g., Eagles et al., 1990; Kinney et al., 1994; Kinney et al., 1998).

The interaction between biological and environmental influences results in considerable variability for behavior and development. Infants who are born prematurely or low gestational age are at risk for developmental delays, difficult temperament (irritability and unresponsiveness) and inattention as infants and

toddlers and experience more learning difficulties and emotional problems as children compared to children born within the range of normal weight (Saigal, 2000).

Gestational age may be conceptualized as an indirect index of fetal growth attributed to the prenatal environment. The impact of preterm delivery on child development, has received a great deal of attention and links between preterm birth and neonatal complications, developmental delays, difficult temperament (irritability and unresponsiveness) and inattention in infancy and emotional problems as children have been reported (see reviews Lou et al., 1994; Paarlberg et al., 1995; Saigal , 2000; Taylor, Klien, Schayschneider, & Hack, 1998; Wolke & Meyer, 1999). Importantly, the long-term development outcome of prematurity, and/or with low birth weight, largely depends on the environment.

A brief overview of the twin method and a review of current literature utilizing the twin design to explore genetic and environmental influences on developmental outcomes are provided next. Then an overview of findings based on molecular genetics designs is presented as additional evidence of the importance of the prenatal environment for development.

The Twin Approach

Twin studies compare similarities between MZ and DZ twin pairs to estimate the effects of nature and nurture, partitioning variance accounted for by genetic, shared and nonshared environmental effects (Plomin, DeFries, McCleanr, & Rutter, 1997). After conception, the cells of the zygote divide repeatedly.

Sometimes it separates and develops into MZ, or identical twins, which are two individuals that result from one fertilized egg, and thus, inherit identical genetic information. DZ or fraternal twins, derive from two separate fertilized eggs. This results in two individuals who share genes, but are no more alike genetically than ordinary siblings. A comparison of the similarities and differences between MZ and DZ twins provides valuable information about the contributions of genetic and environmental factors to infant behavior and development. Heritability is the statistic that represents the extent to which individual differences on an outcome or behavior are genetic influenced. The greater the difference between MZ and DZ twins, the higher the heritability.

To describe environmental influences on behavior, twin studies divide the environment into two types, shared and non-shared or unique environment. The shared environment refers to any environment that makes individuals more similar. These influences can occur inside or outside the home, for example, two siblings who grow up and live in the same house, go to the same school, and share some of the same friends. In a twin study, the influence of the shared environment is suggested when MZ twins are no more similar than DZ twins, most likely because the shared environment is underlying similarities between twins. In addition, utilizing the twin method allows researchers to examine the influence of the non-shared environment on behavior or psychological outcomes, which refers to anything that differentiates family members. For example, although twins share the same parents, parents may treat their twins differently leading to differences in

behavior. Moreover, twins may go to the same school, yet they may have different educational experiences and have different friends. The non-shared environment is best measured by examining the differences between MZ twins. Since MZ twins share 100% of the same genes, any differences between MZ twins must be attributed to two sources of variance, the non-shared environment and sources of measurement error (Petrill, 2001).

There also can be interactions between genes and environments that result when the shared and/or non-shared environment interacts with genetic influence. Accordingly, individual differences arise from gene-environment interactions as opposed to stemming from additive genetic effects on behavior or development and the environment. Thus, any trait or behavioral outcome can be described as a function of genetics, shared environment, and non-shared environmental influences (Petrill, 2001).

Not only do twin analyses partition variance in behavior into genetic, shared environmental, or non-shared environmental influences, the twin method extends beyond estimating these influences and describes shared influences among behaviors. Multivariate behavior genetic methods are based on cross-twin correlations, for example, a comparison is made between one twin's child anxiety score and the co-twin's depression score. If MZ twins are more similar than DZ twins, then genes may play a role in the relation between anxiety and depression. If MZ twins are no more similar than DZ twins, then the environment is responsible for the association between anxiety and depression.

Applying the Twin Method to Studying the Prenatal Environment

In prenatal twin studies, genetic influences, pregnancy experiences, and the postnatal environment can be estimated to determine relative influences on infant behavior and development. To date, there is very little research that employs a twin design to separate relative influence of genes and prenatal environmental factors on infant behavior and development. Applying the twin method to prenatal research affords the opportunity to evaluate differences in the shared intra-uterine environment that may contribute to the differing trajectories of early behavioral development (Piontelli, Bocconi, Boschetto, Kusterman, & Nicolini, 1999). The literature exploring whether the influences of prenatal risks occur independently of a genetic predisposition is sparse.

Of research employing behavioral genetic techniques to study prenatal risks, such as maternal smoking, and perinatal influences on developmental outcomes, results have illustrated maternal smoking during pregnancy significantly increased the risk of ADHD or conduct problems in twin offspring, controlling for genetic influences (Knopik et al., 2005; 2006; Koppen-Shomerus et al., 2000; Maughn et al., 2004; Thapar et al., 2003; Wichers et al., 2002; Wison, 1993). By using a twin design, these studies were able to account for the effect of genetic factors on behavioral outcomes in children. Thapar et al. (2003) studied a sample of 1,452 twin pairs, aged five to 16 years, whose mothers retrospectively reported on maternal smoking behavior during pregnancy. Although genetic influences accounted for most of the variance in child ADHD, maternal smoking

during pregnancy remained a significant influence on ADHD when controlling for genetic influences. Similarly, Maughan et al. (2004) evaluated conduct problems in a sample of 1,116 twin pairs, between the ages of five and seven years, and found the strong association between maternal prenatal smoking and more conduct problems in children. However, when accounting for the genetic influence on conduct problems in children, the associations between maternal prenatal smoking and conduct behavior were reduced, yet prenatal smoking remained a significant predictor of more child conduct problems. These studies highlight the importance of considering both genetic and environmental risks, such as maternal prenatal substance use, on developmental outcomes.

Each of the studies presented next are genetically-informative designs and are useful in understanding gene and environment influences on behavior. Because genetic influences are important contributors to social and emotional development and behavior, it is imperative to utilize genetically informative designs that enhance our understanding of relations between prenatal risk and development. Currently there is a lack of research exploring prenatal risk influences on infant development in twins. Therefore a review of the literature exploring the influences of maternal pregnancy and birth complications on development in twins is provided. These studies illustrate how twin designs can explain links between maternal prenatal risk and developmental outcomes, controlling for genetic predispositions on traits and behavior. As an additional

example, research documenting the influences of maternal smoking and alcohol use during pregnancy and genetics on ADHD in children and adolescents is presented.

One obstetrical complication receiving attention in twin research is low birth weight. It is important to enhance our understanding as to whether being born with low birth weight elevates the risk for developmental delay or behavioral problems, or whether the established links between low birth weight and developmental outcome are driven solely by genetic factors or aspects of the environment. Birth weight itself is subject to both genetic and environmental influence (Vlietnick et al., 1989). One study revealed discordance in psychopathology increased with the degree of birth weight discordance and that this effect was similar for MZ and DZ twins, validating birth weight as an important environmental risk (Van Os & Selten, 1998).

Longitudinal twin studies have provided valuable information concerning the relation between low birth weight and later cognitive ability (Wilson, 1993). In one of the first twin studies to evaluate birth complications and later development, The Louisville Twin Study (Wilson, 1993) sampled 450 pairs of MZ and DZ twins, assessed with the Bayley Scales of Infant Development (BSID) at the ages of six, 12, 18, and 24 months, the Stanford-Binet at age three, and the Preschool and Primary Scale of Intelligence at age six years. Findings illustrated that low birth weight (LBW) infants scored lower on these measures of cognitive ability. Moreover, LBW MZ twins scored lower than their heavier co-

twin as infants and toddlers, yet at the age of six years showed similar cognitive ability indicating an environmental influence on cognitive outcomes at this age (Wilson, 1993).

To rule out the possibility that associations between other birth complications and cognitive ability and temperament were attributed to prematurity or low birth weight, the MacArthur Longitudinal Twin Study sampled nearly 480 pairs of twins at the ages of 14, 20, 24, and 36 months of age (Emde et al., 1992). Difference score correlations revealed associations between differences in birth weight and later cognitive ability at 20 and 24 months of age were due to common genetic influence, as opposed to environmental influences. Temperament was not significantly related to birth weight in infants within the normal range. Although both prominent infant twin studies, the LTS and MALTS, assessed perinatal influences and cognitive outcomes, findings revealed different etiologies. Differences in findings may be due to the sample selection. Unlike the LTS which included twins with a wide-range of birth weights, gestational ages, and perinatal complications, the MALTS selected preferentially for infants born with normal gestational age and birth weights appropriate for gestation age, providing the MALTS with tighter control over perinatal complications. Infants who are born with normal birth weights and gestational ages and healthy birth weights for gestational age are less likely to have perinatal complications than infants born prematurely or with low birth weight, such as the infants in the LTS.

This may explain why the LTS found evidence for environmental influences and the MALTS revealed genetic influences on cognitive development.

In addition to low birth weight, prematurity also has received attention among the few genetically informative studies. Prematurity is associated with a range of neonatal complications and increased risk of adverse developmental outcomes in childhood (Taylor, Klien, Schayschneider, & Hack, 1998; Wolke & Meyer, 1999). It is unknown whether genetic factors play a major role in the development of individual differences in cognitive development in preterm infants. Mechanisms thought to underlie the link between prematurity and cognitive development include exposure to prenatal environment risks (Brook, Brook, & Whiteman, 2000; Brooks, Johnson, Steer, Pawson, & Abdalla, 1995), perinatal or neonatal experiences (Laucht, Esser, & Schmidt, 1997) or postnatal family environment (Bendersky & Lewis, 1994).

To date, researchers exploring genetic and environmental influences on developmental outcomes have found important gene-environment interactions between obstetrical complications and cognitive and behavioral outcomes (Koeppen-Schomerus et al., 2000; Wichers et al., 2002). In one study exploring prematurity and cognitive development twins were divided into three groups according to degree of prematurity: very preterm or high-risk (<32 weeks), moderately preterm or medium-risk (32-33 weeks), and mildly preterm or low-risk (>34 weeks). Significant gene-environment interactions were revealed with heritability differing by environmental risk group. Specifically, shared

environmental factors accounted for a significant portion of variance (84%) of verbal and nonverbal cognitive development in high-risk toddlers, with non-significant additive genetic effects (9%). For both medium-and low-risk toddlers, additive genetic effects explained 33% and 22% of the variance, respectively, whereas shared environment accounted for 65% and 73%, respectively, of verbal and nonverbal cognitive development. Results suggest for toddlers born very prematurely, verbal and nonverbal cognitive development outcomes were not attributed to genetic factors. Rather, shared environmental factors influenced verbal and non-verbal cognitive development for toddlers born very premature at the age of 24 months.

Other prospective research indicated gene-environment interactions between pregnancy and birth complications (mode of delivery, mode of induction of ovulation, fetal presentation, and LBW) and behavior problems in 760 twin pairs between the ages of six and 17 years of age (Wichers et al., 2002). Specifically, additive genetic factors accounted for increased problem behaviors in heavier birth weight children, controlling for gender and gestational age, whereas shared environmental influences accounted for increased problem behavior in lower birth weight children (Wichers et al., 2002). These gene-environment interactions are consistent with previous research indicating interactions between genetic factors, low birth weight, and verbal and non-verbal cognitive development in toddlers (Koeppen-Schomerus et al. 2000). Importantly, findings highlight the importance of considering both genetic and environmental

influences in relation to cognitive and behavioral development in toddlers, children, and adolescents as genetic predispositions may be modified by environmental factors at birth.

Most recently genetic and environmental influences on prenatal and neonatal factors (maternal smoking during pregnancy, maternal alcohol use, duration of labor, gestation, birth weight, days in NICU, time in hospital, and days breastfed) and associations with symptoms of autism were explored in a sample of 13,690 twin pairs between the ages of seven and eight years old (Ronald, Happe, Dworzynski, Bolton, & Plomin, 2010; Ronald & Hoekstra, 2011). Prenatal and neonatal factors were strongly related to social impairments of autism as compared to nonsocial autistic features, although these significant associations were weak. Interestingly, neonatal problems showed modest heritability (13-14%) and significant shared (55% -59%) and nonshared (28% - 31%) environmental influences. This research is the first to examine associations between prenatal and neonatal obstetric factors and symptoms of autism controlling for the variance in autism attributed to genetic influences. A greater number of studies have examined relations between maternal prenatal smoking or alcohol use and ADHD in children and adolescents using the twin design. Maternal smoking and alcohol use during pregnancy are common obstetrical complications; thus, a review of this work is provided next.

To address the issue that mothers who smoke or drink alcohol during pregnancy are more likely to have underlying trait or psychological problems,

such as a history of ADHD, that are then transmitted to their children genetically, a few twin designs have been employed to control for genetic predispositions underlying the risk for ADHD (Thapar et al., 2003; Knopik et al., 2005; 2006). A failure to control for these confounding influences may account for inconsistency of results across previous studies evaluating associations between maternal alcohol use and child externalizing behavior.

It is known that alcohol use or smoking during pregnancy is more common among women with antisocial traits (Bardone et al., 1998). Thus, maternal antisocial behavior increases the risks of antisocial behavior in children through genetics (by means of the transmission of heritable traits) and environmental factors (via the prenatal environment and through exposure to later risks) including harsh parenting styles (Fagot, Pears, Capaldi, Crosby, & Leve, 1998), or maternal alcohol and drug use (Robbins, 1998). As a result, associations between prenatal smoking and child outcomes may be confounded. Antisocial behavior is partly heritable (Rhee & Waldman, 2002), including nicotine use and dependence (Kendler, Neal, Sullican, Corey, Garnder, & Prescott, 1999), and prenatal smoking habits (D'Onofrio, Turkheimer, Eaves, & Corey, 2003), raising the possibility that children who are exposed to smoking prenatally would be at risk for antisocial behavior regardless of whether their mothers smoked during pregnancy. Failure to control for heritable factors may account for the suggested relationship between prenatal exposure and development and behavior outcomes.

Children of alcoholics are significantly more likely to have been exposed to high-risk environments, including prenatal exposure to alcohol or nicotine, and more likely to display externalizing behavior. Recent research from Knopik and colleagues exploring prenatal risks and ADHD suggested the links are not the results of gene-environment interactions (Knopik et al., 2005; 2006). Rather, the influence of genetic risk for ADHD is independent and not related to the prenatal or obstetrical environmental risks associated with maternal alcohol use during pregnancy or low birth weight. Knopik et al. (2005) interviewed alcoholic parents of adolescent female twin pairs (1091 MZ and 845 DZ pairs) between the ages of 11 and 23 years to investigate the relative contributions of maternal prenatal and postnatal smoking and drinking behavior to an increased risk for ADHD. In order to account for any differences in genetic and environmental effects on ADHD in adolescents whose mothers reported having smoked during pregnancy as compared to those whose mothers drank heavily during pregnancy, twin pairs were divided into two groups depending on whether their mothers reported to having smoked (yes or no) or drank alcohol (yes or no) heavily during pregnancy.

