THE ASSOCIATION BETWEEN PRENATAL AND NEONATAL COMPLICATIONS AND AUTISM SPECTRUM DISORDER IN THE CHILD

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ABSTRACT

Christina Cordero: The Association Between Prenatal and Neonatal Complications and Autism Spectrum Disorder in the Child
(Under the direction of Julie Daniels)

The documented prevalence of autism spectrum disorder (ASD) has increased in recent years. ASD is a heterogeneous group of neurodevelopmental disorders with multiple factors involved; however, specific causal factors remain relatively unknown. Previous studies have shown associations between prenatal and neonatal complications and neurodevelopmental disorders. However, they are limited by small samples sizes, the use of administrative data, or by not accounting for confounding or interaction.

To address these limitations, this study utilized data from the Study to Explore Early Development, a population-based case-control study with data from medical records and maternal report collected from six study sites located in the United States. A clinical evaluation classified each child as meeting criteria for ASD (ASD n=698), having a developmental disorder or delay without traits of ASD (DD n=887), or as a population control (POP n=979). In Aim 1, we evaluated the association between maternal diabetes (n=246) and hypertensive disorders (n=386) and ASD, two common complications of pregnancy. In Aim 2, we evaluated the association between neonatal jaundice (n=1239) and ASD, which is variably associated with ASD in previous literature. In our analysis, we controlled for confounding and assessed whether the observed associations were modified by other factors such as maternal body mass index and gestational age. We evaluated how associations varied when comparing ASD to POP versus DD to POP, allowing us to determine if associations are specific to ASD. In Aim 1, we found an

association with maternal hypertensive disorders and ASD (aOR=1.67[95% CI 1.25, 2.23]), and similarly with DD; but no association with diabetes and ASD. In Aim 2, neonatal jaundice was associated with ASD in infants born 35-37 weeks (aOR=1.83[95% CI 1.05, 3.19]) and also with DD, but not in infants born ≥38 weeks.

Our findings suggest that maternal hypertensive disorders and neonatal jaundice are associated with ASD. Future studies should investigate the role of timing and severity of these disorders in association with ASD. These results can help identify children at higher risk of developmental disorders for whom developmental screening at younger ages may allow for identification at younger ages and potentially earlier intervention.

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LIST OF ABBREVIATIONS

ACOG	American Congress of Obstetricians and Gynecologists
ADA	American Diabetes Association
ADI-R	Autism Diagnostic Interview- Revised
ADOS	Autism Diagnostic Observation Schedule
aOR	Adjusted odds ratio
ASD	Autism spectrum disorder
BMI	Body mass index
BP	Blood pressure
CDC	Centers for Disease Control and Prevention
CGI	Caregiver interview
DD	Developmental disorder or delay
DSM	Diagnostic and Statistical Manual of Mental Disorders
GDM	Gestational diabetes
HELLP	Hemolysis, elevated liver enzymes, and low platelets
HTN	Hypertension
ICD	International Classification for Disease
OGTT	Oral glucose tolerance test
OR	Odds ratio
OSS	Obstetric suboptimality
PIH	Pregnancy induced hypertension
POP	Population control group
RRB	Restricted interests and repetitive behaviors
SCQ	Social Communication Questionnaire

SEED	Study to Explore Early Development
SES	Socioeconomic status
T1DM	Type 1 diabetes
T2DM	Type 2 diabetes
US	United States

CHAPTER 1. INTRODUCTION AND SPECIFIC AIMS

A. Introduction

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders characterized by impaired socialization and communication, and repetitive and stereotyped interests and behaviors. The documented prevalence of ASD has increased in recent years; ASD affects one in 68 children, and 1 in 42 boys, aged 8 (1). Causes of ASD remain unknown, but it is believed to be a complex disease involving both genetic and environmental factors (2, 3). Recent studies provide evidence for gestation and the early postnatal period as sensitive time windows for ASD. One study suggests that formation of the cortical layer *in utero* may be disrupted among children with ASD (4). Other studies have shown that deficits in eye contact and ability to follow the parent's eye gaze (joint attention) are present in infants less than a year old (5, 6).

Perinatal complications have been associated with an increased risk of developmental difficulties in children including ASD (7-13). Several studies have reported associations with the most common prenatal complications (maternal diabetes and hypertension) and most common neonatal complication (neonatal jaundice) and ASD (14-18). We hypothesize that the changes to the perinatal environment associated with these conditions through pathways such as increased inflammation and oxidative stress, over-nutrition (with diabetes) and nutritional insufficiencies and growth restriction (with hypertension) (19), and increases in potentially neurotoxic substances (12, 20-22). This may lead to alterations in gene expression patterns or neural connections and synaptic development that can have a deleterious effect on brain development

and lead to ASD (23-25). More recently, observational studies have begun evaluating these specific conditions, but have been limited by: 1) the sample size to study a rare exposure and rare outcome, 2) the exposure and outcome definitions are obtained from a secondary source without confirmation, and 3) specific complications are not modeled independently, but instead are modeled in aggregate with other conditions, without properly accounting for confounding or interaction.

We address these limitations from previous studies utilizing a large, community-based, case-control study, the Study to Explore Early Development (SEED). We evaluate the association of the most common complications in pregnancy, maternal diabetes and hypertension, with ASD, adjusting for confounding and assessing for interaction by body mass index (BMI). We also evaluate the association of the most common complication in the neonatal period, neonatal jaundice, with ASD, adjusting for confounding and assessing for interaction by gestational age.

SEED is the largest study of children with ASD in the United States to-date and provides the optimal data to evaluate these relationships (26). SEED was designed to identify etiological risk factors for ASD and collected data on potential risk factors from multiple sources, including prenatal and neonatal medical records and maternal interview and questionnaires. SEED has also carefully characterized ASD through in-depth clinical assessment and allows comparison of children with ASD to children with other developmental delays (DD) and a population-based sample (POP) of children of the same age and location from the community. The ability to also compare associations among children with DD to POP children allows us to determine the specificity of any observed associations. We take advantage of this large, rich existing data to address the following specific aims:

B. Specific aims

Specific Aim 1: To examine the association between prenatal complications and autism spectrum disorder in the child. The most common complications in pregnancy, diabetes and hypertension, have been observed in relation to the development of ASD in the child. We expand upon recent findings to evaluate whether this association was specific to ASD, or also observed with DD. We also assessed the complex role of BMI and whether having both conditions increased risk, as multiple conditions can confound or modify these associations, or act as mediators on the causal pathway.

Specific Aim 1a: To examine the association between diabetes in pregnancy and autism spectrum disorder in the child. The classification of mothers with diabetes in pregnancy includes those with a new diagnosis of gestational diabetes or diabetes that was diagnosed prior to pregnancy and continued into their pregnancy.

Hypothesis: We hypothesize that maternal diabetes during pregnancy will be positively associated with ASD in the child. *Rationale:* Diabetes affects maternal inflammatory response, oxidative stress, fetal nutrition, and fetal metabolism, all which may affect fetal neurodevelopment.

Specific Aim 1b: To examine the association between hypertensive disorders in pregnancy and autism spectrum disorder in the child. Hypertensive disorders in the mother are classified as either a diagnosis of hypertension prior to pregnancy with elevated blood pressures during pregnancy, or as a hypertensive disorder that was induced by pregnancy, including pregnancy induced hypertension, preeclampsia, eclampsia and HELLP syndrome.

Hypothesis: We hypothesize that hypertensive disorders in pregnancy are positively associated with ASD in the child. *Rationale:* Maternal hypertension is associated with reduced placental perfusion, changes in fetal oxygen and nutrient consumption, and inflammation and oxidative stress impacting the neurodevelopment of the fetus.

Specific Aim 2: To examine the association between neonatal complications and autism spectrum disorder in the child. We evaluated the association between neonatal jaundice and hyperbilirubinemia in association with the development of ASD in the child, and assessed for interaction with gestational age at birth. We assessed the strength of the association by the method used to define jaundice. We used bilirubin measures from the medical record to assess if the association is stronger among infants that present with more severe jaundice. We evaluated if this association was specific to ASD, or also observed with DD.

Hypothesis: We hypothesize that jaundice and hyperbilirubinemia will be positively associated with ASD in the child. *Rationale:* Neonatal jaundice may be indicative of high levels of bilirubin that may have toxic effects in the brain, particularly in early development.

CHAPTER 2. BACKGROUND

A. The etiology of autism spectrum disorder is relatively unknown

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders that are typically diagnosed in early childhood, usually before the age of three, and characterized by impaired social interactions, impaired verbal and non-verbal communication, and restricted interests and repetitive behavior patterns. ASD affected one in 68 children aged 8 in 2010, with a higher prevalence of 1 in 42 observed in boys, according to the most recent estimates from the Centers for Disease Control and Prevention (CDC) (1). The documented prevalence of ASD has increased dramatically over the past few decades, in part because of better diagnosis and increased awareness (27, 28). A child with ASD requires constant and long-term care from family members and healthcare professionals and therefore carries a large emotional and financial burden. It is estimated that a child with ASD incurs an additional $17,000 per year in medical costs compared to a child without ASD (29) and additionally requires intensive behavioral interventions estimated at $40,000 to $60,000 per child per year (30). Despite the large burden of ASD, little is known about its causes. Early studies in families estimated that about 90% of ASD were heritable (31-34), yet more recent estimates suggest the heritability to hover around 50% (2, 3). Earlier studies do not seem to distinguish between a condition being heritable versus genetic, using these terms interchangeably while heritable diseases can have environmental components. Few gene loci have been consistently identified in association with ASD (2), leading researchers to believe that ASD may more likely be heritable due to shared environment versus there being a single gene that is responsible. In the last few decades,

etiological research focus has shifted to investigate the interaction between genes and environment, where environmental factors trigger or mediate ASD phenotypes in individuals that are genetically vulnerable (35). Twin studies are often used to evaluate the influence of genetic and environmental factors on individuals. The largest population-based twin study to date found that shared environmental factors account for 55% of the variance of risk for ASD among twins, while genetic factors only account for approximately 37% (2). A common theory is that multiple causes are likely to operate in the etiology of ASD, which can include several combinations of genes and environmental factors, broadly defined as any factor that is not genetic (9). By definition, this would classify ASD as a complex disorder where multiple factors contribute to its development. Epidemiological investigations are needed to determine which specific factors are involved.

B. Critical window of brain development

The brain begins to develop *in utero* and continues through early childhood (36). Brain imaging studies using MRI have found distinct differences in brain volume (having more gray and white matter) and atypical growth patterns in early life of children with ASD (37, 38). Etiologic studies of ASD have started to focus on events during the prenatal and perinatal period, as this is known to be of critical importance to brain formation and development. A study recently published in the New England Journal of Medicine identified that areas of the prefrontal and temporal cortex, which begins to develop *in utero*, displayed disrupted growth and layer-specific neuronal differentiation in children with ASD (4). These results support findings from an earlier brain autopsy study that reported abnormalities in the structure of the frontal and temporal cortex in individuals with ASD (39), emphasizing the importance of conditions that can impact early brain development (39-42). Clinical studies have also shown early developmental

differences in infants who are later identified to have ASD. One study found that infants have eye gaze deficits characteristic of ASD as early as 2-6 months of age (6), suggesting early deviation in brain development. Another study further reported decreased joint attention initiation in infants 8-12 months that later develop ASD (5).

C. Environmental exposures during the prenatal and neonatal period

A number of prenatal exposures are associated with an increased risk of the child developing ASD. Exposures such as maternal smoking during pregnancy (43), maternal medication use and exposure to infections (44-49) and related complications such as threatened abortion before 20 weeks (50, 51), bleeding during pregnancy (43), and intra-uterine growth retardation (43, 50, 52, 53), have all been associated with an increase risk in ASD. Obstetric factors and indicators of poor neonatal health such as preterm birth (43, 46, 52-58), low birth weight (43, 50, 53, 59), and cesarean section (43) have also been associated with ASD.