Maternal smoking during pregnancy was significantly associated with low birth weight and increased risk for ADHD, controlling for postnatal maternal and paternal smoking. Interestingly, when considering the influence of both maternal smoking and alcohol use during pregnancy on ADHD, maternal prenatal smoking was no longer a significant predictor of risk for ADHD. Additive genetic effects explained 86% of the variance in ADHD, whereas the non-shared environment

accounted for the remaining 14%, controlling for low birth weight, parental alcohol disorder, and frequent heavy drinking during pregnancy (Knopik et al., 2005). Importantly, the genetic influence on ADHD remained significant after controlling for these prenatal and perinatal risk factors, indicating much of risk for ADHD may not be explained by exposure to smoking or alcohol prenatally or being born with low birth weight. Rather, results suggest an important genetic influence on risk for ADHD and that the influence of the prenatal environment is independent of the genetic risk for developing ADHD, rather than working interactively.

Children of alcoholic parents may be at higher risk because they are more likely to inherit increased genetic risk of ADHD as well as experience prenatal exposure to alcohol and smoking that further increases their risk. Knopik et al., (2006) examined the role of genetic risk, maternal chronic alcohol use and prenatal smoking and alcohol use in determining risk of ADHD using a children-of-twin design, where at least one twin has a history of alcohol abuse or dependence and at least one twin had children aged 13-21 years participated. To disentangle genetic and environmental risk for ADHD, participants were divided into five groups based on maternal alcohol disorder or use history of the twin parent and the parent's co-twin and created risk categories according to genetic risk (low/high) and environmental risk (low/high).There was no evidence for a gene-environment interaction; rather, ADHD was more likely to be diagnosed in children with mothers in the high genetic risk (maternal alcohol disorder and use)

groups and in those whose mothers reported heavy smoking beyond the first trimester of their pregnancy (Knopik et al., 2006). Importantly, maternal heavy smoking during pregnancy remained a significant predictor of ADHD, controlling for maternal genetic risk of alcohol use disorder. However, the strength of the association between maternal prenatal smoking and risk of ADHD was reduced once maternal genetic risk was considered. Further, there was a strong genetic influence on the association between maternal alcohol disorder and increased risk of ADHD. These findings are consistent with earlier research with adolescent female twins (Knopik et al., 2005); it appears prenatal risk factors combine additively, rather than work together, with genetic risk in determining risk for ADHD in children. All together, these findings underscore the importance of considering genetic factors and maternal smoking during pregnancy simultaneously when considering ADHD. In the current study, maternal smoking and alcohol use during pregnancy were considered as risk factors as part of a broader environmental obstetrical risk composite.

A brief review of current research utilizing a molecular genetics design is provided next as an introduction to another method to studying genetic and environmental influences on behavior and development.

Molecular Genetic Studies: Interactions between Genotypes and Environments

Studies of genotype-environment interactions illustrate how inherited characteristics make some individuals more susceptible to both negative and

positive effects of environments (Moffitt, Caspi, & Rutter, 2005). A few molecular studies have yielded important roles of genetics underlying the associations between maternal prenatal risk and offspring behavior and development (Kahn, Khoury, Nichols, & Lanphear, 2003; Neuman, Lobos, Reich, Henderson, Sun, & Todd, 2007; Price, Grosser, Plomin, & Jafee, 2010; Wang et al., 2002). Molecular genetics approaches are based on a number of genetic markers, a piece of DNA that varies between individuals in a population that have been identified within the human genome (McGruffin & Thapar, 1997). For example, genetic markers in the serotonin system have been explored in relation to anxiety and depression (Lesch et al., 1996), bipolar disorder (Collier, Stoberg, & Li, 1996), obsessive-compulsive disorder (Bengel et al., 1999), and seasonal affective disorder (Rosenthal, Carpenter, James, Parry, Rogers, & Wehr, 1998). Molecular genetic studies reveal gene-environment interactions that illustrate how genetics and environmental risks work together and influence developmental outcomes. Applying a molecular genetics approach to research exploring links between prenatal risk and offspring developmental outcome offers a new degree of complexity in unraveling the influences of genetics and environmental risk and the interactive effects that predispose children to behavior and development.

Recently, important gene-environment interactions between maternal prenatal smoking and developmental outcomes have been documented. The role of metabolic genes in determining genetic susceptibility to low birth weight following exposure to maternal smoking during pregnancy was explored and

findings indicated influences of smoking on infant birth weight and prematurity varied depending on maternal genotype (Wang et al., 2002). Metabolic gene polymorphisms CYP1A1 and GSTT1 modified the associations between maternal prenatal smoking and prematurity and infant birth weight; mothers who smoked during their pregnancy with either CYP1A1 or GSTT1 gave birth to babies who were premature or low birth weight but mothers with both the CYP1A1 and GSTT1 genotypes gave birth earlier to babies with the lowest birth weight, controlling for pregnancy and birth complications and mode of delivery (Wang et al., 2002).

In addition, five-year-old children who inherited two copies of the 10-repeat DAT1 allele and who were exposed to prenatal smoking displayed significantly more inattentive, hyperactive-impulsive, and oppositional-defiant behaviors as compared to children who inherited either one copy of the 10-repeat DAT1, and children who inherited either the 9-repeat, 8-repeat or 7-repeat alleles, controlling for household income, marital status, child age and sex, postnatal smoke exposure, and home environment (Kahn et al., 2003). Important relations between prenatal smoking and polymorphisms DAT1 440 and 480 alleles and DRD4 7-repeat allele and development of ADHD, controlling for SES, ethnicity, and pregnancy, labor, delivery, and birth complications, in children and adolescents have been documented (Neuman et al., 2007). Together findings highlight the importance of considering genetic influences on offspring behavior and development.

Although the evidence consistently shows that maternal smoking and alcohol use during pregnancy is associated with undesirable birth outcomes in infants and ADHD and psychopathology in children (Hill, 2002; Hill, Lowers, Locke-Wellman, & Shen, 2000; Kahn et al., 2003; Knopik et al., 2005; Linnet et al., 2003; Mick, Beiderman, Faraone, Sayer, & Kleinman, 2002; Mick, Beiderman, Prince, Fischer, & Faraone, 2002; Milberger et al., 1996; 1998; Price et al., 2010; Thapar et al., 2003), the underlying biological processes are not well understood. ADHD is a highly heritable, commonly diagnosed psychiatric syndrome with onset in early childhood. The attempt to establish a biological mechanism for ADHD has led to an outstanding number of candidate-gene studies of ADHD (Bobb, Castellanos, Addington, & Rapoport, 2005; Faraone et al., 2005). This work stems from research investigating the known effects of stimulant medications on increasing synaptic dopamine concentration; most attention in these studies has been directed to polymorphisms relating to the dopaminergic system. The strongest support is for an association between ADHD and the 7-repeat polymorphism in exon-3 of the DRD4 gene. This research is important and findings illustrate how some children are genetically vulnerable to ADHD, yet exposure to maternal smoking or alcohol use during pregnancy exacerbates this risk. These studies highlight the importance of investigating the interplay between genetics, exposure to prenatal and obstetrical risk, and developmental outcomes.

Currently there is a lack of research exploring links between maternal prenatal risk and social and emotional development and behavior, controlling for shared genetic etiology on behavior. The present study explored the aforementioned associations in infant twins. Previous research suggests maternal prenatal stress and anxiety are associated with adverse pregnancy and infant developmental outcomes by means of abnormal activity of the maternal HPA-axis that then may influence infant physiological and behavioral development of infants (de Weerth & Buitelaar, 2005). Maternal prenatal stress influences fetal neurodevelopment, cognitive functioning, and possibly emotion-regulation in infancy and childhood, therefore is an important factor necessitating more research (Buitlaar et al., 2003; DiPietro et al., 1996a; 1996b; 1998; 2002; 2004; Gunnar, 1998; Huizink et al., 2002; Van den Bergh, 1990). This study utilized a prenatal twin design and estimated influences of maternal prenatal stress risk and obstetrical complications on infant behavior and developmental maturity, controlling for genetic predispositions. Further this study enhanced our knowledge of the complex relations between genetic vulnerability, prenatal stress risk and obstetrical complications, and later development during infancy.

Goals of the Current Study and Hypotheses

Given findings from studies that have examined links between pregnancy stress and obstetrical risk and impaired neural, cognitive, and behavioral development in infancy and childhood (Mayes et al., 1996; 1998; Weiss, John-Seed, & Harris-Muchell, 2007), infant dysregulation (e.g., sleep problems, eating

pathology, & sensory sensitivity), behavioral competencies (e.g., compliance, attention, mastery motivation, & imitation), and developmental maturity, were utilized to capture a broad range of functioning at 12 months of age. The main objective of this study was to use a genetically-informative design to examine the putative influences of maternal prenatal perceived stress, obstetrical complications, and gestational age on infant functioning. Given a developing fetus obtains information about the prenatal environment from nutrients, hormones, and other chemicals that cross the placental barrier, it is likely the interplay between genes and environmental stress during pregnancy may modify heritability of traits in such a way that prepares the infant to adapt and respond appropriately to the demands of the future postnatal environment (Glover, 2011). Thus, a specific aim of the present study was to examine whether or not maternal prenatal perceived stress, obstetrical complications, and gestational age modified the genetic influence on individual differences in infant outcomes. It was expected that as environmental risk increased, the heritability of infant outcomes would decrease because the role of stressful environments would become more important in predicting individual variation in behavioral outcomes as compared to genetic influences. Therefore, it was hypothesized that higher maternal prenatal perceived stress, more obstetrical complications, and lower gestational age would decrease the heritability of dysregulation, competence, and developmental maturity in 12-month-old infants. These relations have not been investigated in the literature to date.

Method

Participants

A total of 291 mother-infant twin pairs residing in the state of Arizona participated in this study. The sample was recruited through collaboration with the Vital Records Office- Department of Health Services who sent recruitment letters to mothers (at least 18 years of age) who had given birth to live twins in an Arizona hospital. With regard to participation, a total of 291 mothers completed one or both of the 12-month interviews and 268 mothers provided consent to obtain medical records. Of the women who agreed to release pregnancy and birth medical records, a total of 236 and 231 medical records were obtained for each twin, respectively, and 203 pregnancy and labor and delivery medical records were obtained. Missing pregnancy and birth medical records were due to incorrect personal information on HIPPA authorization forms or participant mistakes on medical records release forms (i.e., missing signature or date of birth), issues related to hospital medical records department, such as inability to locate one or more members within a family, or hospital medical records department provided insufficient/incorrect information to code pregnancy and twin birth records.

Independent samples *t*-tests were conducted to determine if there was selective attrition between the women who participated in the 12-month interviews and consented to providing pregnancy and twin birth medical record data and the women who only completed the 12-month interviews. The results of the *t*-tests revealed there were no significant differences between these two groups

of women on infant developmental outcomes: infant dysregulation, $t(564) = -.12$, $p = .87$; infant competence, $t(568) = 1.15$, $p = .91$; and infant developmental maturity, $t(576) = -1.29$, $p = .13$. Moreover, there were no significant differences between these two groups of women on a number of demographic variables, including mothers' age $t(260) = 1.63$, $p = .18$; utilization of prenatal care, $t(258) = .82$, $p = .41$; marital status, $t(263) = 1.59$, $p = .12$; mothers' educational level, $t(263) = .63$, $p = .46$; and mothers' employment status, $t(263) = 1.10$, $p = .23$.

With respect to demographic variables and sample characteristics, 96% of the primary caregivers were mothers, 3% were fathers, and 1% were the infants' grandmother; the majority of mothers self-reported their race as Caucasian (64.3%); 26.8% were Hispanic; 4.3% were Asian, Asian-American, or Pacific Islander; 2.5% were African-American; 1.4% were American Indian or Alaskan Native; 1.8% self-reported their race as mixed. With regard to education, 3.3% of mothers did not complete high school, 15.0% had a high school diploma or equivalent, 28.7% had some college and 36.9% had a college degree, and 16.1% of mothers had graduate or professional degrees. A total of 54.9% of mothers were employed during their pregnancy with their twins. Of mothers who were employed, 68.3% were employed full-time and 31.7% were employed part-time.

At the time of the pregnancy, the majority of women were married (81.0%) or in a partnership (9.3%). With regard to household annual income, 10.9% of families earned less than $20,000 annually; 17.1% earned $20,000-

40,000; 16.2% earned $40,000-60,000; 18.1% earned $60,000-80,000; 13.6% earned $80,000-100,000; and 23.4% of families earned $100,000 or greater.

Measures

Medical records were collected from hospitals in the state of Arizona. Maternal pregnancy and twin birth medical records were used to complete measures of pregnancy risks, obstetrical complications, and neonatal risk factors.

The Obstetrical Complications Scale (OCS; adapted from Littman & Paralee, 1974) measured specific prenatal (e.g., alcohol use or smoking during pregnancy) and obstetrical risks that were either experienced during pregnancy or at birth. Previous researchers using the OCS have found it helpful in predicting neurodevelopmental impairment outcomes in children (Kunugi, Nanko, & Murray, 2001), schizophrenia (Cannon et al., 2002), and major affective disorders in women (Jablensky, Morgan, Zubrick, Bower, & Yellachich, 2005). The OCS included a wide range of items indicating potential obstetrical risks for pregnant mothers that are coded on a 0-1 scale of 'no risk" or "risk" for the following variables: pre-term labor, previous history of stillbirth, duration since last pregnancy, maternal blood pressure, Rh antagonism (blood group incompatibility), preclampsia, bleeding during each trimester, maternal prenatal smoking, prenatal alcohol use, prenatal chronic drug use, bed-rest during pregnancy, infections during pregnancy, labor and delivery information, such as administration of magnesium sulfate for preterm labor, medications provided during labor, stimulation of contractions, duration of stages of labor, premature

rupture of membranes, mode of delivery (vaginal or C-section), and post-birth information, including fetal presentation at delivery (e.g., vertex, breech, transverse), meconium staining, fetal heart rate, cord information (e.g., cord around infants neck, cord insertion). In addition, the OCS measured other maternal characteristics, such as maternal parity, blood type, blood pressure, maternal gravidity, history of abortion, prescribed medications, and chronic maternal disease. The OCS had high validity and had been used in diverse samples (see review Cannon, Jones, & Murray, 2002). The obstetrical complications composite was created by summing all the items on the OCS. Inter-rater reliability for the obstetrical complications sum composite, based on 25% of the records, was kappa = .95 between the first author and research assistant.

The Neonatal Complications Scale (NCS; adapted from Littman & Paralee, 1974) was used to examine the state of the infant and potential risks experienced during the first few days after birth. Variables included gestational age (weeks), size (length in inches), birth weight (grams), and 1- and 5-minute Apgar scores. The Apgar score rates five characteristics of neonatal health immediately after birth, including the neonate's heart rate, respiratory effort, muscle tone, color, and reflex irritability. Each characteristic of overall health is rated on a scale of 0 to 2, with higher scores indicating a better overall condition. Other variables included admission to the neonatal intensive care unit (NICU), type and length of resuscitation, birth order, heart rate, respiratory rate, temperature, days spent in the hospital, infection, and illness. The neonatal

complications composite was created by summing all the items on the NCS. The NCS has been used as a reliable screening tool with predictive validity for infant health outcomes, such as risk for autism (Wilkerson, 2002), and risk for other psychiatric disorders, such as schizophrenia and affective disorders (Jablensky, Morgan, Zubrick, Bower, & Yellachich, 2005).