Yet, results across studies have been inconsistent and vary based on study design and methodology. Many studies have grouped multiple complications of pregnancy, labor, and the neonatal period together to create an overall obstetric suboptimality score (OSS), which has also been associated with an increased risk in ASD (60, 61). Others have examined specific complications of pregnancy, but have not presented results adjusted for confounding (58, 62). Recent studies have reported maternal metabolic conditions to be associated with ASD. Specific factors such as high body mass index (BMI) (12, 63-65), maternal weight gain (35), hypertensive disorders (12, 43, 66), and diabetes (12, 35, 64, 67) were included in these studies. However, inferences are limited due mainly to small sample sizes and possibility of bias. For example, many studies have not adequately adjusted for confounding—some adjust for mediators on the pathway to the outcome while others may not have data on covariates and simply report crude

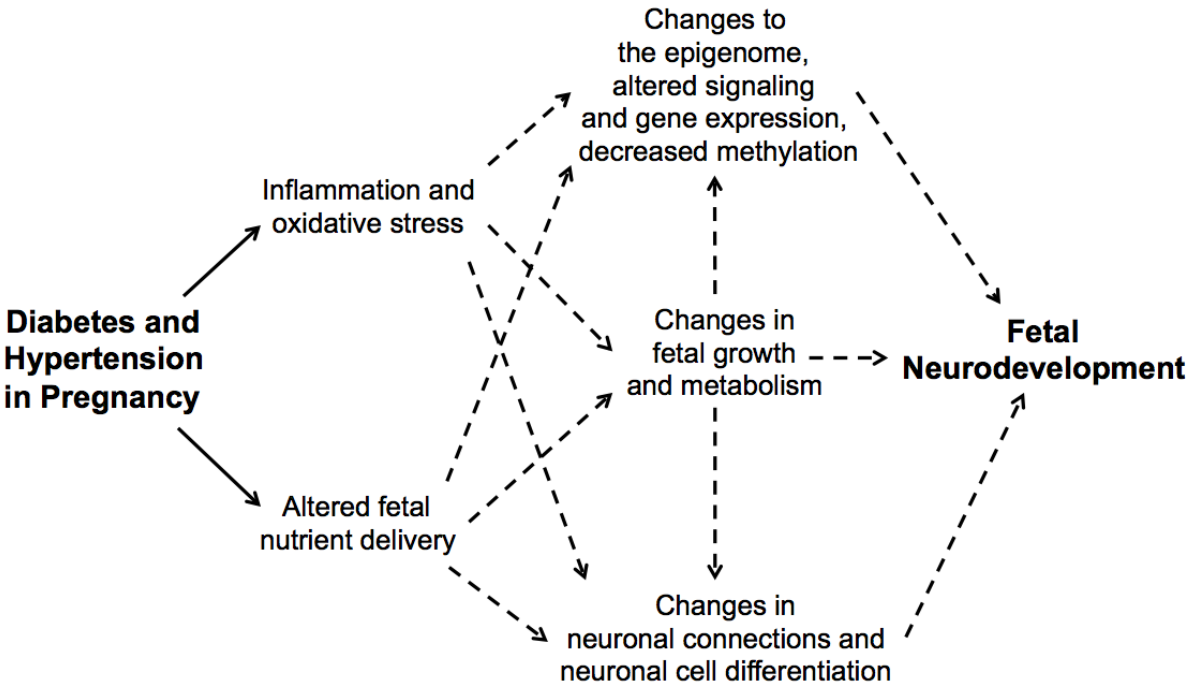
measures (43, 52, 68). Nonetheless, early results from these studies suggest these conditions may be important and warrant more rigorous investigation.

Diabetes and hypertension, the two most common complications in pregnancy, can impact the development of the fetus. The pathogenicity of these conditions in relation to ASD include the role of obesity on endogenous hormones such as leptin (69-72) and testosterone (64, 73, 74), the effect of inflammation and oxidative stress on the fetus (75, 76), and a difference in maternal nutritional stores such as folic acid (77, 78) and fatty acids necessary for brain development such as docosahexaenoic acid (DHA; 79) (see Figure 2.1). Table 2.1 describes studies that have evaluated the association between diabetes or hypertension in the mother and the development of ASD in the child. These are further discussed in Section D and E, which describe in detail the role of maternal diabetes and hypertension on the early neurodevelopment of the child.

Several exposures during the early postnatal period have been shown to affect neurodevelopment. Events during birth, such as labor complications (80-82), hypoxia (83-87), or low Apgar score (88-92) have been implicated as causal factors for psychiatric or neurodevelopmental disorders. Being born preterm (86, 93), with low birth weight or small for gestational age (94, 95), has also been associated with developmental delays or disorders. In infancy and early childhood, toxic exposures such as lead (96-98), iron deficiency anemia (99, 100), or inflammatory responses to infections also affect brain development (101-104). Some of these birth outcomes and neonatal exposures have also been associated with an increased risk of ASD, such as Apgar score or small for gestational age (43, 52), preterm birth (52, 53, 71, 105), asphyxia and hypoxia (105), and congenital anomalies (52, 71, 106).

Insufficient studies have been conducted to evaluate the role of neonatal complications with ASD. Most studies to date include all prenatal, obstetric, and neonatal complications under one category (i.e. obstetric complications or obstetric suboptimality) or in combination of different complications in the same statistical model, adjusting for all factors in each reported measure even if they are not confounders. Few studies have analyzed the role of neonatal jaundice independently with appropriate assessment of confounding. Because jaundice is not typically reported in the birth record, exposure assessment in previous studies has relied on maternal report or access to some medical records. Results from these studies, shown in Table 2.2 and discussed in Section F, are difficult to interpret due to limitations with inadequate sample sizes, inappropriate selection of comparison groups, differing definition of jaundice, and potential for bias by not adjusting for confounders or assessing for interaction with gestational age. This study addresses these limitations through use of the SEED data set.

Figure 2.1. Mechanistic pathway for diabetes and hypertension in pregnancy and fetal neurodevelopment



D. The role of diabetes in pregnancy on neurodevelopment

Gestational diabetes (GDM), defined as any degree of glucose intolerance with onset during pregnancy, is one of the most common complications during pregnancy. The Agency for Healthcare Research and Quality pooled data from different studies to estimate a prevalence of 6- 7% of pregnancies complicated by GDM, with a range of 1 to 25% depending on sociodemographic factors (15, 16). Screening for GDM occurs during 24-28 weeks of gestation, or earlier for women with clinical characteristics consistent with high risk of GDM (107). GDM is often difficult to distinguish from type II diabetes mellitus (T2DM) as T2DM can go unrecognized until the woman receives medical care during pregnancy. Accounting for diabetes existing prior to pregnancy, approximately 10% of pregnancies are complicated by these disorders (108). With the rise in obesity, these estimates are increasing (109).

Changes in insulin production and glucose metabolism normally occur during pregnancy to promote fat storage to sustain the growing fetus and to prepare for lactation (110). Fatty acids are also necessary for synapse formation during fetal brain development, with changes in fatty acid availability directly affecting neuronal pathways (111). An imbalance of insulin and glucose levels can make the body insulin resistant. Insulin resistance induces dyslipidemia and chronic inflammation in adipose, muscle, and liver tissue detected by the presence of inflammatory markers (112). Many of these inflammatory markers have been shown to cross the placenta and enter fetal circulation (22, 110, 113-115). Fetal brain development can be disrupted by the presence of inflammatory markers that cross the placenta and affect neural pathways and gene expression. In animal models, increased levels of maternal inflammatory markers result in offspring with seizures, impairments in learning and cognitive development, and abnormalities in hippocampal regions (21, 22, 116, 117). Studies in children with ASD have found increased

markers of inflammation and oxidative stress as well as lipid mediators in the blood, all of which can cross the blood-brain barrier and affect neurodevelopment (118-122). A recent study using lymphoblastoid cell lines obtained from children with ASD compared with their typically developing siblings found a significant increase in cell necrosis when exposed to oxidative and nitrosative stress (123).

Additionally, maternal insulin resistance may adversely affect fetal development, particularly if the fetus develops hyperglycemia and increases insulin production due to the mother's hyperglycemic state (124). Mothers with diabetes often give birth to macrosomic infants, increasing the risk for obstructed labor, perinatal hypoxia-ischemia, and birth injury (125). Diabetic mothers are also more likely to suffer from placental insufficiency resulting in fetal growth restriction, which can adversely impact brain development (126, 127).

Fetal hyperinsulinemia can also lead to an increase in fetal metabolism and oxygen consumption, resulting in hypoxia of the uterine tissue and if prolonged–fetal iron deficiency, which has been associated with behavioral and developmental disorders (128-131). The insulin-induced high glucose uptake may also cause relative cellular hypoxia in the *in utero* environment (132, 133). Clinical studies have also shown that maternal insulin resistance during pregnancy is associated with deficits in fine and gross motor function in the child, as well as with language impairments, low verbal IQ, greater inattention and hyperactivity, and poorer cognitive function (134-140). Maternal diabetes during pregnancy has also been associated with neonatal complications such as congenital anomalies (141, 142) and neonatal jaundice (143).

Several epidemiological studies have reported positive associations between maternal diabetes and ASD (60, 62, 68, 144). However, others have not reported statistically significant associations with ASD (12, 43, 52, 61, 71). These studies, described in Table 2.1, appear to be

inconclusive due to small numbers of exposed cases and potential bias from study design and statistical modeling approach. There is also great variation in the assessment of diabetes and outcomes that may have led to misclassification and impacted study results.

E. The role of hypertension in pregnancy on neurodevelopment

Like diabetes, maternal hypertension may exist prior to pregnancy, develop during the pregnancy, or be associated with other complications such as preeclampsia, eclampsia, or hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. Preeclampsia affects about 3% of pregnancies while all hypertensive disorders complicate 5-10% or pregnancies (17, 18, 145). These rates have been increasing in the past few years, likely attributed to the rise of obesity and maternal age at time of conception (145). As with diabetes, hypertension in pregnancy can produce a low-level continuous state of inflammation in the fetus that can cause brain damage, even in the absence of hypoxia (146). In fact, because blood pressure is independently related to plasma insulin, many of the adverse fetal effects from maternal hypertension are similar to those from diabetes (147).

Hypertension during pregnancy can result in reduced placental perfusion, limiting nutrients and iron to the fetus, and typically results in intrauterine growth restriction, preterm delivery, and infants that are small for gestational age (20, 148-151). Hypertensive disorders have also been reported to cause asphyxia of the fetus (20, 152). Hypoxia-ischemia while *in utero* has shown the strongest association with brain injury (153-156). Both intrauterine growth restriction and asphyxia are associated with altered brain development and have been associated with several neurodevelopmental disorders (8, 105, 157-159). Conditions of fetal hypoxia and fetal iron deficiency are often present together when the mother is hypertensive, and has been associated with specific changes in the formation of the hippocampus as well as broad changes in

neurodevelopment (130, 131). Previous studies have reported an increased risk of several developmental disorders and delays with hypertensive disorders during pregnancy (160-162). Positive associations have also been reported between hypertension in pregnancy or preeclampsia/eclampsia and ASD (12, 67, 68, 71, 163).

While obstetric suboptimality scores (OSS) that include diabetes and hypertensive disorders in combination with a variety of problematic obstetric conditions have been associated with ASD, it is necessary to study each exposure apart from the other factors. Most of the studies to date (Table 2.1) included maternal diabetes and hypertension along with all other prenatal and perinatal complications in association with ASD, without accounting for the other conditions. This may be because they lacked sufficient data or the large sample size required to have sufficient power to assess both a rare exposure and a rare outcome. Further, many of these studies only have data from one source that does not include information on the timing or severity of disease.

To address these limitations and those previously discussed in Section D, we investigate the association between maternal diabetes and hypertension with ASD in SEED. SEED is a large multi-site case-control study with detailed data on ASD and perinatal conditions, as well as important covariates related to maternal characteristics and health history. Utilizing SEED, we can study rare exposures such as maternal diabetes and hypertensive disorders independently, and also subclassifications of these conditions based on onset. We are also able to assess for interaction with BMI and evaluate the specificity of this association by comparing it to that with DD.