The Neonatal Morbidity Scale (NMS; Minde, Whitelaw, Brown, & Fitzliardinge, 1983) is a comprehensive screening tool with high validity in predicting central nervous system dysregulation in infancy, psychomotor and cognitive developmental outcomes in children and developmental outcomes assessed with the BSID (Medoff-Cooper & Gennaro, 1996). The NMS evaluated neonatal health complications, including bradycardia, tachypnea, and whether infants (twins) were prohibited from feeding. The presence, length, and type of treatment administered were scored depending on severity (3-point scale) of the following complications: apnea, respiratory distress syndrome, and hyperbilirubunemia. All items were scored on a 3-point scale with a not applicable option and summed to form the neonatal morbidity sum composite. In most cases, the medical records department provided insufficient information to code the NMS from twin birth records; thus, we coded 108 NMS in the current study; however due to sample size neonatal morbidity was not included in genetic analyses.

Maternal perceived prenatal stress (Rice et al. 2007) was assessed with one 11-point item that asked mothers how stressed they were during their twin pregnancy on a scale from 0-10, where 0 is defined as, "you were completely relaxed, no stress or worry at all" to 10 which was defined as, "you were as stressed or worried as you can possibly imagine?" Mothers were asked about stress during pregnancy in a telephone or online interview when their twins were 12 months of age (adjusted for prematurity). Previous research has used this measure to assess maternal prenatal stress retrospectively specific to each trimester (early, mid, and late pregnancy) as well as a measure of overall stress during pregnancy (Rice et al., 2007; 2010). This measure of maternal prenatal stress, although subjective and retrospective was significantly correlated with multiple objective birth outcome measures obtained from medical records (Rice et al., 2010).

Gestational age of each twin was coded from medical records. In the current study, gestational age was used as a moderator variable.

The Zygosity Questionnaire (Goldsmith, 1991) determined whether twins were identical or fraternal. As part of the interview, mothers responded to several detailed questions asking about physical similarities and differences between their twins. This questionnaire yields over 95% agreement with zygosity determined via genotyping (Forget-Dubois et al., 2003). Zygosity was further verified with infant birth medical records, especially the placenta lab report..

The Infant-Toddler Social-Emotional Assessment (ITSEA; Carter & Briggs-Gowan, 2001) assessed both normal variation in social-emotional and behavioral development as well as abnormal behaviors that may reflect psychopathology in young children between the ages of 12 months and three years of age. Mothers were asked to indicate on a scale of 0 (Not true/Rarely) to 2 (Very True/ Often) whether each statement described each twin's feelings or behaviors in the last month when their twins were 12 months old (adjusted for prematurity). The ITSEA has high internal consistency and validity across a variety of samples with toddlers and young children and high inter-rater agreement (Carter, Briggs-Gowan, Jones, & Little, 2003). The ITSEA has been used as a screening tool in early intervention and head-start programs and is predictive of childhood social, emotional, and behavioral problems.

In the current study, two mean composites reflecting infant dysregulation and competence were used as outcome variables. Dysregulation was created with five items tapping aspects of infant sleep, (e.g., "wakes up at night and needs help to fall asleep again" or "must be held to go to sleep"), nine items reflecting eating and eating pathology (e.g., "is a picky eater" or "holds food in cheeks"), and seven items tapping sensory sensitivity (e.g., "is bothered by loud noises or bright lights" or "won't touch some objects because of how they feel"). Competence was formed with eight items reflecting compliance (e.g., "puts toys away after playing" or "follows rules"), five items tapping infant attention (e.g., "pays careful attention when being taught something new" or "plays with toys for 5 minutes or

longer"), six items reflecting imitation and play (e.g., "Pretends that objects are something else" or "hugs or feeds dolls or stuffed animals"), and six items reflecting mastery motivation (e.g., "likes figuring things out, like stacking blocks" or "keeps trying even when something is hard"). The reliabilities for the broader mean constructs of infant dysregulation (alpha = .68) and infant competence (alpha = .82) were acceptable.

Developmental Profile II (Alpern, Boll, & Shearer, 1984) is a comprehensive assessment of infant developmental level of functioning in the following developmental areas: physical, language, self-help, social, intellectual, and communication development. Mothers indicated (yes, no, or not yet) whether each twin had achieved particular capabilities in one of the core developmental areas to statements such as, “Does your baby go from crawling or sitting position to a standing position, holding on to something like a coffee table?” “Does your baby understand what “my” means?” or “Does your baby use hand motions or gestures as a way of talking?” or “Has your baby stopped drooling, except when chewing, teething or eating?” A mean composite reflecting physical, self-help, social, intellectual, and communication development formed the developmental maturity outcome variable. The Developmental Profile II has high-reliability and predictive validity for physical and social development, self-help and academic achievement in preschool, kindergarten, and throughout childhood (Quay & Steele, 1997). In the present study, alpha for the mean-score developmental maturity composite was .70.

Procedure

The Division of Public Health Services, Office of Vital Records mailed letters to mothers who were at least 18 years of age and gave birth to live twins in an Arizona hospital between July 2007 and July 2008. Recruitment letters were mailed two months prior to the twins' 12-month birthday, including a postage-paid return response letter. Interested families returned response letters indicating their interest in the research study. The recruitment letter was mailed again one month later as a follow-up if we had not yet heard back from the family.

Participation involved two 40-minute interviews, completed by the primary caregiver (96% were mothers) over the telephone or online using Survey Monkey. The first interview measured general family demographics, twin zygosity, and twin developmental maturity and behavior. The second interview tapped aspects of the family environment and characteristics of the twins' primary caregivers. Additionally, to obtain hospital pregnancy and twin birth medical records, standard HIPAA authorization forms were mailed and returned completed prior to accessing medical records. As a thank you for the families' time, participants received $20.00 for each interview and $10.00 for their completed HIPPA consent (a total of $50.00).

Statistical Approach

The twin method applied in the current study takes advantage of the differing levels of genetic relatedness between MZ twin pairs who are genetically identical, and DZ twin pairs who are, on average, 50% genetically similar.

Specifically, the variance in infant dysregulation, competence, and developmental maturity at 12 months of age was attributed to three different latent factors: additive genetic variance (A), shared environmental variance (C), and non-shared environmental variance (E), which also includes measurement error. Within-pair similarity for the behavioral outcomes was due to genetic factors plus common or shared environment factors. Non-shared or unique environment was a residual term that included environmental factors that make members of a family different from one another and measurement error.

Utilizing Mx structural equation modeling software (Neale, Boker, Xie, & Maes, 2003), a series of nested models were tested to decompose the variance in infant outcomes into these three latent components (A, C, and E). Beginning with the full ACE model, parameters were systematically dropped to test the significance of each influence (E is always included because it includes measurement error) on trait variation, such that ACE, AE, CE, and E only models were fit to the data for each infant outcome. According to simplicity or the rule of parsimony, nested (reduced) models should be estimated to determine if a simpler model can represent the data as well as the full model. To do so, a full model including all ACE estimates was tested first. Fit that is more parsimonious is indicated by the -2 Log Likelihood (-2LL) fit indices of a non-significant difference in chi-square and a negative Akaike's Information Criteria (AIC; Akaike, 1987). The best model reflects the highest probability revealed by the chi-square difference test as well as the lowest AIC value.

Model fit testing began with determining the most parsimonious model with no moderation (ACE, AE, CE, or E only). Next, the full ACE model with moderation on all variance components (additive genetic, shared environmental, and nonshared environmental) was compared to the ACE model with no moderation on variance components. If there was no evidence of moderation, then the model with no moderation was adopted as the most parsimonious model and no other models were fit to the data. However, if the model with moderation fit best, then a series of nested models were compared to the full moderation model to determine the most parsimonious model: (1) only A moderation, (2) only C moderation, (3) only E moderation, (4) A and C moderation (5) A and E moderation, (6) C and E moderation. The best fitting univariate twin models with continuous moderators reflecting latent additive genetic influences, shared environmental influences, and non-shared environmental influences for infant outcomes were selected on the following criteria: -2LL fit statistic, the change in Chi-Square statistic, and the lowest AIC.

To summarize, the focus of this study was to determine gene-environment interactions; specifically, the possible moderating effects of maternal prenatal perceived stress, obstetrical complications, and gestational age on the genetic and environmental variance components for dysregulation, competence, and developmental maturity were examined.

Results

Preliminary Analyses

Frequency histograms were plotted for infant outcomes and environmental moderators to visually inspect the variables for deviations of univariate normality, presence of extreme scores, and skewed distributions. Infant outcome variables and environmental moderators were normally distributed and no transformations were necessary. Analyses to detect potential outliers were conducted using scatterplots and stem and leaf plots (Bollen, 1989). Outliers were considered observations with values that are distinct and distant from the bulk of the data and defined as a value greater than three standard deviations from the mean. No outliers were detected in this dataset.

Descriptive Analysis of Prenatal Risk Factors

The frequencies of individual obstetric and birth risk variables are provided in Table 1. In this birth-record based sample, the majority of the obstetrical risk variables rarely occurred, thus group differences were not examined statistically at the item level. The majority of women did not report smoking (N=4 reported smoking) or drinking alcohol (N=6), and there were no women who reported ingesting illicit drugs (N=0), such as marijuana, cocaine, or methamphetamines during their twin pregnancy. Nearly all of the mothers had prenatal care (N = 172), most mothers were prescribed drugs administered during pregnancy (N = 128) and to stimulate contractions (N = 86). Additionally, some women used fertility treatments (N = 42), experienced bleeding during pregnancy (N = 24), had preeclampsia (N = 46), were prescribed bed rest by medical

professionals (N = 24), and received drugs during labor and delivery (N = 26). Lastly, with regard to mode of delivery, the majority of mothers had a C-section (N = 170) opposed to a vaginal birth (N = 38).

Descriptive statistics for prenatal, obstetrical composite scales, and infant developmental outcomes are presented in Table 2. On average, women reported feeling moderate stress during their twin pregnancy. Infants were born on average 35.40 weeks gestational age (average gestation of twin 1 and twin 2), weighed on average 2528.24 grams (average birth weight of twin 1 and twin 2), and had low rates of neonatal complications and neonatal morbidity risk.

Relations and Main Effects of Prenatal and Obstetrical Variables on Infant Outcomes

Correlations were conducted between prenatal, obstetrical, and neonatal composite variables and infant dysregulation, competence, and developmental maturity (Table 3). In addition, infant age (adjusted for prematurity) and sex were considered as possible covariates. Gestational age was significantly related to more infant competence. Additionally, infant age was positively correlated with infant competence and developmental maturity, whereas infant sex was not significantly related to infant outcomes. Due to the associations between infant age and infant outcomes, age and sex were regressed from all infant outcomes prior to model fitting, which is standard practice in the field of twin model fitting (McGue & Bouchard, 1984).

Multilevel regressions that controlled for twin dependence were then conducted to determine the extent to which maternal perceived prenatal stress, obstetrical complications, and gestational age independently predicted infant dysregulation, competence, and developmental maturity, controlling for the influence of infant age and sex. In addition to testing main effects, two- way interactions between the covariates and predictors were examined. For infant dysregulation, there were significant main effects of maternal perceived prenatal stress and obstetrical complications; $t(102.55) = 2.47$, $p = .02$, & $t(103.24) = 2.13$, $p = .04$, for maternal perceived prenatal stress and obstetrical complications, respectively. There were no main effects of gestational age. Infant competence and developmental maturity were not predicted by maternal perceived prenatal stress, obstetrical complications, or gestational age. There were no main effects of age (adjusted for prematurity) or sex, and no significant interactions between infant age or sex and the predictors in predicting infant outcomes.

Genetic and Environmental Influences on Infant Outcomes

Univariate genetic model fitting results revealed the AE model was the most parsimonious fit for infant dysregulation, whereas the full ACE model fit best for infant competence and developmental maturity (see Table 4). Table 4 summarizes the fit statistics for each univariate model fit, as well as the standardized estimates of genetic (A), shared environment (C), and nonshared environment (E) influences for the full ACE models as well as the reduced models (most parsimonious model is bolded). For infant dysregulation, the best

fitting model was the AE model, with 74% of the variance due to additive genetic influences, and 26% to the nonshared environment. For infant competence, the full ACE model was the best fitting model, with 52% of the variance due to genetic influences, 41% to the shared environment, and 07% nonshared environment. Lastly, for infant developmental maturity the best fitting model was the full ACE model, with 72% of the variance due to additive genetic influences, 25% to the shared environment, and 03% to the nonshared environment. Thus, genetic influences accounted for a substantial portion of the variance in all infant outcomes, with significant effects of the shared environment on competence and developmental maturity.

Prenatal and Birth Moderators of Genetic and Environmental Influences on Infant Outcomes

Univariate genetic models with continuous moderators were used to test maternal perceived prenatal stress, obstetrical complications, and gestational age as moderators of genetic and environmental influences on infant dysregulation, competence, and developmental maturity. This approach extends beyond the heritability of traits by capturing "gene-environment interactions," or more specifically, the variability in heritability estimates across environmental conditions. Tables 5-13 provide the model fits for the full moderation ACE model, models with some moderation components and not others, and a no moderation model with the best fitting model bolded. Findings indicated maternal perceived prenatal stress, obstetrical complications, and gestational age

differentially moderated genetic and environmental influences on infant dysregulation, competence, and developmental maturity, such that the effects of additive genetic influences, shared environmental influences, and nonshared environmental influences on infant outcomes varied continuously as a function of prenatal and obstetrical conditions. Figures 1 through 6 illustrates the change in the ACE estimates for the full ACE moderation model for infant dysregulation, competence, and developmental maturity at three different levels of the prenatal and obstetrical moderators (prenatal stress, obstetrical complications, and gestational age) scaled in standard deviation units (z-scored, -1, 0, 1) from the mean of the moderator. The results of the best fitting models are discussed next.

There was no evidence that maternal perceived prenatal stress moderated genetic and environmental influences on infant dysregulation (Table 5) or infant competence (Table 6); however, prenatal stress moderated genetic, shared, and nonshared environmental influences on infant developmental maturity (Table 7). Figure 1 graphically represents the variance components of infant developmental maturity as a function of maternal perceived prenatal stress. Genetic effects accounted for most of the variance in infant developmental maturity across levels of maternal perceived prenatal stress. As maternal perceived prenatal stress increased, variance in additive genetic effects slightly decreased (58% - 56%), whereas the variance in developmental maturity accounted for by the shared and nonshared environment increased.

Obstetrical complications did not moderate genetic and environmental influences on infant dysregulation (Table 8), yet there was evidence of obstetrical complications moderating the shared environment for infant competence (Table 9) and the nonshared environment for developmental maturity (Table 10). Figure 2 illustrates that as obstetrical complications increased, variance attributed to the shared environment steadily decreased (10% - 4%). Figure 3 depicts the moderation of developmental maturity showing that as obstetrical complications increased, variance accounted for by nonshared environmental influences decreased. Moreover, the variance attributed to the nonshared environment was higher in the extremes of obstetrical complications as compared to at the mean (5% , 1%, and 5% of the variance accounted for in developmental maturity at one SD below the mean, at the mean, and one SD above the mean, respectively).