Table 2.1. Summary of studies that have looked at the association between diabetes or hypertension in pregnancy and ASD

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
J. Piven, 1993 (61)	53 autistic probands and their 63 siblings who were enrolled in a family study at Johns Hopkins	Sibling-matched case-control study; maternal parity	Obstetric suboptimality score using Gilbert Optimality Scale and data from maternal interview or medical records.	n=39 "autistic subjects" matched with n=39 randomly matched sibling controls.	Null
N. Juul-Dam, 2001 (58)	74 children seen at a research center in California.	Case-control study; none.	28 pre-, peri-, and neonatal risk factors obtained from parental interview and available obstetric and neonatal records. Including gestational diabetes (n=6), preeclampsia (n=8), and hyperbilirubinemia (n=22).	Children with PDD (n=74) diagnosed between 2.5 to 4 years old using DM-IV and ADI-R criteria, reevaluated at 5 yrs old where autism n=51 (10 excluded due to incomplete records) and PDD-NOS=13.	Incidence was not statistically significantly higher at an alpha=0.05 level for GDM or preeclampsia, was statistically significantly higher for hyperbilirubinemia.
C.M. Hultman, 2002 (43)	Swedish Birth Register linked to the Swedish Inpatient Register from 1987-1994.	Population-based nested case-control study. Conducted analysis with a crude model and with a model that included all other variables in the analysis.	Maternal, pregnancy, and infant characteristics, including complications during pregnancy, delivery and the neonatal period classified by ICD-8 and ICD-9. Includes diabetes (n=4) and hypertensive diseases (n=24).	Children discharged with a main diagnosis of infantile autism according to ICD-9 from any hospital in Sweden before 10 years of age (n=408).	Diabetes adjusted OR=1.2 (95% CI 0.3-5.7) Hypertensive diseases adjusted OR=1.6 (95% CI 0.9-2.9)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
H. Leonard, 2006 (62)	Linked datasets of 236,964 children born in Western Australia between 1983-1992.	Population-based case-control study; did not adjust for covariates.	Preexisting medical conditions recorded on the midwives' notification form for all births using ICD-9, including diabetes (n=6).	Ascertained educational and disability services sources that link to the birth record using DSM-IV criteria, including children with mild to moderate ID (n=2462), severe or profound ID (n=212), and ASD with ID (n=191). Compared to children without ID.	Diabetes (ASD with ID vs. no ID) OR=2.89 (95% CI 1.28-6.51) Diabetes (Mild-moderate ID vs. no ID) OR=1.69 (95% CI 1.26-2.27)
S. Buchmayer, 2009 (52)	Swedish Birth Register linked to the Hospital Discharge Register from 1987-2002.	Population-based case-control study. Conducted analysis with a crude model and with a model that included all other variables in the analysis.	Several maternal, pregnancy-related, and neonatal factors collected from the Birth Register and Discharge Register including diabetes mellitus (n=18), gestational hypertension (n=21), preeclampsia (n=39).	Children discharged with a main diagnosis of infantile autism according to ICD-9/ICD-10 from any hospital in Sweden before 10 years of age (n=1,216).	Diabetes crude OR=1.13 (95% CI 0.67-1.89)/ adjusted OR=0.90 (95% CI 0.49-1.67) Gestational hypertension crude OR=1.18 (95% CI 0.73-1.92)/adjusted OR=1.04(95% CI 0.59-1.81) Preeclampsia crude OR=1.41 (95% CI 0.98-2.02)/adjusted OR=1.64 (95% CI 1.08-2.49)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
I. Burstyn, 2010 (68)	Provincial delivery records for singleton live births in Alberta, Canada between 1998-2004.	Population-based birth cohort. Conducted analysis controlling for all maternal, obstetric, and neonatal risk factors that were collected.	Maternal and obstetric risk factors extracted from the delivery records, including pre-pregnancy diabetes (n=16) and GDM (n=54), and preeclampsia (n=27).	Children that were assigned an ICD-9 code for ASD by physician billing from at least one claim from any physician (n=1,138)	Pre-pregnancy diabetes RR=1.65 (95% CI 1.01-2.71) GDM RR=1.24 (95% CI 0.94-1.65) Preeclampsia RR=1.49 (95% CI 1.00-2.23)
J. R. Mann, 2010 (67)	Women with singleton pregnancies in South Carolina Medicaid billing records from 1996-2002	Birth cohort from SC; controlling for maternal age, race, alcohol use, and educational attainment, year of birth, child's sex.	Maternal conditions/diseases during pregnancy (Preeclampsia n=52), lifestyle factors reported on the birth certificate, and child anomalies.	ASD determined by all children receiving services from the SC Department of Disabilities and Special Needs or diagnosed with ICD-9 by at least two different providers (n=472)	Preeclampsia/eclampsia OR=1.69 (1.26-2.28)
L. Dodds, 2011 (71)	All live births in Nova Scotia Perinatal Database between 1990-2002.	Retrospective, longitudinal cohort study. Presented univariate results without controlling for confounding.	Pre-pregnancy factors (diabetes n=7), prenatal factors, and pregnancy diseases/conditions (GDM n=32; PIH n=106), labor and delivery variables, and neonatal factors and neonatal diseases/conditions. OSS.	Children that were assigned an ICD-9 code or ICD-10 code for autism and any PDDs. Autism (n=924), no autism (n=128,809).	Pre-pregnancy diabetes RR=1.98 (0.94-4.16) Gestational diabetes RR=1.29 (0.90-1.83) Pregnancy-induced hypertension (PIH) RR=1.24 (1.02-1.52)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
P. Krakowiak, 2012 (12)	CHARGE study, 1,004 children between 24-60 months in California recruited from 2003-2010.	Population-based case-control study; maternal age, race/ethnicity, education level, delivery payer, calendar time.	Metabolic conditions (MC), type 2 diabetes (n=4), GDM (n=44), history of hypertension (n=19), and obesity (n=111). Assessment by questionnaire and medical records (available for half).	Children with a prior diagnosis of ASD were confirmed with the ADI-R and ADOS (n=517). SCQ administered to DD (n=172) and GP (n=315) children to evaluate for ASD.	Diabetes OR=1.52 (95% CI 0.82-2.83) Hypertension OR=2.84 (95% CI 0.94-8.56) Obesity OR=1.67 (95% CI 1.10-2.56) Any MC OR=1.61 (95% CI 1.10-2.37)
K. Lyall, 2012 (60)	Nurses Health Study II, 66,445 parous nurses in the U.S. enrolled from 1989-2003	Cohort Study; maternal age, race, marital status, income, spouse education, and parity. For GDM and HBP: history of miscarriage, prior abortion, pregnancy related HBP, other complications, etc.	Obstetric suboptimality score and individual complications with first birth. Maternal report by questionnaire collected every two years, may have occurred with pregnancy other than index. GDM in first birth n=58, pregnancy-related HBP in first birth n=85.	Maternal report in 2005 asking women if they had a child with autism, Asperger syndrome, or "other autism spectrum disorder", unknown if from first birth where exposure occurred; n=793 (1.2%).	OSS of 4+ OR=2.96 (95% CI 1.74, 5.03) GDM OR=1.88 (95% CI 1.15-3.07) Pregnancy-related HBP OR= 0.96 (95% CI 0.58-1.59)
S. G. Gregory, 2013 (144)	All recorded live births in North Carolina from the North Carolina Detailed Birth Record from 1990-98 linked to educational data from 1997-98 and 2007-08 school year.	Controlled for induced or augmented birth, maternal age, maternal race/ethnicity, birth order, education, marital status, singleton birth, smoking, hypertension, diabetes.	Induction or augmented birth was the main exposure, looked at contributing factors such maternal characteristics and maternal conditions during pregnancy (hypertension n=386; diabetes n=220).	An autism diagnosis from public school educational records (n=5,648).	Hypertension OR=0.95 (95% CI 0.85-1.05) Diabetes OR=1.24 (95% CI 1.08-1.42)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
A. T. Langridge, 2013 (8)	All live singleton births in WA between 1984-1999 linked with data from population-based disability databases	Adjusted for all maternal conditions, birth year, and sociodemographics.	Maternal conditions and pregnancy outcomes (including diabetes and hypertension), labor and delivery complications, neonatal outcomes. No Ns avail.	ASD (with ID and without ID) identified in state-wide disability database (N=752 and 452, respectively)	Diabetes OR= 1.03 (95% CI 0.62-1.69) Hypertension OR= 1.34 (95% CI 1.05-1.71)
P. Polo-Kantola, 2014 (164)	Registry-based case-control study from all singleton births in Finland from 1990-2005	Birth type, Apgar scores, neonatal treatment, smoking during pregnancy, parity, and maternal psychiatric history	Maternal factors including high blood pressure (=56), birth factors, neonatal treatment	Childhood autism obtained from hospital discharge registry (n=1036)	High blood pressure OR=1.49 (95% 1.1-1.21)
C. K. Walker, 2015 (163)	CHARGE study, 1,004 children between 24-60 months in California recruited from 2003-2010.	Population-based case-control study; maternal educational level, pre-pregnancy obesity, and parity.	Preeclampsia (n=32) and placental insufficiency.	Children with a prior diagnosis of ASD were confirmed with the ADI-R and ADOS (n=517). SCQ administered to DD (n=194) and GP (n=350) children to evaluate for ASD.	Preeclampsia OR=2.36 (95% CI 1.18, 4.68))
A.H. Xiang, 2015 (165)	Retrospective longitudinal cohort study including 322,323 singleton children born in 1995-2009 at Kaiser Permanente	Birth year, maternal age, parity, education, household income, race/ethnicity, history of comorbidity, and sex of the child.	From birth record and medical record (GDM n=310, T2DM n=115).	Clinical diagnosis of ASD in the medical record (n=3,388)	T2DM OR= 1.21 (95% CI 0.97-1.52) GDM OR= 1.04 (95% CI 0.91-1.19) GDM <26 wks OR= 1.42 (95% CI 1.15-1.74)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
N. Connolly, 2016 (166)	Medical records of children seen in behavioral clinic associated with Cincinnati Children's Hospital, 2009- 2014	Case-control study; maternal age, maternal race, year of birth, and BMI.	Using ICD-9 codes and natural language processing, GDM (n=52) and preexisting diabetes (n=1), chronic hypertension (n=15), hypertension in pregnancy (n=29).	Clinical diagnosis of ASD in the medical record from the behavioral clinic; non-ASD DD, and POP	GDM OR=1.64 (95% CI 1.22, 2.22) Obesity and GDM= 2.53 (95% CI 1.72, 3.373)
M. Li, 2016 (167)	Medical records, recruited at Boston Medical Center, 1998-2014	Prospective birth cohort; year or birth, sex, maternal age, parity, smoking, and preterm birth.	Using ICD-9 codes, GDM (n=9), preexisting diabetes (n=10)	Using ICD-9 codes, ASD (n=102)	GDM HR=1.86 (95% CI 0.92, 3.76) Preexisting diabetes HR=2.25 (95% CI 1.14, 4.42)

F. The role of neonatal jaundice on neurodevelopment

Both neonatal jaundice and hyperbilirubinemia have been associated with the development of ASD in the child. Jaundice, a yellow discoloration of the skin and the sclera of the eyes, occurs in most newborn infants usually as a result of increased bilirubin levels. During the neonatal period, fetal hemoglobin breaks down as it is replaced by adult hemoglobin, resulting in the release of bilirubin produced in the cells. Bilirubin travels through the bloodstream to be excreted by the liver, but this process may be prolonged due to an immature liver leading to an increase of bilirubin in the blood (168). Increased bilirubin levels may be seen more frequently with conditions characterized by increased hemolysis or other conditions compromising infant liver function. Jaundice may also be seen with breastfeeding due to factors in the breast milk that promote reabsorption of bilirubin after it is secreted into the intestinal tract, and can be more common when feeding times are irregular (168). Visual jaundice is seen in 60% of term neonates and usually resolves within the first week of life (169, 170). However, if bound to albumin, bilirubin can cross the blood brain barrier where it may have toxic effects on the developing brain and may even cause death (Figure 2.2). Animal studies have shown bilirubin in regions of the basal ganglia, hippocampus, substantia nigra, and brainstem nuclei (171). High levels of bilirubin have been shown to cause mental retardation, cerebral palsy, and kernicterus in children (172). Bilirubin can be measured directly from the blood or transcutaneously, neonates with high levels according to the Bhutani curve (Figure 2.4) may be treated with phototherapy or if not successful or in extreme cases, exchange transfusion (173).

Recently, several studies have reported an association between high levels of bilirubin and the development of ASD in the child (52, 105, 174-176). Other studies did not find a statistically significant association (10, 177), but this may have been due to limitations such as

not accounting for confounders (10) and small sample sizes that make it difficult to detect associations at high levels of bilirubin exposure. These studies also have varying methods of collecting data and classifying jaundice, some using parental report, differing bilirubin cut-offs, or administrative codes. Many of these studies also fail to fully address the role of preterm birth and gestational age. For example, neonatal jaundice is more common in infants born preterm and infants may also be more susceptible to high levels of bilirubin at earlier gestational ages, which may affect the association with ASD(14). This study addresses these limitations and helps elucidate whether the association with perinatal complications and ASD is likely due to prenatal complications (maternal diabetes or hypertension) or subsequent neonatal complications (neonatal jaundice).

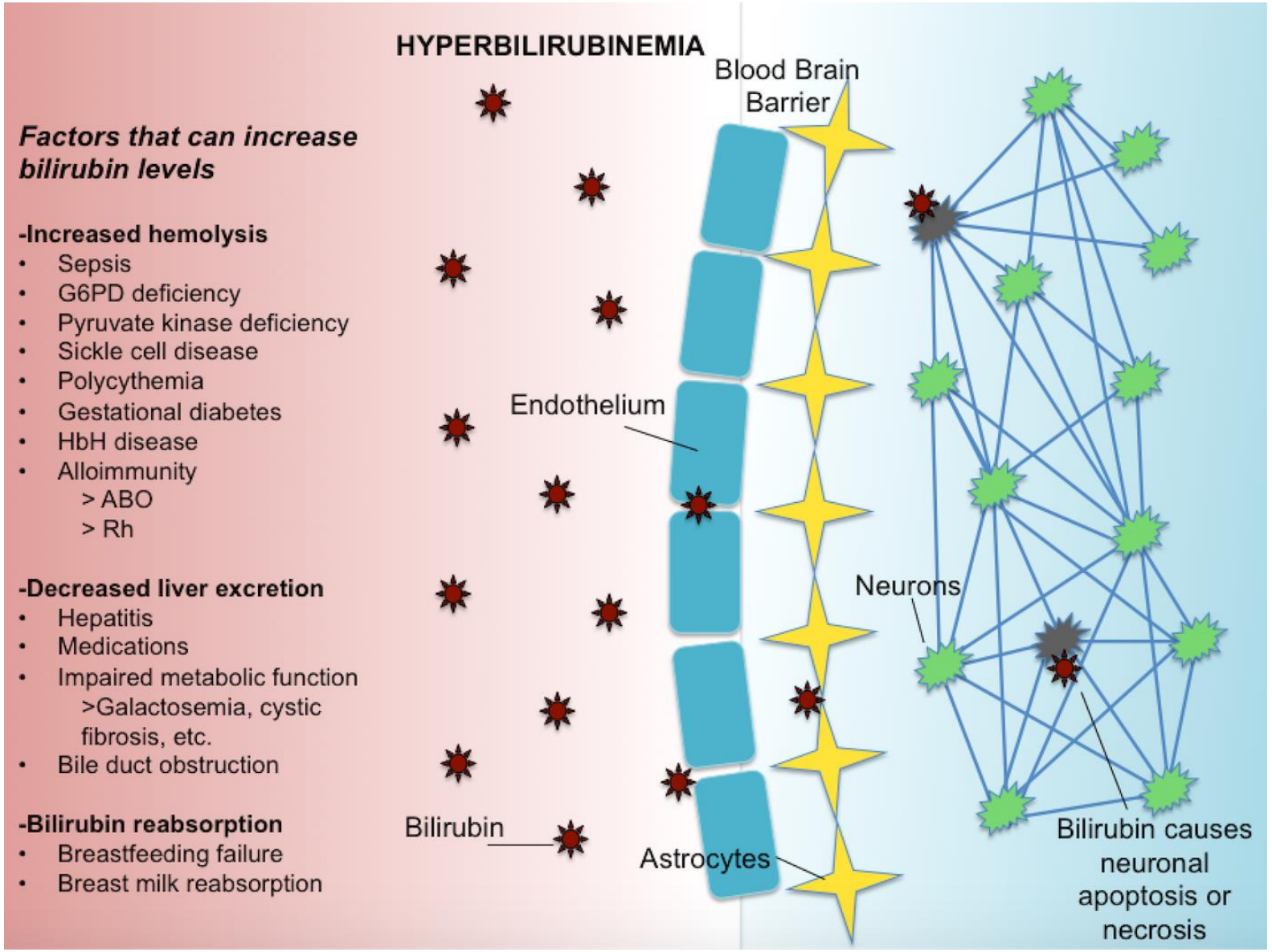


Figure 2.2. Biological mechanism for neonatal jaundice and hyperbilirubinemia in association with the development of ASD

Table 2.2. Summary of studies that have looked at the association between neonatal jaundice or hyperbilirubinemia and ASD

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
L. A. Croen, 2005 (177)	Singleton term infants born between 1995 and 1998 at a northern California Kaiser Permanente Hospital	Nested case-control study; sex, birth facility, maternal age, maternal race/ethnicity, maternal education, gestational age	Maximal neonatal bilirubin levels from medical records: 15-19.9 mg/dL (n=27), 20-24.9 mg/dL (n=6), ≥25 mg/dL (n=1)	Children with an ICD-9 ASD diagnosis recorded in an outpatient database (n=338)	*15-19.9 mg/dL* aOR=0.74 (0.48–1.15); *20-24.9 mg/dL* aOR=0.66 (0.27–1.59); *≥25 mg/dL* aOR=1.12 (0.11–11.15)
K. A. Jangaard, 2008 (178)	Infants with gestational age ≥35 weeks born in Nova Scotia, Canada from Jan 1 1994-Dec 31 2000 in a Perinatal Database.	Population-based cohort study; adjusted for fetal sex and parity.	Infants identified as having hyperbilirubinemia, with moderate hyperbilirubinemia defined as TSB 230-<325 μmol/L (n=19).	ASD diagnosis from Canadian insurance database (n=187).	Unadjusted RR=1.6(1.0–2.6) Adjusted RR=1.6(1.0–2.5)
R. D. Maimburg, 2008 (175)	Children born in Denmark from 1990-1999.	Population-based matched case-control study; adjusted for birth weight, gestational age, mother's age.	Medical record data from the prenatal and perinatal period, hyperbilirubinemia was defined if maximum bilirubin value in μmol/L exceeded 10% of the infant's weight in grams on the same day, if not available birth weight was used. A serum bilirubin test was only ordered if jaundice was visible.	473 children <10 years old with a diagnosis of ASD in the Danish Psychiatric Central Register, and 473 controls from birth records.	Serum bilirubin test: UOR=1.4(1.1–2.1); AOR=1.2(0.8–1.7) Hyperbilirubinemia- (by infant weight): UOR=5.6(2.2–14.5); AOR=3.7(1.3–10.5) (by birth weight): UOR=6.0(2.1–17.3); AOR=3.8(1.2–12.1)
S. Buchmayer, 2009 (52)	Swedish Birth Register linked to the Hospital Discharge Register from 1987-2002.	Population-based case-control study; adjusted for all infant characteristics and neonatal complications of interest.	Several maternal, pregnancy-related, and neonatal factors collected from the Birth Register and Discharge Register including neonatal jaundice (n=74).	Children discharged with a main diagnosis of infantile autism according to ICD-9/ICD-10 from any hospital in Sweden before 10 years of age (n=1,216).	Crude OR=1.32(1.01–1.72) Adjusted OR=1.18(0.86–1.63)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
R. D. Maimburg, 2010 (174)	All children born in Denmark from Jan 1 1994-Dec 31 2004.	Population-based, follow-up study; smoking, irregular fetal presentation, Apgar score, parents' age, mother's citizenship, birth weight, congenital malformations.	Birth and medical record data, jaundice exposure determined by ICD-10	ICD-10 ASD diagnosis (n=532) and diagnosis of other disorders of psychological development	Neonatal jaundice: Crude HR=1.84(1.26–2.69); Adjusted HR=1.56(1.05–2.30)
M. P. Mamidala, 2013 (105)	Obtained via simple random sampling of individuals from 8 major Indian cities from Sept 2010-Dec 2012.	Case-control study; maternal age at delivery, sex, and birth year.	Parent questionnaire regarding parental, prenatal, perinatal, and neonatal characteristics, including neonatal jaundice (n=64).	481 ASD cases aged 2-10 obtained from developmental centers and 481 typically developing controls obtained from schools.	Neonatal Jaundice: Crude OR=3.58 (2.02–6.35) Adjusted OR=2.89 (1.58–5.28)
G. Duan, 2014 (176)	Cases were treated in a hospital in Zhengzhou city, China from Feb 2011-Feb 2013. Controls were healthy children from local kindergartens.	Case-control study; parental age, paternal introversion, passive smoking, premature rupture of the fetal membrane, premature delivery, birth asphyxia.	Neonatal jaundice (n=49) data collected from clinical data and parent report in cases and from parent report in controls.	Pediatric autism cases (n=286) treated by a hospital in China, and 286 controls from schools.	Neonatal jaundice crude RR=11.57 (1.86–34.72) Severe jaundice adjusted RR=21.81 (12.22–35.54)
W. Froehlich-Santino, 2014 (10)	California Autism Twin Study (CATS), obtained from the California Department of Developmental Service from 1987-2004.	Population-based twin study; none.	Parent questionnaire regarding specific complications of pregnancy, labor, and the neonatal period, including jaundice (n=59).	ASD diagnosis verified by ADI-R and ADOS. N=388 [194 twin pairs; 137 individuals with ASD] from age 4-18 yo. One child in the twin pair must have ASD.	Jaundice: OR=1.54 (0.94–2.52)

First author, year	Data source, population, and study period	Design, confounders	Exposures (n with ASD), ascertainment	Outcome (n with outcome), ascertainment	Measures of association
Y. W. Wu, 2016 (179)	Infants born ≥ 35 weeks in Northern California, seen at Kaiser Permanente from 1995-2011.	Retrospective cohort study, TSB levels, treatment, maternal and paternal age, maternal and paternal education, maternal race/ethnicity, sex, gestational age, birth weight, apgar, year of birth.	Total serum bilirubin levels in medical record, n=146 with TSB ≥20, n=848 with 15<TSB<20, n=580 with phototherapy.	ASD diagnosis either by ICD-9 code, n=5,979	TSB≥20 OR=1.09 (95% CI 0.89, 1.35) 15<TSB<20 OR=1.07 (95 CI 0.98, 1.17) Phototherapy OR=1.10 (95% CI 0.98, 1.24)

CHAPTER 3. METHODS

A. Study design

The Centers for Autism and Developmental Disabilities Research on Epidemiology (CADDRE) Network were established as regional centers of excellence for research on ASD and developmental disabilities in 2002. The CADDRE Network designed the Study to Explore Early Development (SEED) to better understand ASD phenotype and etiology. SEED is a multisite case-control study with population-based, multiple-source ascertainment of children with ASD and two comparison groups, children with developmental delays or disorders other than ASD (DD) and children sampled from the general population (POP). Case children (ASD) were those confirmed to have ASD based on DSM-IV criteria for autistic disorder, pervasive developmental disorder-not otherwise specified, and Asperger syndrome. Potential participants were recruited from a catchment area near the study sites in six states (California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania)(26).

SEED was designed to be the largest multi-site epidemiological investigation of etiological and risk factors contributing to ASD. A clinical evaluation was conducted to confirm ASD diagnosis and assess all children's neurodevelopment. SEED collected detailed data from maternal and child medical records, maternal interview, and birth certificates for the period covering the first maternal prenatal care visit through the child's third birthday. Data collection was uniform across all study groups. A summary of data collection sources and questions used for this study are described in more detail below. More details on methods specific to each aim are presented in Chapter 4 and 5.

B. Study population

SEED required eligible children to have been born in and reside in one of six study catchment areas between September 1, 2003 and August 31, 2006 and to be between the ages of 30 and 68 months of age at the time of in-person assessment. SEED aimed to obtain a population-representative sample. The catchment areas were of similar population size in California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania. SEED also required the children to live with a knowledgeable caregiver who was at least 18 years of age and spoke English (or Spanish if participating in CO or CA)(26). For the purposes of this analysis, we also require that the caregiver be the biological mother because of the focus on prenatal data.

Children in the ASD and DD groups were ascertained through multiple agencies that evaluate or serve children with developmental challenges, even if they did not already have a specific diagnosis of ASD or other developmental disability. Recruitment targeted agencies focused on early intervention, special education, and related service programs for toddlers and young children, as well as hospitals, clinics, and individual providers. Parents also might have contacted the study directly and the child enrolled if eligible. This "broad diagnostic net" for ascertainment ensures that previously diagnosed and undiagnosed ASD children were identified (26). SEED's recruitment procedures across multiple agencies and multiple sites ensured sample diversity that should be more generalizable to the broad US population than single clinic-based studies.

State birth records were randomly sampled to identify children from the general population to invite for participation. POP children were required to be born in the specified date range, whose mothers were residing in the study catchment area at time of delivery, and were still alive at time of invitation to participate in the study. Families in all three recruitment groups

were invited to participate in the study via mail and, if interested, received a follow-up call. During this call, the Social Communication Questionnaire (SCQ) (180) was administered to the child's primary caregiver to screen the child for ASD. SEED ascertainment and evaluation procedures are described in more detail below and in Figure 3.1. This analysis is composed of children enrolled in SEED that have data available from either the caregiver interview or medical records on the exposure of interest and met criteria for one of the three outcome groups discussed in more detail below.