Lastly, the significant moderating impact of infant gestational age on infant dysregulation, competence and developmental maturity is presented. Specifically, there was evidence that gestational age moderated genetic and nonshared environmental influences on infant dysregulation (Table 11). Figure 4 illustrates how gestational age moderated variance components of additive genetic effects and nonshared environmental influences on infant dysregulation. As gestational age increased, the variance accounted for by genetic effects decreased (77% to 66%) as well as variance due to nonshared environments (7% to 4%). Heritability of dysregulation was higher for low-gestational age infants as compared to high-gestational age infants. Further, gestational moderated shared

and nonshared environmental influences on infant competence (Table 12); Figure 5 illustrates as gestational age increased, the variance attributed to the shared environment increased dramatically (30% to 80%) whereas variance accounted for by the genetic factors decreased (68% to 28%). Finally, gestational age moderated nonshared environmental influences on infant developmental maturity (Table 13). Figure 6 graphically depicts the variance decomposition of developmental maturity showing the moderating impact of infant gestational age on nonshared environmental influences on developmental maturity. As gestational age increased, variance attributed to the nonshared environment decreased (24% - 6%) and variance accounted for by genetic effects decreased (74% - 52%) whereas variance accounted for by the shared environment increased (1% - 42%). Thus, the nonshared environment has a greater impact for infants with lower gestational age and the shared environment has a larger impact for infants with higher gestational age.

Summary of Findings

To summarize, the current study included a relatively low risk sample with minimal exposure to illicit drugs or alcohol during pregnancy, although mothers reported moderate stress during pregnancy, and there were high rates of some obstetrical complications such as use of legal drugs during pregnancy and stimulation of contractions. Gestational age was positively related to more infant competence. Prenatal and obstetrical variables had few main effects on infant outcomes. Specifically, infant dysregulation was significantly predicted by

maternal perceived prenatal stress and obstetrical complications, but infant competence and developmental maturity were not independently predicted by prenatal and obstetrical variables. Infant outcomes were heritable, with competence and developmental maturity also influenced by the shared environment (Table 4). Utilizing behavioral genetic models with moderators, results indicated maternal perceived prenatal stress moderated genetic and environmental influences on developmental maturity whereas obstetrical complications moderated shared environmental influences on infant competence and nonshared environmental influences on developmental maturity. Gestational age moderated the heritability and nonshared environment of infant dysregulation, shared and nonshared environmental influences on competence, and nonshared environmental influences on developmental maturity. Taken together, prenatal stress, obstetrical complications, and gestational age were important nonlinear influences on infant outcomes.

Discussion

There is very little research that employs a twin design to separate relative influence of genes and prenatal environmental factors on infant behavior and development. To date, there is a sparse literature evaluating the relative influence of maternal prenatal risk, such as smoking or alcohol use during pregnancy, or perinatal environmental risks, including prematurity or low birth weight, to offspring developmental outcomes, controlling for genetic predispositions (Knopik et al., 2005; 2006; Koppen-Shomerus et al., 2000; Maughan et al., 2004;

Thapar et al., 2003; Wichers et al., 2002; Wilson, 1993). The goal of the current study was to evaluate the complex interplay of genetic and environmental factors contributing to infant behavior. The focus was to determine whether or not maternal prenatal perceived stress, obstetrical complications, and gestational age modified the genetic influence on individual differences in infant dysregulation, competence, and developmental maturity. It was hypothesized that higher maternal prenatal perceived stress, more obstetrical complications, and lower gestational age would decrease the heritability of dysregulation, competence, and developmental maturity in 12-month-old infants. This approach extends beyond the heritability of traits by capturing gene-environment interactions or more specifically, the variability in heritability estimates across prenatal and obstetrical environmental conditions. Thus, these models afforded the opportunity to understand how maternal perceived prenatal stress, obstetrical complications, and gestational age change the impact of genetic factors on infant behavior. These relations have not been investigated in the literature to date and several important findings emerged.

All infant outcomes were heritable, with competence and developmental maturity also influenced by the shared environment. Importantly, maternal perceived prenatal stress and obstetrical complications moderated the heritability of infant competence and developmental maturity, whereas gestational age moderated the heritability of infant dysregulation, competence, and developmental maturity. Given the impact of genetic factors on infant behavior

and developmental maturity varied depending on prenatal and obstetric conditions, identifying these moderators adds to the understanding of the etiology of infant behavior. Taken together, maternal perceived prenatal stress, obstetrical complications, and gestational age were important nonlinear influences on infant outcomes.

Estimating Heritability of Infant Outcomes

Univariate genetic model fitting results revealed infant outcomes were heritable and the relative contributions of genetic and environmental influences on infant outcomes are discussed next. With regard to the etiology of infant dysregulation, 74% of the variance was due to additive genetic influences and 26% to the nonshared environment. For infant competence, the full ACE model was the best fitting model, where 52% of the variance was due to genetic influences, 41% to the shared environment, and 07% to the nonshared environment. Lastly, for infant developmental maturity 72% of the variance was due to additive genetic influences, 25% was attributed to the shared environment, and 03% to the nonshared environment. Thus, genetic influences accounted for a substantial portion of the variance in all infant outcomes, with significant effects of the shared environment on competence and developmental maturity, which is consistent with previous research. Twin studies exploring the etiology of young children's behavior have found that dysregulation is highly heritable whereas behavioral competencies were more environmentally influenced (Knafo, Van Hulle, Zahn-Waxler, Robinson, & Rhee, 2008; Saudino, Carter, Purper-Ouakil, &

Gorwood, 2008; Van Hulle, Lemery-Chalfant, & Goldsmith, 2007). It was not surprising that infant dysregulation was highly heritable because ITSEA Dysregulation assesses infant sleep, eating, and sensory sensitivity, behaviors that have been shown to be heritable (Goldmisth, Van Hulle, Schrieber, & Gernsbacher, 2006; Lemery-Chalfant, Goldsmith, Schmidt, Arneson, & Van Hulle, 2006; Petit et al., 2011; Tremblay et al., 2008). For example, reactivity to food has high heritability with low contributions from the environment (Plomin & Rowe, 1977), eating behaviors and problems in young children have a substantial genetic influence (Saudino et al., 2008), as does eating disorders in adolescents and adults (Bulik, Sulllivan, Wade, & Kendler, 2000; Gorwood, Kipman, & Foulon, 2003).

Prenatal and Obstetrical Risk Moderates Heritability of Infant Outcomes

Maternal perceived prenatal stress, obstetrical complications, and gestational age differentially moderated genetic and environmental influences on infant dysregulation, competence, and developmental maturity, such that the effects of additive genetic influences, shared environmental influences, and nonshared environmental influences on infant outcomes varied continuously as a function of prenatal and obstetrical conditions. Next, a discussion of the interplay between genetic and environmental influences on infant behavior considering varying prenatal and obstetrical risk conditions is provided. Findings that were consistent with hypotheses are discussed first, followed by discussion of

unexpected results indicating higher heritability of infant outcomes under greater environmental risk.

Findings Consistent with Hypotheses

Maternal perceived prenatal stress significantly moderated the heritability of developmental maturity, but not dysregulation or competence. Genetic effects accounted for most of the variance in infant developmental maturity across maternal perceived prenatal stress, such that as maternal perceived prenatal stress increased, the influence of additive genetics decreased (58% - 56% of the variance in infant developmental maturity) and the shared environment increased (35% - 38% of the variance in infant developmental maturity). This finding was consistent with hypotheses, predicting that with increasing levels of maternal perceived prenatal stress, the variance attributed to the genetic factors would decrease and variance accounted for by the environment would increase. One reason for these subtle changes in proportions of variance in developmental maturity may be that proximal processes actualize genetic potentials by enhancing functional competence and reducing developmental dysfunction (Brofenbreener & Ceci, 1994). Thus, the extent to which environmental stress modifies the genetic expression of behavior varies as a function of the characteristics of the overall environment of the child and the developmental nature of the outcome. In the case of overall developmental maturity, there is evidence that exposure to stress prenatally operates in a u-shaped function, where at high and low levels stress prenatally may increase the likelihood of immaturity but average levels of

stress may benefit development by providing the capabilities to respond appropriately to environmental stress postnatally (DiPietro, 1996; 2010). For example, the magnitude of change in heritability for developmental maturity at low levels of stress to high levels of stress is small and may reflect that maternal prenatal stress interacted with genetic influences less so than gestational age or obstetrical complications because of the nonlinear nature of the impact of stress prenatally on development. In the current study, women reported average levels of stress which may have activated gene expression necessary for optimal infant development. Given current findings, a noteworthy goal of future research would be to explore a quadratic relation in a sample with more variability in levels of maternal prenatal stress. Taken together, this study extends beyond previous research exploring prenatal risk and development by capturing the dynamic interplay between genetic susceptibility and environmental stress in the etiology of behavior in infancy.

Given that early environmental stressors may shape the development of the HPA-axis prenatally and in the early postnatal period (Lupien, McEwn, Gunnar, & Hein, 2009), it is important to investigate how stress during pregnancy impacts developmental outcomes by interacting with genetics. Growing evidence from research in the animal and human literatures suggests maternal prenatal stress can affect offspring physiological and behavioral development (see reviews by Charil, Laplante, Vaillancourt, & King, 2010; Gutteling, de Weerth & Buitelaar, 2005; Weinstock, 2001). Stress during human pregnancy poses a risk

for pre-term delivery (<37 weeks gestation), lower birth weight (<2500 grams), smaller head circumference, lower Apgar scores, newborn irregularity of biological functions, poor soothability shortly after birth, more irritability (Bergman, Sarkar, Gloverm & O'connor, 2010; Bolton, Wurmser, Buske-Kirschbaum, Papousek, Pirke, & Hellhammer, 2011; Charil et al., 2010; Crandon, 1979; Dunkel-Schetter,1998; Lobel, Dunkel-Schetter, & Scrimshaw, 1992; Lou, Hanson, Nordentoft, Pryds, Jensen, & Nim, 1994; Paarlberg, Passchier, Dekker, & Ven Geign, 1995; Van den Bergh, 1990), lower mental and psychomotor development (Bergman et al., 2007; Brouwers, van Barr, & Pop, 2001; Buitelaar, Huizink, Mulder, Robles de Medina, & Visser, 2003; Davis et al., 2007; 2001; Davis & Sandman, 2010; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), difficult temperament (Austin, Pavlovic, Leader, Saint, Parker, 2005; Gutteling et al., 2005), negative behavioral reactivity, controlling for postnatal maternal depression (Davis, Glynn, Schetter, Hobel, Chicz-DeMet, & Sandman, 2007; Davis, Snidman, Wadhwa, Glynn, Dunkel-Schetter, & Sandman, 2004) and inattention (Buitelaar et al., 2003) in infancy and toddlerhood, with effects persisting into late childhood (Gutteling et al., 2005; O'Connor, Heron, Golding, Beveridge, & Glover, 2002; Van den Bergh & Marcoen, 2004). The aforementioned research suggests important links between stress during pregnancy and developmental outcomes, however, the impact of genetic factors on behavior and development was not considered in these studies. Failure to control for heritable factors may account for the considerable variation in results

across studies exploring the relations between prenatal exposure to risk and developmental and behavior outcomes. To my knowledge, the current study is the first study to utilize a genetically informative design revealing how different levels of maternal prenatal stress environments modify the heritability of developmental maturity in 12 month old infants.

Multiple gestation pregnancies are at an increased risk of obstetrical complications including maternal hypertension, excessive bleeding in labor and delivery, and need for tocolysis, a drug treatment for preterm labor that results in suppression of uterine contractions and inhibition of the birth process (Luke & Brown, 2007). Stressful pregnancies as determined by more obstetrical complications and delivery complications (duration of labor, fetal heart function, and lower 5-min Apgar scores) have predicted more crying and fussing as well as more difficulties in regulating infant behavior (deWeerth & Buitelaar, 2005). In the present study, obstetrical complications independently predicted dysregulated behaviors. Other research has indicated obstetrical and neonatal complications have been related to ADHD, conduct disorder and oppositional defiant disorder (ODD) in seven and eight year old twins (Wagner et al., 2009), and increased risk for schizophrenia, personality disorders, antisocial behavior, and mood disorders, including bipolar disorder in adults (e.g., Eagles et al., 1990; Kinney et al., 1994; Kinney et al., 1998).

Interestingly, obstetrical complications significantly moderated the impact of genetic and environmental influences on infant behavior depending on the

specific infant outcome. In line with hypotheses, the heritability of infant developmental maturity decreased with greater obstetrical complications; however, the heritability of competence was higher with greater obstetrical complications, a finding that is not in line with predictions and will be discussed in later sections.

As predicted, obstetrical complications significantly moderated the impact of the nonshared environment on infant developmental maturity. Specifically, the variance attributed to the nonshared environment was higher in the extremes of obstetrical complications as compared to at the mean (5% , 1%, and 5% of the variance accounted for in developmental maturity at one standard deviation below the mean, at the mean, and one standard deviation about the mean, respectively). The relative contributions of genetic effects steadily decreased with greater levels of obstetrical complications, which is consistent with predictions. Previous prospective research indicated pregnancy and birth complications (mode of delivery, mode of induction of ovulation, fetal presentation, and LBW) moderated heritability of behavior problems such that the genetic influence was lower with greater obstetrical complications in twin pairs between the ages of six and 17 years of age (Wichers et al., 2002).

Findings Inconsistent with Hypotheses

Surprisingly, maternal perceived prenatal stress did not moderate genetic and environmental influences on infant dysregulation or competence. Obstetrical complications did not moderate the genetic and environmental influences in infant

dysregulation. Dysregulation was significantly predicted by prenatal stress and obstetrical complications in the regression analyses; findings that were consistent with previous literature documenting associations between prenatal stress and adverse developmental outcomes and behavioral problems (Chassnoff et al., 1998; Davis & Sandman, 2010; Glover, 2011; O'Connor et al., 2002). With regard to competence, there was no evidence that obstetrical complications moderated genetic and environmental influences on competence in infants. With regard to stress prenatally, it is likely maternal prenatal stress impacts the developing fetus via release of cortisol or other biological factors that influence or moderate dysregulated and competence behavior. Moreover, perhaps due to the nonlinear nature of maternal prenatal stress, it may be that the moderate levels of maternal prenatal stress reported in this study operated in such as way that benefited infants' outcomes by providing development of capabilities necessary to adapt and successfully respond to the demands of the postnatal environment (DiPietro, 2010).

Unexpectedly, findings revealed that as obstetrical complications increased, the variance in infant competence attributed to the shared environment steadily decreased (10% - 4% of variance in infant competence). At the highest level of obstetrical complications, 82% of the variance was accounted for by genetic factors while the environment accounted for the remaining 28%. Although this finding is in the opposite direction of my hypothesis, it is consistent with the theoretical framework of the bioecological model whereby the heritability of

infant behavior and developmental maturity increased with increasing levels of some environmental stressors (Bronfenbrenner & Ceci, 1994).

The bioecological model posits behavior is more heritable under stressful conditions and may explain the findings that were contrary to hypotheses. Unexpected results indicated the heritability of behavior was higher with more environment stress. Brofenbrenners's bioecological systems theory (1994) explains the potential impact of natural environments that influence a developing person. In particular, the bioecological theory puts forth that an individual's heritable characteristics interact with the immediate environment to shape developmental outcomes. Thus, development is the result of a series of interactions between the changing individual and the changing environment. Accordingly, the interaction between genetic influences and the immediate environment produce differences in behavior by means of proximal processes. Proximal processes are environmental factors that afford the opportunity to impact the expression (activate or block) of genes involved in inherited developmental outcomes, including coping abilities under stress, acquiring knowledge and learning, and establishing and maintaining relationships with others (Brofenbrenner & Ceci, 1994). Thus, proximal processes constitute the basic mechanisms that produce effective developmental functioning.

Maternal prenatal stress may be viewed as a proximal process that penetrates the intrauterine environment potentially giving rise to expression of inherited traits that are necessary to adapt in response to the stressful prenatal

environment. One component of the bioecological model is that heritability is expected to be higher when proximal processes are strong and lower when such environmental processes are weak (Bronfenbrenner & Ceci, 1994). Further, it posits that under environmental stress or adversity, heritability estimates of behavior will increase in a linear manner with the increasing or decreasing magnitude of the environmental factor. Thus, proximal processes are the mechanisms by which genotypes transform into phenotypes subsequently fostering effective psychological development given environmental demands.

In the current study, low gestational age may have created an environmental affordance for the expression of heritable traits (dysregulation, low competence, and developmental immaturity). For example, heritability of dysregulation, competence, and developmental maturity was substantially higher for infants with low gestational age and more obstetrical complications perhaps because in more stressful environments the potential for gene expression for adaptive behavior was activated rather than blocked. Likewise, for infants born with higher gestational age and those exposed to low rates of obstetrical complications, the environment was more important in predicting individual differences in behavior and developmental maturity because in the absence of a stressful environment, the genes may have stayed dormant, lacking the potential to be expressed, giving rise to greater influences on behavior due to the environment. This model may explain why heritability of dysregulated behavior, behavioral competencies, and developmental maturity was lower in more

protective prenatal and perinatal environments, such as being born full-term with few obstetrical complications.

Environmental stress and obstetrical complications are risk factors for a variety of behavioral and psychiatric outcomes (Gutteling et al., 2005; Kinney et al., 1994; Kinney et al., 1998; O'Connor et al., 2002; Van den Bergh & Marcoen, 2004). There are sensitive periods in the biological development of an individual that begin in the prenatal period, continue during the first few years of life when rapid developmental growth takes place, and slow down later in childhood and adolescence until one reaches full development in early adulthood (Fox, Leavit, & Nelson, 2010). An evolutionary perspective may help clarify underlying mechanisms involved in the interplay between prenatal stress and obstetrical complications and infant outcomes (Glover, 2011). A critical component to evolutionary theory is that variation in genes contributes to reproductive success of a species in changeable environments. It has been suggested that symptoms of inattention and hyperactivity, both associated with maternal prenatal stress, may be adaptive and reflect fetal programming of the brain to prepare the infant to respond appropriately to changeable or harsh environments postnatally. Accordingly, one mechanism for which prenatal environmental stress may affect infant and child development is whether the genes related to behavior were expressed given environmental demands during prenatal development. Epigenetics is the study of biochemical modifications of deoxyribonucleic acid (DNA) influencing gene expression without altering the structural sequence of the

DNA (van Ijzendoorn et al., 2011). Epigenetic changes have the potential to make permanent alternations in gene expression, such as the silencing of genes through methylation. Research exploring the role of gene methylation in development from animal and human work may be one mechanism by which prenatal and obstetrical risks modified heritability in outcomes.

Recent compelling evidence from animal studies have highlighted the immediate environment as having epigenetic control over gene expression. Long-term changes in responses to stress in offspring rats exposed to adverse rodent parenting behaviors, reflecting permanently altered methylation patterns affecting the expression of the glucocorticoid receptor gene that were inherited and influenced parenting behavior and stress regulation in the next generation offspring have been documented (Champagne, 2008; Meany & Szfe, 2005; Szfe, Weaver, Champagne, Diorio, & Meany, 2005). Research exploring the impact of rearing conditions (highly sensitive versus neglectful parenting) on methylation patterns in Rhesus monkeys has found highly sensitive rearing conditions were related to lower levels of methylation in DNA cells located in the brain and more extreme neglectful rearing conditions lead to changes in methylation of DNA, which may reflect gene expression of traits that will prepare offspring to respond and adapt to the expected state of the environment.

Only a few studies have explored the impact of early developmental adversity and DNA methylation in humans. Recent prospective research has explored the epigenetic impact of postnatal stress during infancy on DNA

methylation in adolescents (Essex et al., 2011). Significant differences in DNA methylation patterns involved in regulation of stress were revealed among adolescents who were exposed to high levels of stress during infancy and early childhood. Taken together, the prenatal environment and early postnatal experiences may impact gene methylation and subsequently the expression of genes related to development (Essex et al., 2011; Oberlander et al., 2008).

Thus far, researchers exploring the role of methylation in child development have suggested that child development and behavior may be viewed as a dynamic interplay between environmental experience and the child's DNA through methylation; thus, a series of epigenetic modifications of specific genes result in individual differences in physiology, emotion and behavior (van Ijezendoorn, Bakermans-Kranenburg, & Ebstein, 2011). For example, the stress hormone cortisol can block a gene from expressing proteins (Lee et al., 2010). Chronic exposure to cortisol in the cellular environment impacts gene expression by turning genes on and off which is reflected in individual differences in infant behavior. Thus, one possible mechanism for understanding the interplay of exposure to prenatal stress and obstetrical risk on developmental outcome given genetics is prenatal methylation. It may be the case that some individuals were prenatally programmed in such a manner that increases their susceptibility to negatively respond to stress in the postnatal environment (Oberlander er al., 2008). Interestingly, exposure to positive psychosocial environments, including a positive caregiving environment, may give rise to the process of demethylation, a

reversal of DNA methylation, thereby enhancing developmental outcomes and reactivity to stress (Bakermans-Krenenburg, van Ijzendoorn, Mesman, & Juffer, 2008).

It appears epigenetic mechanisms, including methylation, may explain why exposure to stressful environments early in life, such as stress, increased this risk of adverse physical and psychological outcomes (Miller et al., 2009). Through methylation, exposure to stressful conditions early in life affects the up-regulation of adrenergic receptors and the down-regulation of glucocorticoid receptors and may underlie how exposure to early environmental stress impacts developmental outcomes later in life. Methylation can be viewed as a process of environmental priming that prepares the organism to either adapt or not to future environments. Epigenetic studies help clarify how the environment influences biochemical regulation that impacts the expression or non-expression of genes resulting in lasting positive or negative effects on developmental outcomes (van Ijzendoorn et al., 2011).

The impact of preterm delivery on child development has received a great deal of attention and consistent documented links between preterm birth and neonatal complications, developmental delays, difficult temperament (irritability and unresponsiveness) and inattention in infancy and emotional problems as children have been reported (see reviews Lou et al., 1994; Paarlberg et al., 1995; Saigal, 2000; Taylor, Klien, Schayschneider, & Hack, 1998; Wolke & Meyer, 1999). The present study adds to the extant body of literature focused on links

between maternal psychological states and physical health during pregnancy and developmental risks by considering how prenatal stress moderates the role of genetics on infant outcomes.

Unexpectedly, the risk factor of low gestational age did not lower the heritability of infant outcomes as hypothesized. Rather, the variance attributed to genetic factors on infant outcomes was highest in infants born with low gestational age as compared to infants born with higher gestational age. Most interesting, gestational age moderated the heritability on all infant outcomes. To summarize, there was significant moderation of gestational age on genetic and nonshared environmental influences on infant dysregulation, shared and nonshared environmental influences on infant competence, and nonshared environmental influences on infant developmental maturity.

Gestational age moderated the genetic and nonshared environmental influences on infant dysregulation, thus as gestational age increased, the variance accounted for by genetic effects decreased (77% to 66%) as well as the nonshared environment (7% to 4%) while the shared environment increased (18% to 31%). Dysregulation reflects the infant's capacities to sleep, eat, symptoms of eating pathology, and sensory sensitivity. Gestational age can be thought of as a proxy for prenatal growth. Development is a series of interactions between activating expression of genetic factors and the impact of the environment. Mammals have developed the capacity to respond appropriately in utero, to improve biological fitness, and prepare offspring for adaptation and success in the postnatal

environment (Gluckman, Hanson, & Spencer, 2005). Under stressful environments, such as low gestational age, heritability is likely to be higher for certain traits that help the individual adapt to their predictive environment. Thus, dysregulation may be adaptive for infants born early as dysregulated characteristics in sleep, eating, or sensory sensitivity behavior may better prepare them to adapt appropriately to a stressful environment. For example, sensitivity to changes in the sensory environment may alert infants to the presence of danger in the environment and dysregulated patterns of sleep and eating may illicit more maternal attention. Predictive adaptive responses may help clarify how early adaptations in behavior due to fetal programming have aided in human evolution (see review, Glover, 2011). Thus, fetal development is altered in a way that adapts the future infant to succeed in the postnatal environment and may explain why dysregulated behaviors in infants low for gestational age may enhance the infants' success in a stressful environment postnatally.

Similarly, for infants born with higher gestational age (38-40 weeks gestation) and developed fully in the womb, individual differences in infant dysregulation were less strongly the result of genetic factors and largely impacted by the environment. Given the interplay between genetic factors and gestational age, it appears infants in certain environmental conditions may be more sensitive to the effect of preterm birth on later dysregulation than others. This study highlights the importance of sensitive periods in development and the need to explore gene-environment interactions in understanding the etiology of

dysregulated behavior. It may be an evolutionary benefit to inherit dysregulated traits in times of environmental stress to prepare infants to adapt successfully to changing environments. More research is needed to examine how the interaction between biological and environmental influences results in variation in behavior and development. Applying a genetically-informative approach will illustrate how inherited characteristics make some individuals more susceptible to the negative or positive effects of specific environments (Moffitt, Caspi, & Rutterm, 2005).

With regard to infant competence, there was evidence gestational age moderated the shared and nonshared variance components. As gestational age increased, the variance attributed to the shared environment increased dramatically (30% to 80%). Low gestational age reflected a stressful prenatal environment, thereby perhaps activating additional genes that may contribute to competence in infants. Previous work has posited mechanisms thought to underlie the link between prematurity and aspects of cognitive development which include exposure to prenatal environment risks (Brooks, Johnson, Steer, Pawson, & Abdalla, 1995), perinatal or neonatal experiences (Laucht, Esser, & Schmidt, 1997) or postnatal family environment (Bendersky & Lewis, 1998). One component of competence in this study is attention. Studying pregnancy and obstetrical influences on aspects of attention during infancy is important because inattention may enhance the risk of later cognitive challenges and behavior. Further, the ability to allocate attention flexibly allows children to regulate their

exposure to stressful situations or contexts (Rothbart & Bates, 2006; Rothbart, Posner, & Rosicky, 1994; Ruff & Rothbart, 1996).

Contrary to present results, previous findings indicated the heritability of behavioral problems was lower when children were born with low birth weight whereas behavioral problems were highly heritable for children who were born with normal birth weights (Wichers et al., 2002). Similarly with regard to cognitive outcomes, research has shown the impact of genetic factors on cognitive development was stronger when infants had heavier birth weights than infants with lower birth weights (Koeppen-Schomerus et al., 2000). Findings from the present study indicated for preterm infants, genetic factors largely contributed to behavior outcomes whereas the environment had more of an impact for full-term infants. Although previous work has explored to the role of birth weight and this study examined the impact of gestational age, results highlight how individual differences in genetic predispositions may be modified by various environmental conditions at birth.

Lastly, gestational age significantly moderated the heritability of infant developmental maturity. Inconsistent with predictions, as gestational age increased the variance attributed to genetic factors decreased (74% - 52%) and variance attributed to the environment dramatically increased (1% - 42%). Thus, the environment had a greater impact on infant developmental maturity for infants with higher gestational age (full-term) while genetic factors strongly contributed to developmental maturity in infants born preterm. Similar to dysregulation and

competence outcomes, the impact of the proximal process low gestational age may have provided the opportunity for genetic influences to be actualized and raised the proportion of variance attributed to genetic factors in developmental maturity. Developmental maturity reflects physical development, self-help behaviors, and capacities in social, communication, and intellectual development. Previous studies have found important gene-environment interactions between preterm delivery and cognitive and behavioral outcomes in toddlers (Koeppen-Schomerus et al., 2000; Wichers et al., 2002). Specifically, shared environmental factors accounted for a significant portion of variance (84%) in very preterm toddlers, with non-significant additive genetic effects (9%) in verbal and nonverbal communication whereas for both average gestational age and full-term toddlers, additive genetic effects explained 33% and 22% of the variance, respectively, and the shared environment accounted for 65% and 73%, respectively. Findings from this study showed the opposite pattern whereby genetic factors were especially important in predicting developmental maturity at 12 months for those with low gestational age. Differences in the patterns of results may have emerged due to measurement, for example, communication development was only one aspect of the developmental maturity composite used in the current study and not a focal aspect of cognitive development. The developmental maturity composite is beneficial to researchers because it assessed several aspects of development, providing a comprehensive measure of development during infancy.

Study Limitations

Some limitations of this study must be acknowledged. First**,** concerning data obtained from medical records there was some missing data (21%). Reasons for missing medical records included insufficient or incorrect information on medical records consents forms (ie., missing date of birth), medical or hospital staff sent partial information inadequate to code obstetrical scales, and some women did not consent. Thus, it was only possible to include a sub-sample of mothers whose medical records were collected in the analyses. However, missing data did not appear to have a large impact on results (see Methods).

Secondly, findings may be open to the criticism of informant bias since measures of infant behavior were obtained by maternal report (Kagan, 1998). In relation to focal aspects of infant temperament, caregiver report questionnaires are useful to researchers (Bates, Wachs, & Emde, 1994; Rothbart & Derryberry, 2002). Caregiver report offers a unique perspective of infant temperament as parents have seen their infants behave and respond to naturally occurring stimuli in a variety of situations, including infrequent responses or behaviors that may be particularly important in studying a specific dimension of temperament (Thomas & Chess, 1977; Thomas, Chess, Birch, Hertzig, & Korn, 1963). In addition, caregiver reports of infant behavior have established a fair degree of objectivity and validity. Mother report of infant temperament and independent observations, including naturalistic settings and structured contexts, were positively correlated and agreed moderately (r=.53-.66) or strongly (r=.83) across most studies

(Gerardi-Caulton, 2000; Goldsmith & Rothbart, 1991; Hagekull, Bohlin, & Lindhagen, 1984; Matheny, Wilson, Thoben, 1987). All together, agreement between caregiver-report measures and independent observations of infant behavior provide validation for the use of caregiver-report measures.

Ideally, measures of maternal prenatal stress would have been collected prospectively over the course of pregnancy. Subsequently, the measure of maternal perceived prenatal stress may be open to criticisms of retrospective report. Previous research has used this measure to assess maternal prenatal stress retrospectively specific to each trimester (early, mid, and late pregnancy) as well as a measure of overall stress during pregnancy (Rice et al., 2007; Rice et al., 2010). This measure of maternal prenatal stress, although subjective and retrospective, has good construct validity from evidence showing significant correlations with multiple objective birth outcome measures obtained from medical records (Rice et al., 2010). Specifically, it was significantly correlated with lower birth weight, shorter gestation, smaller head circumference, and shorter length at birth. In the current study, results were consistent with previous work; maternal perceived prenatal stress was correlated with lower birth weight, shorter gestation, and smaller head circumference, although it was not significantly associated with shorter length at birth. Thus, retrospective report appeared to be a valid method for the assessment of maternal perceived stress in this study, although comparing this method to prospective assessments across the course of pregnancy has yet to be studied.