C. Outcome assessment

ASD ascertainment

During the initial recruitment call, a positive screen was defined as an SCQ ≥11. This lower cut-point was used to increase the sensitivity of the screener because of children's young age (26). Children who screened positive or had a previous diagnosis of ASD were placed in an ASD workflow and received a standardized in-clinic assessment by trained research-reliable clinicians. ASD was confirmed by both the Autism Diagnostic Observation Schedule (ADOS) (181, 182) and the Autism Diagnostic Interview-Revised (ADI-R) (183) as meeting standard criteria for both instruments, or relaxed criteria for the ADI-R if criteria for the ADOS is met as described in Table 3.1. Children in the ASD workflow that were untestable or refused to take the ADOS/ADI-R during clinical evaluation were placed in a separate "Possible ASD" group; children in this group are excluded from this study.

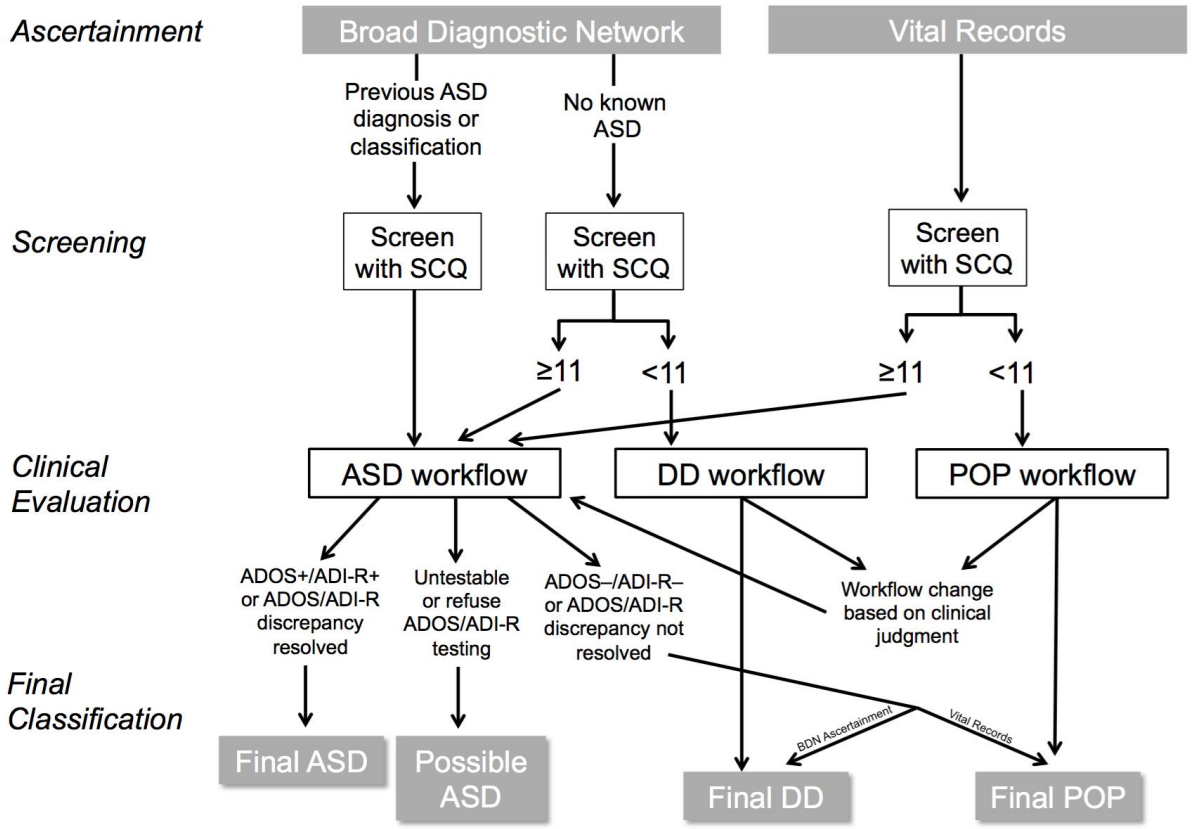


Figure 3.1. SEED recruitment and final classification of participants.

The general development of all children was assessed in the clinic using the Mullen Scales of Early Learning (184). All parents also completed several instruments to characterize their child's development including the Child Behavior Checklist (185) and the Social Responsiveness Scale (186). All children in the ASD workflow, and those in DD or POP workflow with an Early Learning Composite score of ≤78 on the Mullen Scales, also completed the Vineland Adaptive Behavior Scales (187). If the clinician suspected that a child in the DD or POP had possible ASD at the time of evaluation, the child was moved to the ASD workflow and the ADOS/ADI-R was administered.

All SEED clinicians have advanced degrees in developmental pediatrics, developmental psychology, clinical psychology and related fields and experience with the assessment and

diagnosis of children with ASD (188). Clinicians who administered the ADI-R and ADOS participated in pre-data collection exercises to establish reliability, at least quarterly exercises to maintain reliability, and at least yearly exercises to verify administration fidelity (188).

Table 3.1. Instruments administered in SEED and criteria for ASD classification

Instrument	**Domains**	**Criteria**
Autism Diagnostic Observation Scale-2 (ADOS) (181, 182)	Social effect Restricted interests and repetitive behaviors (RRB)	Module 1 with no words=11 Module 1 with some words=8 Module 2 less than 59 months=7 Module 2 more than 59 months=8 Module 3=7
Autism Diagnostic Interview- Revised (ADI-R) (183)	Social Communication RRB	Standard: Social=10; Communication=8 for verbal children or 7 for nonverbal children; RRB=3 Relaxed (when child meets the ADOS criteria but not standard ADI-R) Social=10 and Communication=6 for verbal children or 5 for nonverbal children; Communication=8 for verbal children or 7 for nonverbal children and RRB=8; Social=10 and RRB=2

DD and POP comparison group ascertainment

Two different comparison groups were included in SEED: 1) children with developmental delays or disorders other than ASD (DD), and 2) children sampled from the general population (POP). Comparisons between the ASD and POP group identify risk factors in children with ASD relative to children from the general population. Comparisons between the ASD and DD group can identify unique risk factors to ASD independent of risks common to other developmental disorders.

Children with an SCQ <11 were grouped according to their recruitment source, with those entering through the Broad Diagnostic Network placed in the DD workflow and those sampled from vital records placed in the POP workflow. If the clinician suspected that a child in the DD or POP workflow had possible ASD at the time of in-person evaluation, the child was moved to the ASD workflow and the ADOS/ADI-R was administered. Otherwise, they were

classified as Final DD or Final POP. Children in the ASD workflow that were ADOS and ADI-R negative or had a discrepancy in their ADOS/ADI-R that could not be resolved were classified as Final DD if recruited from the Broad Diagnostic Network, or as Final POP if recruited from vital records.

The DD group includes children that may have a previous ASD diagnosis or with observed or reported ASD characteristics. Sub-classifications were created in the DD group to designate children that fall into these categories. For the purposes of this analysis, these children are excluded in order to have a DD comparison group that is distinct from ASD and more representative of other developmental disorders. While children with these characteristics may also be found in the POP group, all POP children are included to maintain its representativeness of a true population control group.

D. Exposure assessment

Exposure assessment included data from available sources: medical record (including hospital stays and clinic visits) and maternal report (from interview and health history forms). This is described in more detail in more detail below.

Medical records

At enrollment, all participants were mailed medical records release forms. Once the signed release forms were obtained, hospital and clinic medical records were obtained from the prenatal, labor/delivery, neonatal, and pediatric periods, from which data was abstracted into four corresponding data collection forms by trained data abstractors. Quality control steps were implemented including training exercises for data abstraction, and testing initial and ongoing quality control standards for all data collected from medical records. Data from the medical records were abstracted to multiple forms arranged by topic such as Prenatal Care, Labor and

Delivery, Neonatal/Birth Hospital, and Pediatric. Questions used for this analysis from these forms are described below in Tables 3.2, 3.4, and 3.5 below.

Caregiver interview (CGI)

Following enrollment, the caregiver was interviewed via telephone by a trained interviewer with computer assistance. If the mother was the caregiver being interviewed, questions were asked regarding sociodemographic information, maternal health history and medical conditions, and regarding the pregnancy with the index child including complications, and conditions or complications during pregnancy, delivery and the early postnatal period. Questions from the CGI that are used for this analysis are included below in Tables 3.2, 3.4, and 3.5 below.

Autoimmune disease survey

Following the caregiver interview, a questionnaire packet was mailed to the caregiver. Two of the forms included were a family autoimmune disease history survey, in which the mother would indicate whether she has ever had an autoimmune condition and her age of onset. Gestational diabetes and diabetes (either requiring insulin or with no insulin use) were included in this form. Data used for this analysis from this questionnaire are described in Tables 3.2, 3.4, and 3.5 below.

Diabetes in pregnancy

Mothers with diabetes in pregnancy include those with a diagnosis of gestational diabetes (GDM), Type I diabetes (T1DM), Type II diabetes (T2DM), or diabetes-not otherwise specified (diabetes- NOS). Any report of diabetes in the caregiver interview, the autoimmune disease survey, or the prenatal medical record, is used to classify diabetes. In those with a date of diagnosis available, a new diagnosis of diabetes-NOS before the 20^{th} week of pregnancy was

classified as T2DM. A new diagnosis of diabetes-NOS at or beyond 20 weeks of gestation (in those with a date of diagnosis available) is classified as GDM. In addition, results from an oral glucose tolerance test (OGTT) are used when available to identify women with GDM (see below). Because it is often difficult to distinguish undiagnosed T2DM and GDM, we have a broad exposure category of 'Any diabetes' that includes GDM, T2DM, T1DM, and diabetes-NOS. Mothers previously diagnosed with diabetes with an active diagnosis in their prenatal medical record are also included.

Table 3.2 describes the sources of data and specific information collected regarding maternal diabetes. From these sources we determined if the mother had diabetes during or before her pregnancy, the type of diabetes diagnosed, results from glucose tolerance tests during the pregnancy, and if she was taking medications for the diabetes.

Table 3.2. Sources of data in SEED pertaining to diabetes in pregnancy

Data Source	Condition	Section *Variable Name*	Question	Possible Responses
Maternal Interview	Diabetes, not specified	CGIE2727_MedCondition *MI_E27_DIB*	Did you take any medications or receive any treatment for diabetes during the time period of 3 months before pregnancy and index child's date of birth?	0 = 'No' 1 = 'Yes' Missing
	GDM	CGIF0101_Intro *MI_F1_GDB*	Did you have gestational diabetes during your pregnancy with index child?	0 = 'No' 1 = 'Yes' Missing
Autoimmune Disease Survey	GDM	AID_Condition_09_16 *AI_GDI_MX*	Gestational diabetes in the mother?	0 = 'No' 1 = 'Yes'
		AI_GDIAGE_MX	Age of onset of GDM Numeric (years)	Restricted to 1 yr before age at birth
	Diabetes requiring insulin	AID_Condition_09_16 *AI_DBI_MX*	Diabetes requiring insulin in the mother?	0 = 'No' 1 = 'Yes'
		AI_DBIAGE_MX	Age of onset of diabetes Numeric (years)	Restricted to 1 yr before age at birth
	Diabetes with no insulin	AID_Condition_09_16 *AI_DBN_MX*	Diabetes with no insulin use?	0 = 'No' 1 = 'Yes'
		AI_DBNAGE_MX	Age of onset of diabetes Numeric (years)	Restricted to 1 yr before age at birth
Prenatal Medical Record	GDM	MRA_PRE_C_PregHistoryDetail1 *PR_C10L9_GEST*	Complications with mother–GDM?	0 = 'No' 1 = 'Yes'
		PR_C_GEST	Indicate whether GDM occurred in index pregnancy or one or more past pregnancies	1='Index' 2='Past' 3='Both'
	GDM Diabetes, all types;	MRA_PRE_E_BloodType *PR_E9A_FBS* *PR_E9A_OTHSP*	Glucose tolerance tests- FBS	1 = 'NL (1)' 2 = 'ABNL (2)' Missing

	GDM	*PR_E9C_1HOUR* *PR_E9C_OTHSP*	Glucose tolerance tests- 1 hour	1 = 'NL (1)' 2 = 'ABNL (2)' Missing 30 = 'Type I Diabetes' 33 = 'GDM' 32 = 'Diabetes-NOS' 31 = 'Type II Diabetes
		PR_E9D_2HOUR *PR_E9D_OTHSP*	Glucose tolerance tests- 2 hour	
		PR_E9E_3HOUR *PR_E9E_OTHSP*	Glucose tolerance tests- 3 hour	
		MRA_PRE_M_Med Cond *PR_MA_COND*	Medical conditions during pregnancy and date or age at diagnosis	
	Diabetes, not specified	MRA_PRE_Q_All Meds *PR_QB_CODE*	Medications taken during index pregnancy	2 = 'Antidiabetes (10)'

GDM was also classified based on results from an oral glucose tolerance test (OGTT). The OGTT is typically performed during 24-28 weeks of gestation, but may be used as early as the 20th week to diagnose gestational diabetes. The results from the OGTT are recorded as "Normal" or "Abnormal" for each hour that blood glucose was measured. Two abnormal measurements are needed to classify the mother as having gestational diabetes. In the instances where "Normal" or "Abnormal" are not specified in the medical record, the numerical value under "Other" for the OGTT are used if available. To classify a woman as having GDM, these results from the OGTT must meet criteria for guidelines published by Carpenter and Coustan (189) (see Table 3.3), that are recommended by the American Diabetes Association (ADA) (190) and the American Congress of Obstetricians and Gynecologists (ACOG) (191). In some clinical practices, a glucose screen may be conducted to identify women at high risk for diabetes who should be followed-up with an OGTT. However, glucose screens are not used for diagnosis of GDM, the standard is the use of OGTT.