Another consideration is potential sample influences on developmental outcomes. The sample was comprised of twin infants from primarily Caucasian and Latino educated families. Given twins are at higher risk for obstetrical and developmental outcomes as compared to single born infants, one potential implication is that the findings of this study may not generalize beyond twins (Pollack, Lantz, & Frohna, 2000). For example, twins tend to be born early (about 35 weeks gestation) with lower birth weights (on average about five pounds) and subsequently are at a higher risk for admittance to the NICU and respiratory problems (Demissie, Ananth, Martin, Hanley, MacDorman, & Rhoads, 2002; deWeerth & Buitelaar, 2007; Zuppa, 2003). Despite the twin sample, obstetrical data from medical records indicated this is a low risk sample. Regular prenatal care is one of the most important ways women can protect their unborn child (Johnson, Walker, & Niebly, 1991).

Further, research documenting associations between obstetrical complications and risk for developmental delay and psychopathology (Keenan, Grace, & Gunthorpe, 2003; Kinney et al., 1998; Taylor, Fisk, & Glover, 2000) may be particularly important when applied to twin development. Multiple gestation pregnancies and deliveries are more challenging than a single pregnancy and delivery and twins are increased risk for adverse developmental outcomes due to the nutritional and placental challenges associated with sharing a common uterine environment (Demissie et al., 2002; deWeerth & Buitelaar, 2007; Zuppa, 2003). Twins also have different biological and psychological developmental

trajectories than singletons (Rutter & Redshaw, 1991). Not only are twins born earlier with low birth weights and experience more obstetrical complications than singletons, twins are at risk for developmental delay, social-emotional problems, and psychopathology (deWeerth & Buitelaar, 2007). The psychopathological hypothesis posits twins are at risk for increased behavioral problems as compared to singletons due to pre and peri-natal risk among twins. In contrast to hypotheses, research has indicated having a cotwin facilitates development of adaptive social behaviors (e.g., compliance and prosocial behaviors) as compared to singletons, and rates of problems behaviors were similar among singletons and twins (Pulkkinenem, Caalamo, Hietala, Kaprio, & Rose, 2003). One reasonable interpretation is that growing up with a twin may create a favorable social environment that affords the opportunity for acquiring adaptive social and emotional behaviors. Future studies should test the assumptions of the twin design with infant samples and generalization to singleton populations.

It is likely aspects of a protective postnatal social environment, not measured in the current study, affords the opportunity for acquiring adaptive competent social and emotional behaviors. Given the developing brain is a result of a series of interactions between genetic influences and environmental factors (Fox et al., 2010), components of the construct of infant competence in this study, for example, were likely influenced by postnatal experiences that were not captured in this analysis. For example, maternal stress and anxiety in the postpartum period may increase risk for psychological maladjustment to the

transition to parenthood and disruption in marital quality (Figueiedo, Field, & Diego, 2008). In addition, prematurity affects parents indirectly through their infants' behavior and parental anxiety or worry regarding infant development and health (Allen, Manuel, Legault, Naughton, Pivor, & O'Shea, 2004). Specifically, infants born preterm tend to be more difficult and less alert, attentive, active, and responsive than infants born at full term (Crnic, Ragozin, Greenberg, Robinson, & Basham, 1983), and mothers tend to be more intrusive, controlling, or psychologically withdrawn (Forcada-Guex, Pierrehumbert, Borghini, Moessinger, & Muller-Nix, 2006). However, some researchers have indicated infants born prematurely demonstrate social and emotional competencies by six months of age, corrected for prematurity (Gerner, 1999). Positive aspects of parenting may be particularly important for infants' born prematurely in developing socially adaptive behaviors. Researchers have indicated maternal sensitivity was the strongest predictor of development of socially-adaptive behaviors, including cooperative and compliance behaviors in infants' born with low gestational age (Fuertes, Faria, Soares, & Crittenden, 2009). Thus, it is necessary to identify the potential impact of possible risks during pregnancy and the early influences on infant behavior and development because these characteristics seen in infancy may extend beyond this period and influence future mental health.

Future Directions

Future studies should continue to explore mechanisms underlying the association between maternal prenatal and obstetrical risk and behavior and

developmental outcomes with twin samples in order to consider the impact of genetic factors on behavior under certain environmental conditions. One aim of future research is to apply a molecular genetics approach to explore underlying biological mechanisms and identify candidate genes associated with outcomes. Studies of genotype-environment interactions illustrate how inherited characteristics make some individuals more susceptible to the negative or positive effects of specific environments (Moffitt, Caspi, & Rutterm, 2005). The role of metabolic genes in determining genetic susceptibility to low birth weight following exposure to maternal smoking during pregnancy is one example. Mothers with metabolic gene polymorphisms CYP1A1 or GSTT1 and who smoked during their pregnancy gave birth to babies who were premature or low birth weight (Wang et al., 2002). Additionally, five-year-old children who inherited two copies of the 10-repeat DAT1 allele and who were exposed to prenatal smoking displayed significantly more inattentive, hyperactive-impulsive, and oppositional-defiant behaviors as compared to children who inherited either one copy of the 10-repeat DAT1, or children who inherited either the 9-repeat, 8-repeat or 7-repeat alleles, controlling for household income, marital status, child age and sex, postnatal smoke exposure, and home environment (Kahn et al., 2003). The interaction between biological mechanisms and environmental influences that results in considerable variation in behavior and development is important to investigate and needs more research attention.

Thus far, a few explanations have been put forth in the literature, although theories of possible mechanisms remain unconfirmed in empirical studies. The placenta is a structural and chemical barrier that protects the developing fetus from adversity; however, in situations of a stressful prenatal environment, the placental barrier can become compromised (Seckl, 2004). One explanation is the proposed biological hypothesis, that suggests that maternal prenatal stress may facilitate the release of catecholamines, resulting in vasoconstriction of maternal blood vessels, diminishing blood flow to the fetus and restriction of oxygen and nutrients, which may interfere with adequate development of the central nervous system (Lobel et al., 2002; Monk et al., 2000). Further, maternal cortisol, which as a consequence of stress, is released in high concentrations, passes though the placental barrier and CRH and ACTH are then synthesized from the placenta. Although cortisol is necessary for fetal maturation and the birth process, slight variations in cortisol, particularly in early pregnancy, have the potential to generate changes in the infant's own stress response system (Davis, Glynn, Waffarn, & Sandman, 2011; DiPietro et al., 1996; Wadhwa et al., 1997). Lastly, maternal HPA-axis hormones may stimulate the placenta to produce CRH that enters the fetal circulation (Majzoub & Karalis, 1999) and fetal exposure to increased levels of HPA-axis hormones could affect the infant's development of the HPA-axis, and autonomic and endocrine functioning (Barbazanges, Piazza, le Moal, & Maccari, 1996; Charil, Laplante, Vaillancourt, & King, 2010; Sandman et al., 1999). Because a combination of all three possible mechanisms is most

likely responsible for the effects of maternal prenatal stress (see review, deWeerth & Buitelaar, 2005), a genetically informative design could reveal possible interactions between biological mechanisms and environmental factors on developmental outcomes.

Given the possible biological mechanisms underlying the relations between maternal prenatal stress and infant developmental outcomes, it is also important for future studies to obtain biological measures, namely maternal cortisol, and focus on disentangling underlying mechanisms. Studies with non-human primates have found that both chronic stress during pregnancy (Schneider Roughton, Koehler, & Lubach, 1999) and two weeks of adrenocorticotrophic hormone administration at mid-gestation predicted poorer attention, greater distractibility, and delayed object permanence in offspring (Schneider, 1992a; 1992b; Schneider, Moore, & Becker, 2001). Cortisol may be an important moderator of infant dysregulation and developmental maturity. By applying a twin design, exploring gene- cortisol environment interactions would provide more information with regard to the etiology of infant behavior and development across different stress conditions.

Researchers should also explore the psychosocial explanation, which suggests prenatal and obstetrical risk influences on infant development are mediated through postnatal environmental influences on development. Research indicates maternal stress and anxiety in the postpartum period and psychological maladjustment to the transition to parenthood have adverse effects on

developmental outcomes in infants (Figueiedo & Costa, 2009), children (Van den Bergh & Morcoen, 2004), and significantly disrupts marital quality (Figueiedo, Field, & Diego, 2008). Moreover, parenting practices are an important influence on social and behavioral competencies, namely children's compliance, empathy, and prosocial behaviors (Eisenberg & Fabes, 1998; Krevans & Gibbs, 1996). Maternal sensitivity, in particular, has been shown to moderate the relation between prenatal anxiety and stress and delayed cognitive development in infants (Grant, McMahon, & Austin, 2010). Moreover, a sensitive caregiving environment has an important modifying role on the adverse effects of prenatal drug use and infant health outcomes and cognitive development in children under the age of three years old (Messinger et al., 2004). Further, a secure attachment with the caregiver attenuates the association between exposure to cortisol in utero and infant cognitive development (Bergman et al., 2010). Therefore, future studies should not only focus on maternal prenatal and obstetrical risk factors but also explore postnatal psychosocial factors, such as parenting and the caregiving environment, in relation to infant behavior and developmental maturity, because it is probable that intrauterine exposures to biological effects of maternal prenatal stress or obstetrical complications, including infant low gestational age, may be reinforced by postnatal environmental influences, such as negative or sensitive interactive experiences with parents, during the course of the first year of life.

Conclusions

This is first study to explore how maternal perceived prenatal stress, obstetrical complications, and gestational age modified the heritability of infant dysregulation, competence, and developmental maturity, revealing prenatal and obstetrical risks were important nonlinear influences on infant outcomes. This study adds to the few investigations that have explored genetic and environmental influences on child behavior, development, and considered the impact of pregnancy and obstetrical risk factors (Koeppen-Shomerus et al., 2000; Knopik et al., 2006; Wichers et al., 2002*)*. A particular strength of this study was the use of a genetically informative design to explore gene-environment interactions of prenatal and obstetrical risk, adding knowledge to the extant body of literature showing links between maternal perceived prenatal stress and adverse developmental outcomes and stress regulation (Bergman et al., 2007; 2010; Davis, Glynn, Waffarn, & Sandman, 2011; Davis et al., 2010; Gutteling et al., 2005; Huizink, 2002; Markham & Koeing, 2011; Mulder et al., 2002; O'Connor et al., 2002; Rothenberger, Resch, Dposzpod, & Moehler, 2011; Van den Bergh & Marcoen, 2004) and obstetrical complications and risk for developmental delay and psychopathology (Keenan, Grace, & Gunthorpe, 2003; Kinney et al., 1998; Taylor, Fisk, & Glover, 2000). The present study highlights the importance of investigating relations between prenatal stress and obstetrical complications and infant developmental outcomes in twin samples as a means to determine how the prenatal and obstetrical environments modify the heritability of developmental

outcomes. It may be that the developing fetus' adapt to the demands of the intrauterine environment and different genes may influence infant dysregulated behavior or competence depending on pregnancy and obstetrical conditions, which may increase or decrease the risk of future health and behavioral problems. Thus, it is necessary to identify the potential impact of other possible risks during pregnancy and early postnatal influences on infant behavior and development because these behavioral characteristics seen in infancy may also extend beyond this period and influence future mental health.

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APPENDIX A

IRB APPROVAL FOR DATA COLLECTION

IRB APPROVAL FOR DATA COLLECTION

ASU ARIZONA STATE UNIVERSITY
Research Compliance Office
Office for Research & Sponsored Projects Administration
P.O. Box 873503
Tempe, AZ 85287-3503
Phone
(480) 965-6788
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To:	Kathryn Lemery PSYCHOLOGY
From:	Mark Roosa, Chair Soc Beh IRB
Date:	03/20/2008
Committee Action:	**Expedited Approval**
Approval Date:	03/20/2008
Review Type:	Expedited F5 F7
IRB Protocol #:	0803002735
Study Title:	Arizona Twin Project
Expiration Date:	03/18/2009

The above-referenced protocol was approved following expedited review by the Institutional Review Board.

It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date. You may not continue any research activity beyond the expiration date without approval by the Institutional Review Board.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Soc Beh IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Soc Beh IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

Table 1.

Frequency of Prenatal Risk or Obstetric Complications

Obstetrical Risk Exposure	Frequency (N)
History of Preterm Birth	
Yes	12
No	158
History of Stillbirth	
Yes	4
No	160
Used Fertility Treatments	
Yes	42
No	60
Used Prenatal Care	
Yes	172
No	6
Prenatal Smoking	
Yes	4
No	156
Prenatal Drinking	
Yes	6
No	140
Prenatal Illicit Drug Use	
Yes	0
No	150
Bleeding during Pregnancy	
Yes	24
No	92
Preeclampsia	
Yes	46
No	80
Bed Rest	
Yes	24
No	36
Drugs administered during pregnancy	
Yes	128
No	4

Obstetrical Risk	Frequency (N)
Drugs administered during labor and delivery	
Yes	28
No	126
Given Magnesium Sulfate	
Yes	36
No	84
Stimulation of Contractions	
Yes	86
No	38
Placental Previa	
Yes	2
No	126
Premature Birth (< 36.5 wks)	
Yes	114
No	82
Mode of Delivery	
Vaginal	38
C-Section	170
Prolonged Unwanted Sterility	
Yes	18
No	132
Length of Time Since Last Pregnancy	
< 12 months	59
> 12 months	128
Cephalopelvic Disproportion	
Yes	13
No	174
Hospitalized Due to Preeclampsia	
Yes	27
No	160
Infections or Acute Illness	
Yes	28
No	162
Chronic Illness or Disease	
Yes	23
No	180
Blood Pressure During Pregnancy	
Exceeding 140/90	27
Not Exceeding 140/90	165

Obstetrical Risk Exposure	Frequency (N)
Rh Antagonism	
Yes	18
No	162
Albuminuria	
Yes (<2)	16
No (absent or less than 2)	172
Hypermesis (Hospitalized)	
Yes	27
No	168
Hemoglobin Levels (Unhealthy)	
Yes	21
No	174
Ruptured Membranes (Assisted)	
Yes	38
No	154
Duration of 1st Stage Labor	
3 - 20 hours	29
> 3 or < 20 hours	168
Duration of 2nd Stage Labor	
< 10 or > 120 minutes	49
10- 120 minutes	154
Duration of 3rd Stage Labor	
< 10 or > 120 minutes	27
10- 120 minutes	164
Amniotic Fluid (Stained)	
Yes	47
No	118
Meconium Staining	
Yes	72
No	152
Episiotomy	
Yes	36
No	172
Abnormal Placental Connections	
Yes	27
No	168
Hemoglobin Levels(Unhealthy)	
Yes	28
No	154

Table 2.

Means and Standard Deviations for Continuous Study Variables

Study Variable	N	Mean	SD	Range
Maternal Perceived Prenatal Stress	270	5.48	2.57	0 - 10
Gestational Age (weeks) Twin 1	291	35.42	2.61	23.5 - 40
Gestational Age (weeks) Twin 2	291	35.38	2.67	23.5 - 40
Infant Age (months, adjusted for prematurity)	291	12.57	1.17	11.0 - 14.49
Infant Birth Weight (grams) Twin 1	228	2516.30	546.16	1020 - 3508
Infant Birth Weight (grams) Twin 2	228	2540.17	544.22	1100 - 3880
Obstetrical Complications	203	9.62	7.87	0 - 47
Neonatal Complications Twin 1	131	.32	.74	0 - 4
Neonatal Complications Twin 2	131	.32	.74	0 - 5
Neonatal Morbidity Risk Twin 1	108	.93	2.12	0 - 10
Neonatal Morbidity Risk Twin 2	108	.86	1.68	0 - 7
Infant Dysregulation Twin 1	285	.75	.22	.24 – 1.76
Infant Dysregulation Twin 2	285	.63	.21	.24 – 2
Infant Competence Twin 1	287	1.05	.34	.06 – 2
Infant Competence Twin 2	287	1.03	.37	.05 – 2
Developmental Maturity Twin 1	291	1.60	.15	1.04 – 1.96
Developmental Maturity Twin 2	291	1.72	.16	1.04 – 1.98

Note: Maternal perceived prenatal stress, gestational age, infant age, developmental maturity, infant dysregulation, and infant competence were obtained with mother report. Infant birth weight, obstetrical complications, neonatal complications, neonatal morbidity risks were obtained by coding twin pregnancy and birth medical records. Twin 1 refers to a sample of randomly selected twins; Twin 2 refers to a sample of the cotwins.

Table 3.

Correlations between Continuous Study Variables

	1	2	3	4	5	6	7	8	9
1. Perceived Prenatal Stress	-	-.24*	.17	-.16	-.03	.05	.02	.08	.01
2. Gestational Age	-.24*	-	-.12	-.15	-.01	.11	.06	.04	.15*
3. Obstetrical Complications	.17	-.03	-	.08	.21*	-.06	-.06	.06	-.03
4. Neonatal Complications	-.16	-.15	.08	-	-.01	.03	-.04	.12	.13
5. Infant Dysregulation	-.12	-.01	.10	.08	-	-.09	-.03	.02	.02
6. Infant Competence	-.16	.12*	-.15	.11	-.04	-	.65**	.12	.32**
7. Developmental Maturity	-.03	.09	-.04	.05	-.03	.64**	-	.12*	.36**
8. Infant Sex	-.01	.09	.04	-.11	-.04	.10	.11	-	-.04
9. Infant Age	.01	.15*	-.03	.16	.02	.25**	.38**	-.06	-

Note: **Correlations significant at the .01 level (two-tailed); * Correlations significant at the .05 level (two-tailed). Correlations for Twins 1 and 2 are displayed in the lower half and upper half of the table, respectively. Twin 1 refers to a sample of randomly selected twins; Twin 2 refers to a sample of the cotwins. Sample sizes for the variables varied; for variable 1, N = 279 twin pairs, for variable 2, N = 291 twin pairs, for variable 3, N = 203 mothers, for variable 4, N = 131 twin pairs, for variable 5, N = 285 twin pairs, for variable 6, N = 287 twin pairs, for variable 7, N = 291 twin pairs, for variable 8, N = 291 twin pairs, and for variable 9, N = 291 twin pairs.

Table 4.

Model Fit and Estimates of Genetic, Shared Environment, and Nonshared Environment Contributions to Dysregulation, Competence, and Developmental Maturity Infant Outcomes.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*p*	*AIC*	*A*	*C*	*E*
Dysregulation	ACE	-258.81	505				-1268.82	.63	.09	.28
	AE	**-256.76**	**506**	**2.05**	**1**	**.15**	**-1268.76**	**.74**		**.26**
	CE	-204.28	506	52.48	1	.001	-1157.28		.35	.65
	E	-233.10	508	28.82	2	.001	-1126.71			1.00
Competence	**ACE**	**-88.99**	**468**				**-1035.99**	**.52**	**.41**	**.07**
	AE	-12.05	469	76.94	1	.001	-956.05	.97		.03
	CE	-9.97	469	79.02	1	.001	-953.05		.96	.04
	E	-3.68	470	86.91	2		-1640.12			1.00
Developmental Maturity	**ACE**	**-790.02**	**471**				**-1732.02**	**.72**	**.25**	**.03**
	AE	-760.14	472	29.88	1	.001	-1704.14	.97		.03
	CE	-110.77	472	79.24	1	.001	-1054.77		.88	.12
	E	-99.84	473	114.77	2	.001	-1266.91			1.00

Note: -2LL = the fit statistic -2 times the log likelihood; df = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; p = probability; AIC = the fit index Akaike's Information Criterion; a^2 = additive genetic; c^2 = shared environment; e^2 = nonshared environment standardized estimates. The best fitting model is bolded.

Table 5.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Dysregulation Moderated by Maternal Perceived Prenatal Stress.

	Model	*-2LL*	*df*	$\Delta\chi^2$	*Δdf*	*p*	*AIC*
Dysregulation	A, C, and E Full Moderation	-916.19	524				-1964.19
	No Moderation	**-910.79**	**527**	**5.40**	**3**	**.00**	**-1964.79**

Note: *-2LL* = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; *Δdf* = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 6.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Competence Moderated by Maternal Perceived Prenatal Stress.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*p*	*AIC*
Competence	A, C, and E Full Moderation	-255.45	494				-1243.45
	No Moderation	**-251.26**	**497**	**4.19**	**3**	**.24**	**-1245.26**

Note: *-2LL* = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 7.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Developmental Maturity Moderated by Maternal Perceived Prenatal Stress.

	Model	*-2LL*	*df*	$\Delta\chi^2$	*Δdf*	*p*	*AIC*
Developmental Maturity	**A, C, and E Full Moderation**	**-156.97**	**512**				**-1180.98**
	Only A moderation	-147.96	514	9.02	2	.001	-1175.96
	Only C moderation	-140.53	514	16.45	2	.001	-1168.53
	Only E moderation	-143.85	514	13.13	2	.001	-1171.86
	A and C moderation	-148.45	513	8.53	1	.001	-1174.45
	A and E moderation	151.65	513	5.23	1	.02	-1177.65
	C and E Moderation	-144.97	513	12.07	1	.001	-1151.87
	No Moderation	-121.87	515	35.11	3	.001	-1151.87

Note: *-2LL* = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; *Δdf* = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 8.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Dysregulation Moderated by Obstetrical Complications.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*P*	*AIC*
Dysregulation	A, C, and E Full Moderation	-331.74	202				-735.74
	No Moderation	**-330.51**	**205**	**1.23**	**3**	**.74**	**-740.50**

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 9.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Competence Moderated by Obstetrical Complications.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*p*	*AIC*
Competence	A, C, and E Full Moderation	-118.99	194				-506.99
	Only A moderation	-103.84	196	15.16	2	.001	-495.84
	Only C moderation	**-118.11**	**196**	**.88**	**2**	**.64**	**-510.11**
	Only E moderation	-115.61	196	3.39	2	.18	-507.61
	A and C moderation	-118.67	195	.32	1	.57	-505.68
	A and E moderation	-115.68	195	3.14	1	.07	-505.68
	C and E Moderation	-118.43	195	.56	1	.45	-508.43
	No Moderation	-102.38	197	16.62	3	.001	-496.38

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 10.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Developmental Maturity Moderated by Obstetrical Complications.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*P*	*AIC*
Developmental Maturity	A, C, and E Full Moderation	-47.12	202				-478.02
	Only A moderation	-44.76	204	29.25	2	.001	-452.76
	Only C moderation	-10.43	204	63.58	2	.001	-418.43
	Only E moderation	**-73.53**	**204**	**.48**	**2**	**.78**	**-481.53**
	A and C moderation	-72.51	203	1.54	1	.36	-491.52
	A and E moderation	-72.61	203	1.42	1	.23	-477.76
	C and E Moderation	-71.76	203	2.25	1	.13	-477.76
	No Moderation	-35.50	205	38.52	3	.001	-445.50

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; AIC = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 11.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Dysregulation Moderated by Gestational Age.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*P*	*AIC*
Dysregulation	A, C, and E Full Moderation	-341.03	202				-745.03
	Only A moderation	-331.24	204	9.79	2	.001	-739.24
	Only C moderation	-332.74	204	8.20	2	.02	-740.74
	Only E moderation	-313.58	204	27.45	2	.00	-721.58
	A and C moderation	-289.29	203	51.78	1	.001	-695.29
	A and E moderation	**-339.96**	**203**	**1.06**	**1**	**.30**	**-745.96**
	C and E Moderation	-337.59	203	3.44	1	.06	-743.59
	No Moderation	-330.63	205	10.41	3	.02	-740.63

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 12.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Competence Moderated by Gestational Age.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*P*	*AIC*
Competence	A, C, and E Full Moderation	-131.14	194				-519.14
	Only A moderation	-109.99	196	21.14	2	.00	-501.99
	Only C moderation	-109.92	196	21.21	2	.00	-501.92
	Only E moderation	-122.90	196	8.23	2	.02	-514.90
	A and C moderation	-116.82	195	14.31	1	.00	-506.82
	A and E moderation	-125.49	195	5.64	1	.02	-515.49
	C and E Moderation	**-130.59**	**195**	**.54**	**1**	**.46**	**-520.60**
	No Moderation	-140.15	197	9.01	3	.001	-518.62

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

Table 13.

Model Fit of Genetic, Shared Environment, and Nonshared Environment Contributions to Infant Developmental Maturity Moderated by Gestational Age.

	Model	*-2LL*	*df*	$\Delta\chi^2$	Δdf	*p*	*AIC*
Developmental Maturity	A, C, and E Full Moderation	-74.02	202				-478.02
	Only A moderation	-44.76	204	29.25	2	.001	-452.76
	Only C moderation	-10.43	204	63.58	2	.001	-418.43
	Only E moderation	**-73.53**	**204**	**.48**	**2**	**.78**	**-481.43**
	A and C moderation	-57.98	203	16.04	1	.001	-411.51
	A and E moderation	-72.60	203	1.41	1	.23	-478.60
	C and E moderation	-71.76	203	2.25	1	.13	-477.76
	No Moderation	-35.50	205	38.52	3	.001	-445.50

Note: -2LL = the fit statistic –2 times the log likelihood; *df* = degrees of freedom; $\Delta\chi^2$ = change in chi-squared from the best fitting full model to reduced model; Δdf = change in degrees of freedom from the best fitting full model to the reduced model; *p* = probability; *AIC* = the fit index Akaike's Information Criterion. The best fit model is bolded.

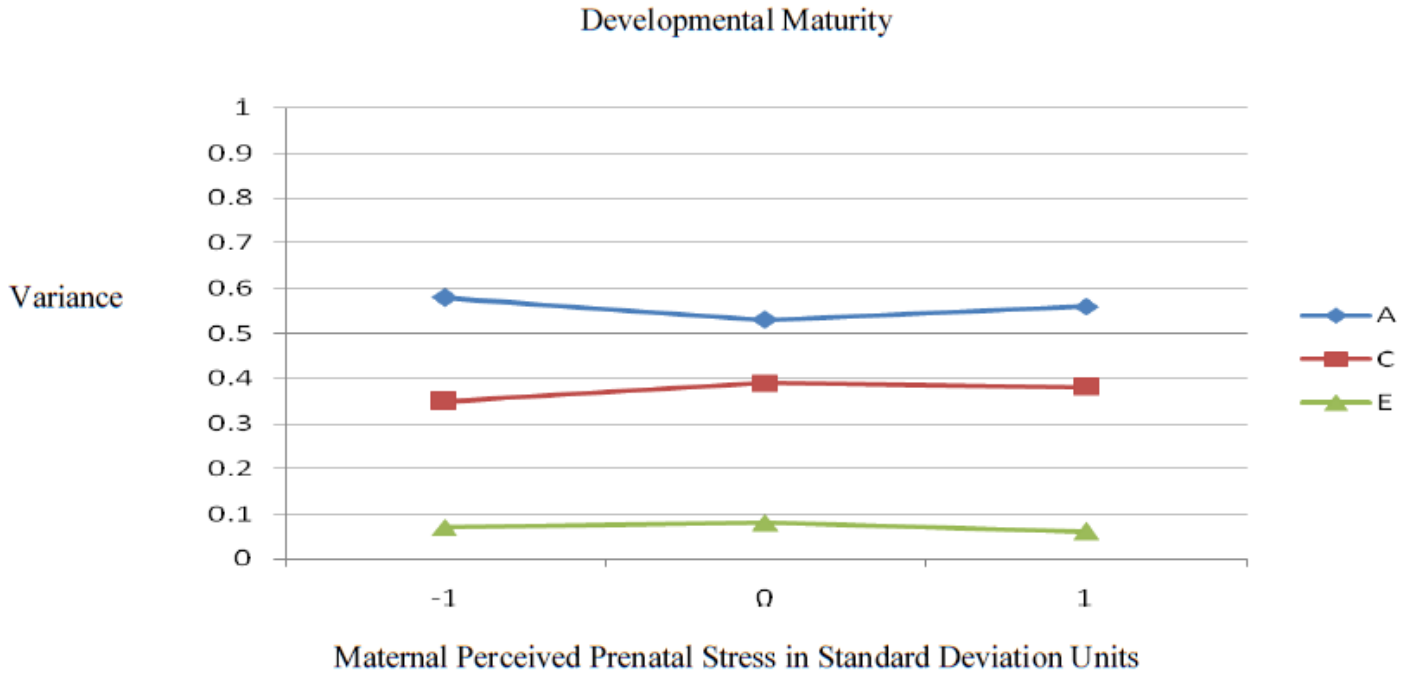

Figure 1. Variance in developmental maturity as a function of maternal perceived prenatal stress. Depicts A, C, and E moderation. A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.