There are two types of OGTT, one uses a 100-g oral glucose load and measures blood glucose at fasting baseline and again over three hours, and the other uses a 75-g oral glucose load

and measures blood glucose at fasting baseline and again over two hours. At least two of the glucose measurements in Table 3.3 must be met or exceeded in order to fulfill criteria for GDM. Mothers with a previous history of diabetes that is not active during the pregnancy with the index child were not excluded from the analysis and were classified as unexposed.

Table 3.3. Criteria for diagnosis of GDM

	100-g oral glucose load		75-g oral glucose load	
	mg/dL	mmol/l	mg/dL	mmol/l
Fasting	95	5.3	95	5.3
1-hour	180	10.0	180	10.0
2-hour	155	8.6	155	8.6
3-hour*	140	7.8		

Hypertension in pregnancy

A mother was classified as having hypertension if she reported it during the caregiver interview or if there was a diagnosis in the medical record of a hypertensive disorder during pregnancy such as pregnancy-induced hypertension (PIH), preeclampsia, eclampsia, or hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Serial blood pressure measurements recorded during clinic and hospital visits were abstracted from the medical record when available. Blood pressure measurements were also used to identify women with PIH (see below).

Table 3.4 describes the data collected regarding maternal hypertension during pregnancy. We used medical record and maternal report to determine the onset of hypertension before or during pregnancy, the type of hypertension diagnosed, and if the mother used anti-hypertensive medications.

In addition to the broader category of 'Any Hypertension,' mothers were classified into three subclassifications of hypertensive disorders: 1) preexisting or chronic hypertension, 2) pregnancy-induced hypertension, or 3) preeclampsia, eclampsia, or HELLP. The first and last categories were allowed to overlap as mothers with chronic hypertension before pregnancy can develop preeclampsia, eclampsia, or HELLP. However, there was no overlap with PIH and the

other subclassifications. Mothers with a previous history of hypertension that was not active during the pregnancy with the index child were not excluded and thus classified as unexposed.

Table 3.4. Sources of data in SEED pertaining to hypertension in pregnancy

Data Source	Conditions	Section *Variable Name*	Question	Responses
Maternal Interview	High blood pressure, not specified	CGIE2727_Med Condition *MI_E27_HBP*	Did you take any medications or receive any treatment for high blood pressure?	0 = 'No' 1 = 'Yes' Missing
	Eclampsia	CGIF0101_Intro *MI_F1_ECLM*	Did you have eclampsia during your pregnancy?	0 = 'No' 1 = 'Yes' Missing
	PIH/Preeclampsia	*MI_F1_HTN*	Did you have pregnancy-induced hypertension or preeclampsia during your pregnancy?	0 = 'No' 1 = 'Yes' Missing
	HELLP syndrome	*MI_F1_HELP*	Did you have HELLP syndrome during your pregnancy?	0 = 'No' 1 = 'Yes' Missing
Maternal Medical History Form	High blood pressure, not specified	MMH_CondInfo2 *MM_21_HBPX*	Did you have the condition during pregnancy with the study child? – High blood pressure	0=’No’ 1=’Yes’ Missing
		MM_43_OTH	Other? Specify.	Write-in string
Prenatal Medical Record	Eclampsia, HELLP syndrome, PIH/ Preeclampsia/ Gestational hypertension	MRA_PRE_C_PregHistory Detail1 *PR_C10L7_ECL*	Complications with mother – Eclampsia?	0 = 'No' 1 = 'Yes'
		PR_C10L10_HELLP	Complications with mother – HELLP	0 = 'No' 1 = 'Yes'
		PR_C10L19_PIH	Complications with mother – pregnancy induced hypertension, preeclampsia, gestational hypertension	0 = 'No' 1 = 'Yes'
		MRA_PRE_C_Comp *PR_C_ECL*	Indicate whether GDM occurred in index pregnancy or one or more past pregnancies	1='Index' 2='Past' 3=’Both’
		PR_C_HELLPL		
		PR_C_PIH		

	Gestational hypertension	MRA_PRE_D_MaternMeasDetail1 PR_D4H_SBP PR_D4I_DBP	D4. Blood pressure – Systolic and Diastolic	2 or more elevated measurements
	Eclampsia, HELLP syndrome, Preexisting hypertension, PIH/ preeclampsia	MRA_PRE_M_Med Cond *PR_MA_COND*	Medical conditions during pregnancy and time period	36= Eclampsia 48= HELLP 55= Pre-existing Hypertension 79= PIH/ Preeclampsia

Pregnancy-induced hypertension is be defined according to ACOG guidelines as having a systolic blood pressure ≥140 mmHG and/or a diastolic blood pressure ≥90 mmHg on at least two occasions that was 1) at least four hours apart (in order to minimize error) 2) taken after the 20th week of pregnancy, and 3) meeting criteria for being previously normotensive with no signs of proteinuria (192). Mothers who are diagnosed with preeclampsia (with or without signs of proteinuria or the development of end-organ dysfunction) are defined as having hypertension as the above for PIH, plus must have evidence of 1) proteinuria or abnormal protein levels in the urine, or 2) taking Magnesium for prevention of seizures.

Neonatal jaundice and hyperbilirubinemia

Unlike hypertension and diabetes, a diagnosis of neonatal jaundice may not require a formal diagnostic test even though it is caused by elevated levels of bilirubin in the blood. Jaundice is often identified by the yellowing appearance of the skin and sclera of the eyes. Some methods used to diagnose jaundice, such as blanching the skin with pressure to reveal underlying skin tone and using an Ingram icterometer (pressing and matching one of 5 shades of transparent plastic against an infant's skin), can be subjective and not accurately representative of bilirubin levels in the blood. However, clinicians often rely on these methods because of convenience and

quick interpretation, particularly when severe jaundice or prolonged hyperbilirubinemia is not suspected. Directly measuring bilirubin in the blood is ideal, but is highly dependent on the timing of the bilirubin measurement. If the blood test is not done at the time jaundice was visible, it may not be indicative of the highest levels of bilirubin the infant was exposed to.

For this analysis, neonatal jaundice is defined as 1) maternal report of neonatal jaundice or treatment for neonatal jaundice in the CGI, and/or 2) diagnosis of jaundice or record of treatment for jaundice within the first 28 days of life in the neonatal or pediatric medical record. Treatment for neonatal jaundice includes phototherapy (bili light or blanket) or exchange transfusion in severe cases. Bilirubin levels are also used to identify jaundice and to identify infants who were at high risk for hyperbilirubinemia during the neonatal period (see below).

Table 3.5 describes the sources of data that were used to identify children who had jaundice or high bilirubin levels. Variables for jaundice and bilirubin levels were created based on the questions in Table 3.5 as described below.

Table 3.5. Sources of data in SEED pertaining to neonatal jaundice

Data Source	Conditions		Question
Maternal Interview	Jaundice	CGIF0816_ObstCompDeliv *MI_F15_JAUN*	F15. During or after delivery of you child, did any of the following occur to your child? (09= Jaundice)
	Jaundice treatment	*MI_F16_PHOTO*	F16. Did your child receive phototherapy or bili lights, bili blanket, or special lights?
Neonatal Medical Record	Hyperbilirubinemia	MRA_Neo_G_Bilirubin *NE_GB1_TOTAL*	Bilirubin levels (see Table 3.2 for cut-offs)
	Hyperbilirubinemia treatment	*NE_GC1_IV* *NE_GC2_PHOTO* *NE_GC3_EXCH*	Treatment of hyperbilirubinemia (including phototherapy, exchange, IV)
	Hyperbilirubinemia/ jaundice	MRA_Neo_J_MedHistory *NE_JA_MEDHX*	Medical history of (145=hyperbilirubinemia/jaundice)
	Hyperbilirubinemia	MRA_NEO_Q_Blood_Product *NE_Q2C_HYPBIL*	Q2c. Blood product transfusion for hyperbilirubinemia
Pediatric Medical Record	Hyperbilirubinemia/ jaundice	MRA_Peds_C_MedHist *PD_CA_CODE*	J1. Medical history (145=hyperbilirubinemia/jaundice)

In addition to the broader category of 'Any neonatal jaundice,' infants were classified into three subclassifications of neonatal jaundice defined as 1) Definite, if treatment for neonatal jaundice was reported in either the medical record or maternal interview, 2) Probable, if a diagnosis of jaundice was available in the medical record, but treatment was not reported, or 3) Possible, if neonatal jaundice was only reported through maternal interview.

Hyperbilirubinemia, defined as a total serum bilirubin measurement above the 95th percentile, was classified according to the Bhutani nomogram (Figure 3.2). The Bhutani nomogram is used among infants born ≥35 weeks gestation and evaluates the measurement relative to the age of the infant (in 12-hour increments). The nomogram defines 95th percentile,

75th percentile, and 40th percentile tracks for bilirubin measurements and designates the zones above as high-risk, high-intermediate risk, and low-intermediate risk (respectively) for any significant hyperbilirubinemia (subsequent measurement in the 95th percentile).(173, 193) We created mutually exclusive subclassifications based on the Bhutani recommended cut-offs (Table 3.6) and defined the referent group as infants with no bilirubin measurement or with bilirubin measurements below the 40th percentile, and no neonatal jaundice indicated in the medical record or maternal interview. A variable encompassing any bilirubin level above the 40th percentile was also created to characterize infants with 'Any elevated bilirubin measurement.'

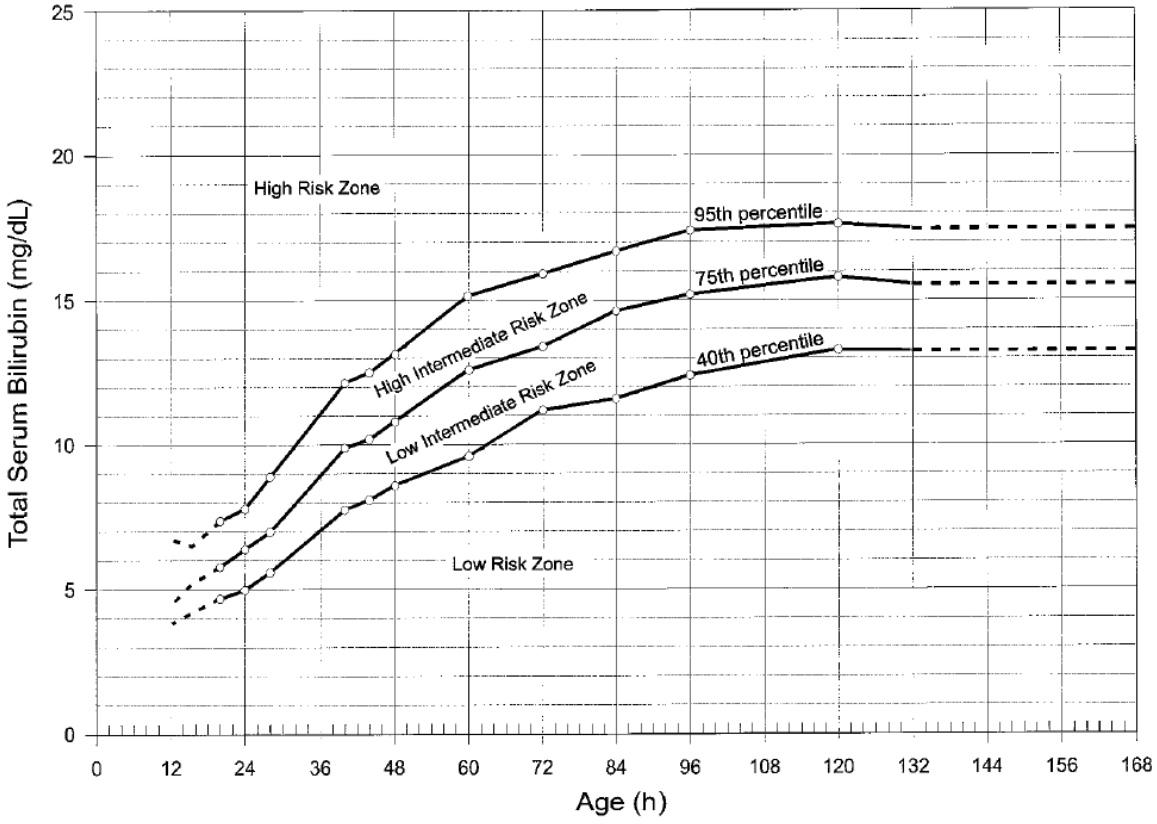


Figure 3.2. Bhutani curve for hyperbilirubinemia cut-offs (193)

Table 3.6. Bilirubin measurement cut-offs for hyperbilirubinemia and visible jaundice for infants ≥35 weeks gestational age (193)

Time since birth (hours)	Bilirubin Measurement Levels (mg/dL)		
	95th Percentile Hyperbilirubinemia	75th Percentile High-Intermediate	40th Percentile Low-Intermediate
0-12	6.7	4.5	3.75
>12-16	6.5	5.25	4.25
>16-20	7.25	5.8	4.75
>20-24	7.75	6.5	5
>24-28	8.9	7	5.5
>28-32	10	8	6.3
>32-36	11	9	7.1
>36-40	12.1	9.9	7.8
>40-44	12.5	10.1	8.1
>44-48	13.1	10.8	8.7
>48-52	13.9	11.25	8.9
>52-56	14.5	12	9.2
>56-60	15.1	12.5	9.7
>60-64	15.3	12.9	10.1
>64-68	15.7	13.1	10.7
>68-72	16	13.4	11.1
>72-76	16.1	13.8	11.3
>76-80	16.5	14.2	11.45
>80-84	16.7	14.6	11.6
>84-96	17.25	15.1	12.3
>96	17.25	15.5	13.2

Concordance between data sources

Preliminary analyses were conducted to evaluate the concordance between data from medical records and maternal report. Agreement was substantial for data regarding maternal diabetes (97% agreement, Cohen's Kappa=0.80) and maternal hypertension (90% agreement, Cohen's Kappa=0.61) during pregnancy, but fair for neonatal jaundice (69% agreement, Cohen's

Kappa=0.35) (194). We believe this may be due to mild jaundice not receiving a formal diagnosis and delayed timing of the bilirubin laboratory test. Based on this, data from either the medical record or maternal report will be used to define the exposures for Specific Aim 1. However, for Specific Aim 2, two separate analyses will be conducted: 1) using neonatal jaundice as the exposure, as reported by maternal interview or a diagnosis in the medical record, or a report of treatment for jaundice in either data source, and 2) using bilirubin measurements abstracted form the medical record, and classified into percentiles using the Bhutani nomogram.

E. Covariate assessment

A thorough literature search was conducted to identify covariates that may be potential confounders in the association of these exposures with ASD or broader developmental delays. Table 3.7 contains all of the potential confounders identified and information on the data source for these variables in SEED and contains links to references that have established an association with maternal diabetes (DM), maternal hypertension (HTN), or jaundice (JN) and ASD.

Table 3.7. Summary of covariates associated with perinatal exposures and with ASD in the child.

Covariate	**Data Source**	**Association with exposure(s)**	**Association with ASD**
Pre-pregnancy BMI	Prenatal medical record	DM: (19, 195-199) HTN: (195, 200-204)	(12, 65, 71)
Maternal race/ethnicity	Maternal interview	DM: (205-208) HTN: (202, 204, 209) JN: (210, 211)	(59, 212)
Maternal education (or other SES proxy)	Maternal interview	DM: (213) HTN: (214) JN: (211, 215) *May also be associated with maternal report*	(59, 215, 216)
Maternal age at delivery	Prenatal medical record	DM: (207, 208, 217) HTN: (202, 203) JN: (211, 218)	(59, 219, 220)
Parity	Maternal interview	HTN: (202, 203) JN: (175, 211) *May also be associated with maternal report.*	(13, 61, 175, 221, 222)
Plurality	Maternal interview	HTN: (203, 223)	(224-226)
Maternal smoking	Maternal interview	DM: (198, 208, 227-229) HTN: (204) JN: (211)	(43, 230, 231)
Infant sex	Neonatal medical record	JN: (211, 218, 232)	(233)
Infant race/ethnicity	Maternal interview	JN: (211, 218, 232)	(59, 212)
Pre-term birth	Neonatal medical record	JN: (211, 218, 234)	(8, 235-237)

DB: Diabetes; HTN: Hypertension; JN: Jaundice

F. Statistical analysis

We hypothesized that prenatal and neonatal complications would be associated with an increased risk of ASD in the child. We used multivariable logistic regression models to obtain odds ratios (OR) for having ASD vs. POP given these exposures. We evaluated the specificity of

this association by comparing these effect estimates to the ORs for DD vs. POP. We used the Mullen Scales of Early Learning Composite Standard Score of identify children with ASD and intellectual disability (ID; score <70) or ASD without ID (score ≥70) and further evaluated for differences between ASD with ID compared to ASD without ID.

For each aim, a Directed Acyclic Graph (DAG) was used to identify potential confounders based on their associations with the exposure and the outcome in the literature (238). We evaluated the association of the confounders identified through the DAG with the exposure and outcome. Those that showed no association were excluded to obtain a more parsimonious model. Confounders were included as covariates in multivariable logistic regression model for each aim. Logistic regression models were used to evaluate the association between 'Any diabetes,' 'Any hypertension,' and 'Any neonatal jaundice.' Separate models were created for sub-classifications of diabetes (GDM and preexisting diabetes), hypertension (preexisting hypertension, PIH, and preeclampsia, eclampsia, and HELLP), and neonatal jaundice (definite, probable, and possible).

Previous studies have shown interaction between BMI and maternal diabetes and hypertension, and between gestational age and neonatal jaundice. We assessed for interaction by including an interaction variable in our model and obtaining a p-value for the interaction term. An alpha level $\alpha=0.20$ was set a priori for significance of the interaction term. We also obtained stratum-specific estimates and evaluated for any departure from homogeneity and tested the assumption of a multiplicative model.

Specific Aim 1a

Factors that have been associated with the exposure of maternal diabetes in pregnancy and the outcome of ASD are shown in the DAG in Figure 3.3. These include: maternal age at

conception, maternal race/ethnicity, maternal education (as a proxy for socioeconomic status [SES]), maternal smoking (any smoking 3 months before conception or during pregnancy), and study site. Maternal hypertension and BMI are variables that depending on the onset of diabetes (which can be difficult to determine) may be confounders, mediators, or moderators. Thus, these variables were added separately into the model and we assessed for interaction between these variables and maternal diabetes. Data on all covariates was obtained from maternal interview, the prenatal medical record, or birth certificate data if the other data sources are missing.

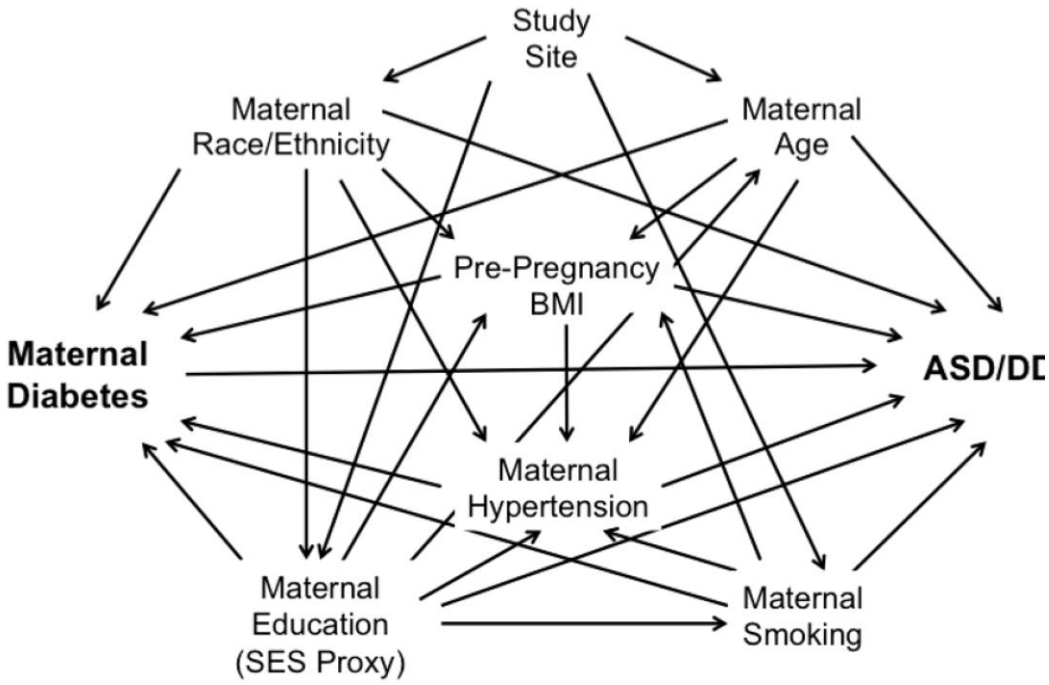


Figure 3.3. Directed Acyclic Graph (DAG) of the relationship between maternal diabetes in pregnancy in Specific Aim 1a and the outcome of ASD or DD

The exposure group of "any maternal diabetes during pregnancy" is broad and heterogeneous. It is possible that the association with ASD is stronger in mothers with preexisting diabetes or GDM. For this reason, we also present results for these two subclassifications as defined above.

Specific Aim 1b

Factors that have been associated with the exposure of maternal hypertension in pregnancy and the outcome of ASD are shown in the DAG in Figure 3.4. These include: maternal age at conception, maternal race/ethnicity, maternal education (as a proxy for SES), maternal smoking (any smoking 3 months before conception or during pregnancy), plurality, and parity. As before, maternal diabetes and BMI are variables that depending on the onset of hypertension (which can be difficult to determine) may be confounders, mediators, or moderators. Thus, these variables were added separately into the model and we assessed for interaction between these variables and maternal hypertension. Data on all covariates was obtained from maternal interview, the prenatal medical record, or birth certificate data if the other data sources are missing.

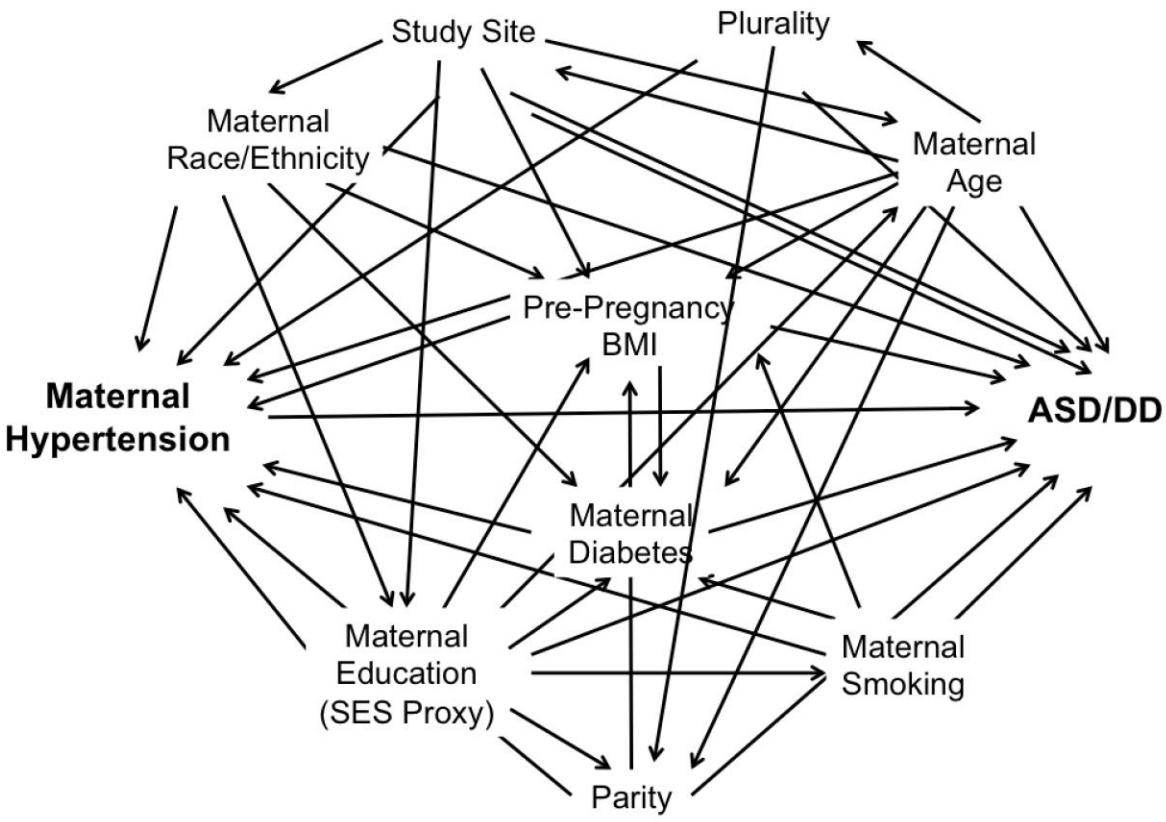


Figure 3.4. Directed Acyclic Graph (DAG) of the relationship between maternal hypertension in pregnancy in Specific Aim 1b and the outcome of ASD or DD

The exposure group of "any maternal hypertension during pregnancy" is broad and heterogeneous. It is possible that the association with ASD is stronger in mothers with preexisting hypertension, PIH, or more severe hypertensive disorders such as preeclampsia, eclampsia and HELLP. For this reason, we also present results for these three subclassifications as defined above.