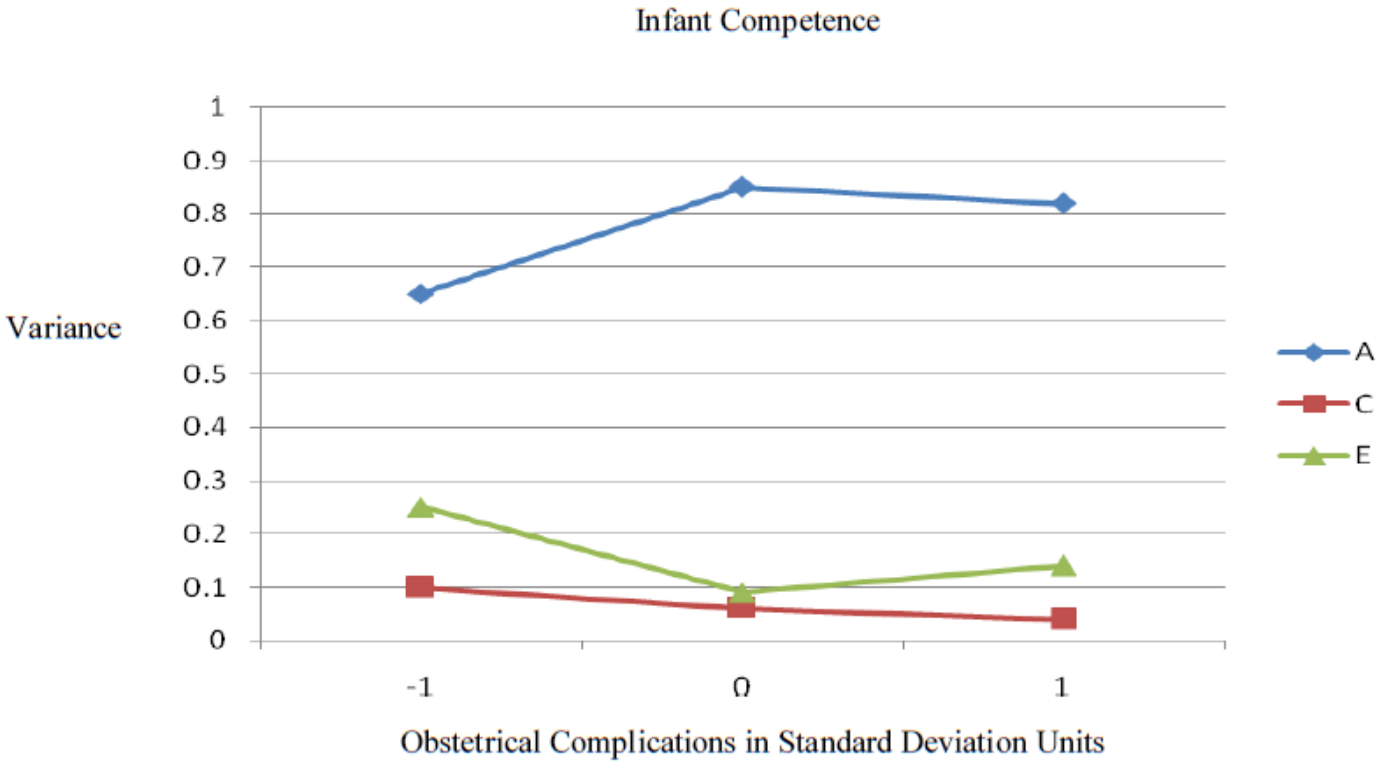

Figure 2. Variance in infant competence as a function of obstetrical complications. Depicts model for C only moderation. A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.

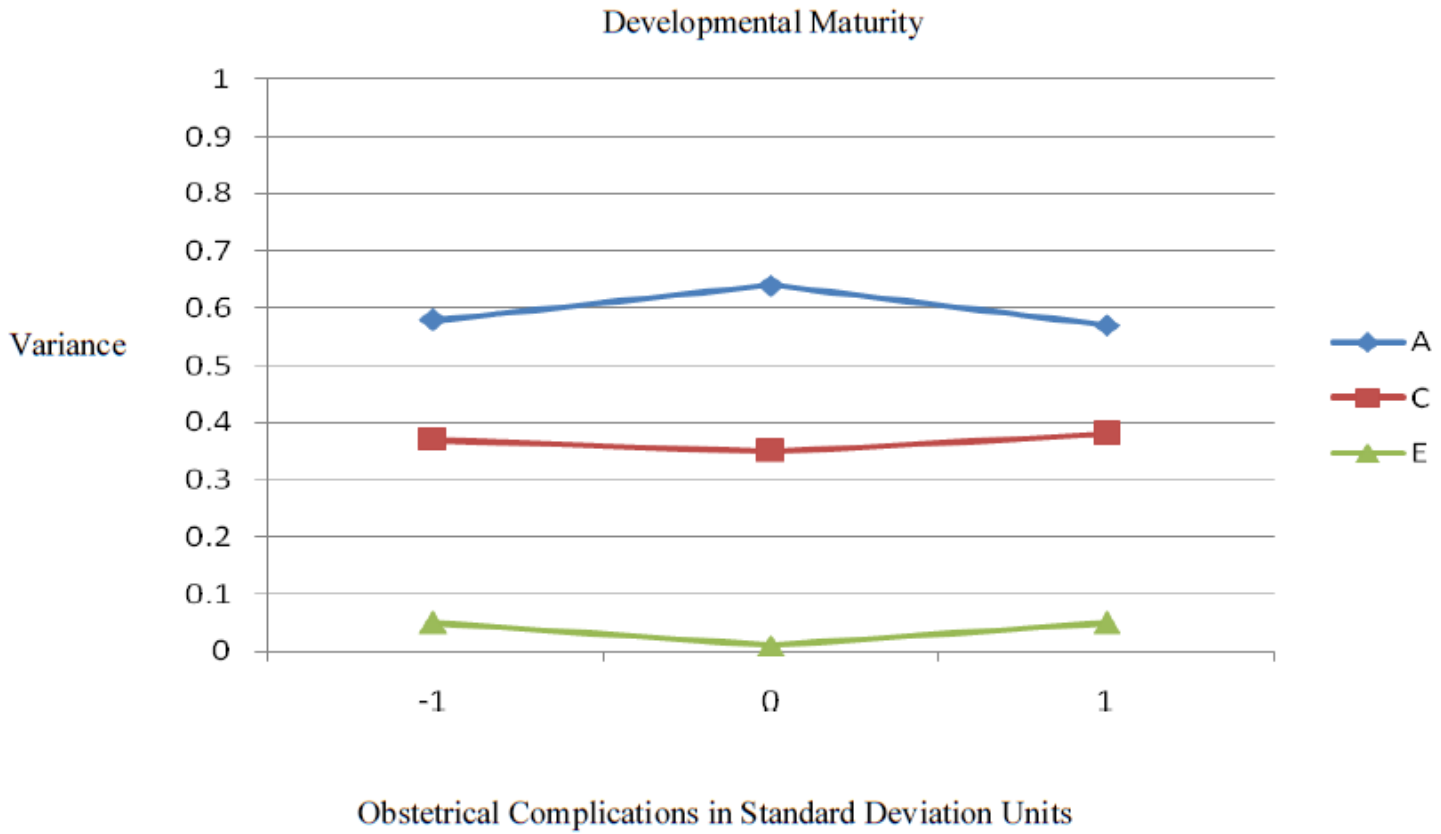

Figure 3. Variance in developmetnal maturity as a function of obstetrical complications. Depicts E only moderation. A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.

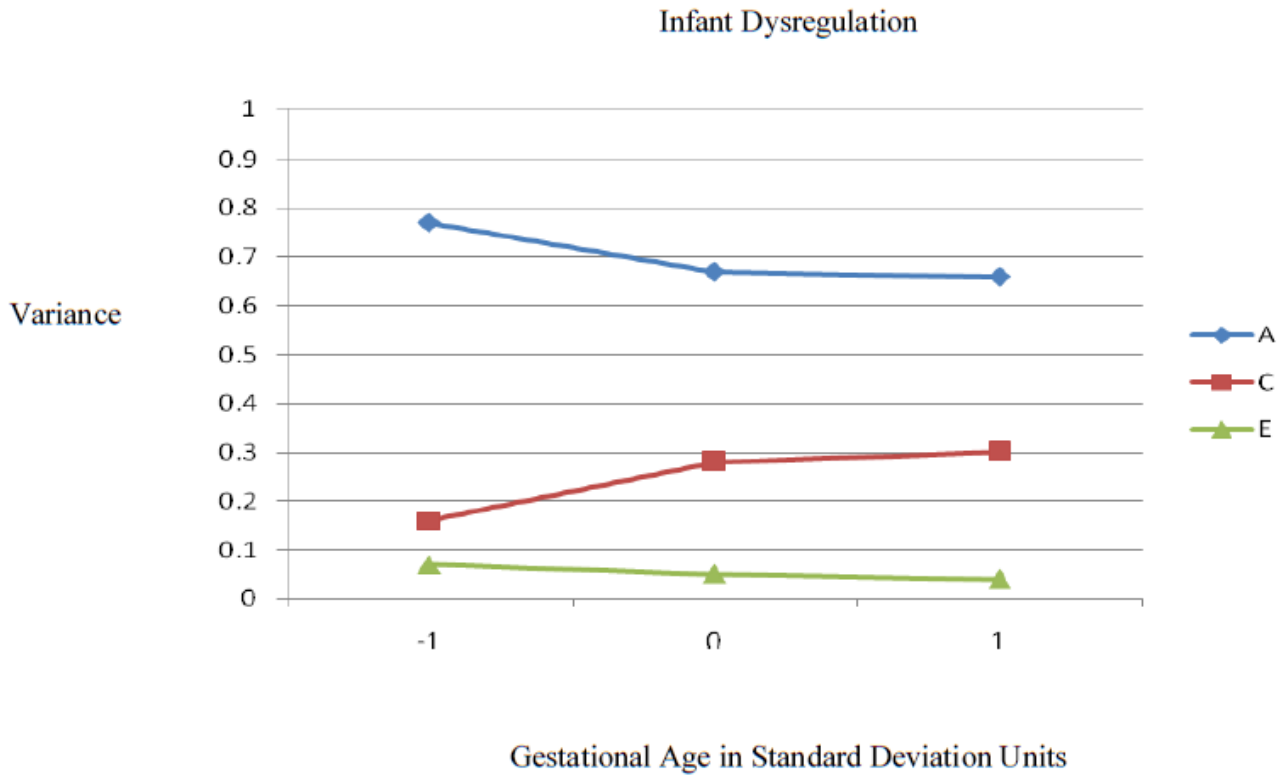

Figure 4. Variance in infant dysregulation as a function of gestational age. Depicts A and E moderation. A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.

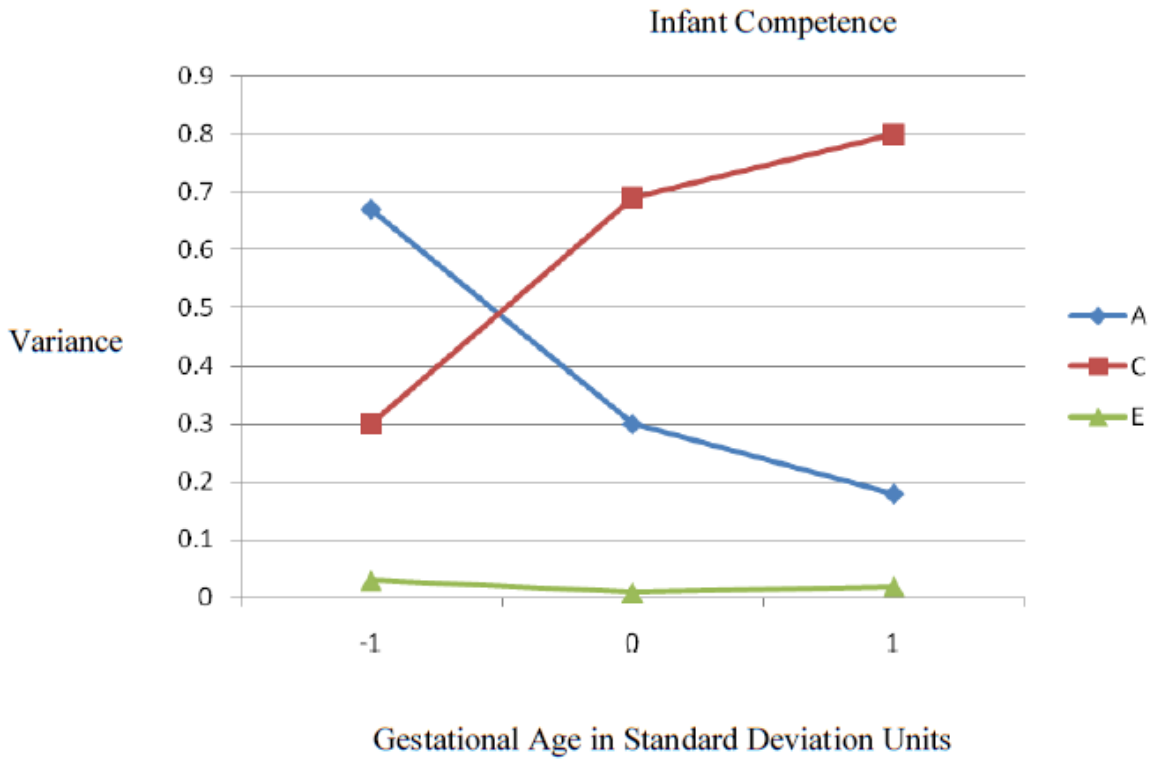

Figure 5. Variance in infant competence as a function of gestational age. Depicts C and E moderation. A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.

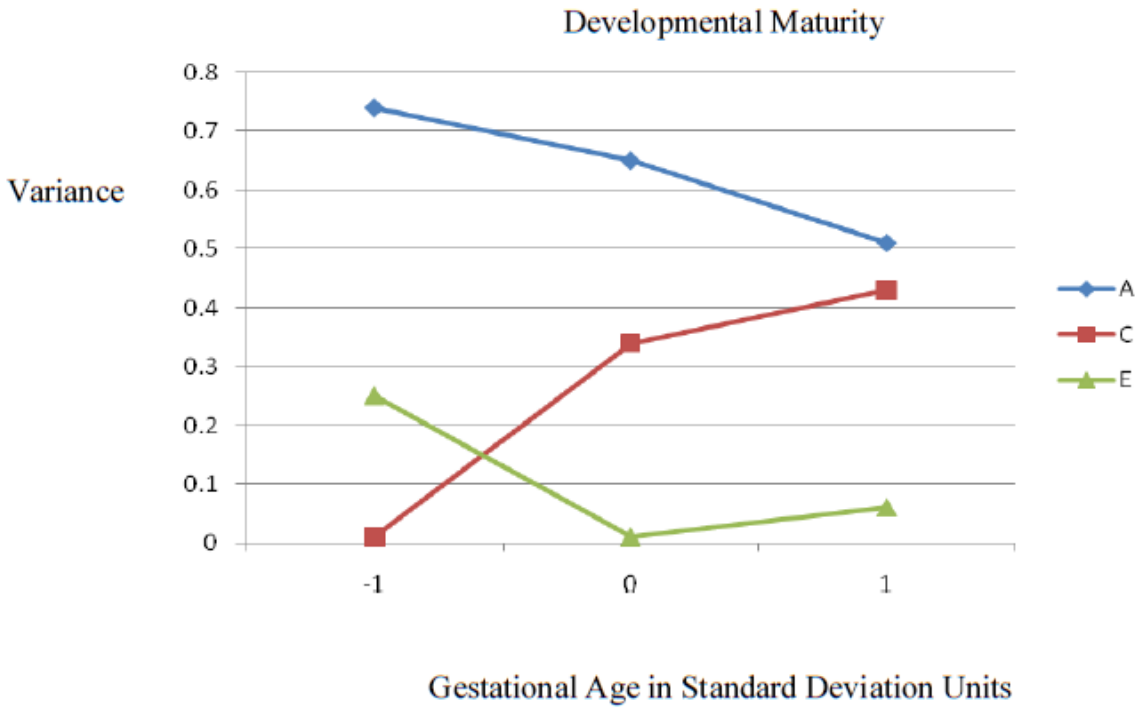

Figure 6. Variance in developmetnal maturity as a function of gestational age. Depicts E only moderation.

A = additive genetic variance, C = shared environmental variance, and E = nonshared environmental influences.