Specific Aim 2

Factors that have been associated with the exposure of neonatal jaundice and the outcome of ASD are shown in the DAG in Figure 3.5. These include: maternal age at conception, maternal education, maternal diabetes during pregnancy, parity, plurality, infant race/ethnicity, infant sex, and gestational age at birth. These covariates were obtained from maternal interview, the prenatal or neonatal medical record, or birth certificate data if the other data sources are missing.

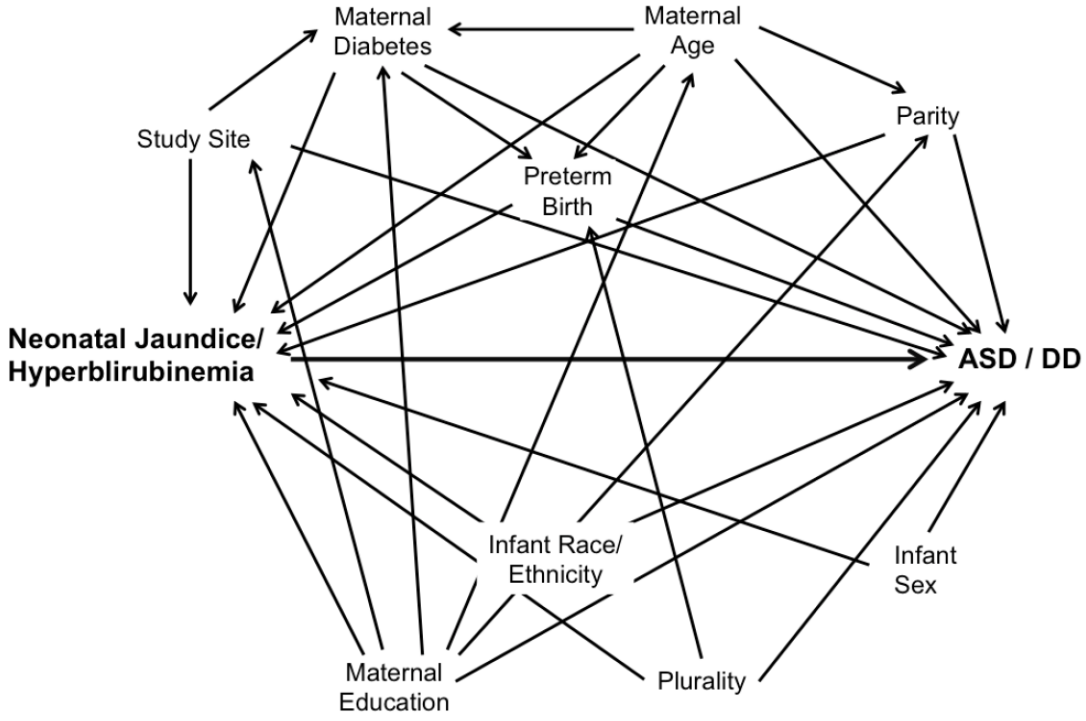


Figure 3.5. Directed Acyclic Graph (DAG) of the relationship between neonatal jaundice and hyperbilirubinemia in pregnancy in Specific Aim 2 and the outcome of ASD or DD

While the exposure variable for neonatal jaundice was inclusive of all reports of jaundice in the CGI and medical record, we also evaluated the association between subclassifications of neonatal jaundice and ASD/DD. As described above, these include 1) Definite jaundice requiring

treatment, 2) Probable jaundice with a diagnosis in the medical record, or 3) Possible jaundice only reported in the CGI. Categories for elevated bilirubin levels according to cut-offs from the Bhutani nomogram were also evaluated in association with ASD/DD.

The role of preterm birth in specific aim 2

Neonatal jaundice and ASD are more common in the preterm infant. For this reason, we carefully considered the role of gestational age in our analysis. First, we assessed for interaction using the <35 weeks gestation, 35-37 weeks gestation, and ≥38 weeks gestation categories used by the American Academy of Pediatrics for the treatment of neonatal jaundice (173). We obtained stratum-specific estimates for jaundice and ASD/DD by these gestational age strata. Because the preterm brain and liver function are less developed, we hypothesized that a stronger association would be observed among infants born <38 weeks gestation.

Sensitivity analysis

Potential misclassification of the exposure

It is possible that the exposure is misclassified due to poor maternal recall or missing medical records. Many of the variables abstracted from the medical records in SEED did not require the abstractor to enter 'No' for the condition if no diagnosis was found, only a 'Yes' if it was. This may lead to misclassification if the participant is classified as unexposed due to a 'Yes' not being entered. Mothers also reported conditions during pregnancy on the CGI up to 68 months after giving birth. For each exposure variable, there were mothers or infants that were discordant based on data from maternal report and medical record. These discordant pairs may be more likely to be misclassified. While we assume that this misclassification is non-differential, it may still have an effect on our results. To evaluate this, we created a 'Confirmed' variable for diabetes and hypertension, which required a diagnosis in both the medical record and

CGI. For jaundice, we created subclassifications of 1) Definite, if the infant received treatment for jaundice, 2) Probable, if there was a diagnosis in the medical record, or 3) Possible, if there was only a report in the CGI and no report of treatment.

Potential misclassification of a confounder

In Specific Aim 1, correct adjustment of maternal hypertension in the maternal diabetes exposure model, and vice versa, requires knowing accurate time of onset for both conditions since one condition can increase risk for the other. With maternal diabetes particularly, a mother may be in a diabetic state for a long period of time before she is screened and diagnosed. Date of diagnosis was not always available for these conditions and may not accurately represent onset. For this reason, we produced two models: 1) where we control for the other condition as confounder if present, regardless of time of onset, and 2) where we do not control for the confounder. Creating these bounds allowed us to evaluate the effect of confounder misclassification on our results.

G. Power calculations

Statistical power was calculated using Stata and a Type 1 error rate of $\alpha=0.05$. Only the ASD and POP groups were used for these calculations. Our analysis included 699 cases of ASD and 979 population controls, for a total sample size of 1672. In the diabetes exposure group, there are a total of 139 mothers exposed according to the CGI. The probability of being exposed was $Pr(X=1)=139/1533=0.083$ and the probability of having the outcome of ASD in the unexposed group was $Pr(Y=1 \mid X=0)=630/1533=0.41$. In the hypertension in pregnancy exposure group, there are a total of 265 mothers exposed according to the CGI. The probability of being exposed was $Pr(X=1)=265/1667=0.159$ and the probability of having the outcome of ASD in the unexposed group was $Pr(Y=1 \mid X=0)=554/1402=0.40$. In the neonatal jaundice exposure, there are a total of 753 infants exposed according to the CGI. The probability of being exposed was

Pr(X=1)=753/1678=0.449 and the probability of having the outcome of ASD in the unexposed group was Pr(Y=1 l X=0)=358/925=0.39. The statistical power achieved by minimum effect sizes is provided in Table 3.8. At some effect sizes, the statistical power is below adequate. This is common when studying rare exposures. While we may be limited in making causal inferences at this level, the associations that are detected and patterns in the data can still be very informative. When we included diagnosis of these complications by medical record and abstracted measurements, the number exposed almost doubled.

Table 3.8. Statistical power obtained to detect an association with ASD by effect size for the exposures of maternal diabetes, maternal hypertension, and neonatal jaundice according to CGI

	Maternal Diabetes			Maternal Hypertension			Neonatal Jaundice		
Minimum Effect Size	Pr(x=1)	Pr (Y=1lX=0)	Statistical Power	Pr(x=1)	Pr (Y=1lX=0)	Statistical Power	Pr(x=1)	Pr (Y=1lX=0)	Statistical Power
OR=1.25	0.083	0.41	24%	0.159	0.40	38%	0.449	0.39	61%
OR=1.5	0.083	0.41	63%	0.159	0.40	86%	0.449	0.39	98%
OR=1.75	0.083	0.41	88%	0.159	0.40	99%	0.449	0.39	~100%
OR=2.0	0.083	0.41	97%	0.159	0.40	~100%	0.449	0.39	~100%

CHAPTER 4: MATERNAL DIABETES AND HYPERTENSIVE DISORDERS IN ASSOCIATION WITH AUTISM SPECTRUM DISORDER

A. Introduction

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders characterized by deficits in social interaction and communication, as well as restrictive and repetitive patterns of behaviors or interests (239). Since the 1990's, the documented prevalence of ASD has steadily increased (240). This may be due in part to better diagnosis and increased awareness (241-243). The most recent statistics estimate that one in 68 children aged 8 years is affected with ASD in the U.S. (244). The etiology of ASD remains unknown, but it is complex and suspected to involve both genetic and environmental factors (2, 3). Recent studies provide evidence for the prenatal period as a sensitive time window for the development of ASD (4, 245, 246). Pregnancy complications have been frequently reported in association with the development of ASD in the child, (43, 68, 71) with the hypothesis that conditions associated with altered fetal nutrient delivery and increased inflammation and oxidative stress can have deleterious effects on *in utero* brain development.

Maternal diabetes and hypertension are the most common complications of pregnancy. With the onset of the obesity epidemic and increasing maternal age in the U.S., the number of pregnancies affected by diabetes and hypertension has also increased (17, 247). A few studies have shown a positive association between maternal diabetes and hypertension and the development of ASD in the child (12, 62, 67, 71, 163, 165-167, 248). However, some studies did not model the conditions independently and were limited by small sample sizes. In this analysis,

we independently examine maternal diabetes and hypertension using data from a large epidemiologic investigation of etiologic risk factors for ASD.

B. Methods

Study population

Participants were from the Study to Explore Early Development (SEED), a multisite case-control study with multiple-source ascertainment of children with ASD, children with developmental delays or disorders other than ASD (DD) and children sampled from the general population (POP) (26). Children in the ASD and DD groups were ascertained through multiple agencies and clinical settings that evaluate or serve children with developmental challenges. From these sources, we selected children with select special education or International Classification for Disease (ICD) codes indicative of autism/ASD or other developmental challenge often seen as precursor or co-occurring diagnoses in children eventually diagnosed with ASD. This broad diagnostic net for ascertainment ensures that previously diagnosed and undiagnosed ASD children were identified (26). State birth records were randomly sampled to identify children from the general population (POP group) who were born in the same time period and catchment area as children with ASD.

SEED required eligible children to have been born in and currently (at time of study enrollment) reside in one of six study catchment areas (located in California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania) (26). Children also had to be between the ages of 30 and 68 months of age at the time of in-person assessment, born between September 1, 2003 and August 31, 2006, and live with a knowledgeable caregiver who was at least 18 years of age, spoke English (all sites) and/or Spanish (California and Colorado), and was able to provide legal consent. SEED recruited 3,769 participants. For this study, we restricted analyses to the 2,564

participants who had a final outcome classification of ASD, DD, or POP and had data available on pregnancy complications.

Data collection

The SEED data collection protocol is detailed elsewhere (26). SEED collected data on family medical history, maternal reproductive health and pregnancy outcomes, and child development and behavioral characteristics through telephone interviews, self-administered forms, an in-person child developmental assessment, collection of biosamples, and abstraction of maternal and child medical records. Limited birth certificate data were also obtained for all enrolled participants; however, birth certificate data did not include information on pregnancy complications. Data collection was standardized across sites and subject to uniform standards for quality data checks.

Outcome assessment

All children enrolled in the study were initially screened for autism symptoms using the Social Communication Questionnaire (SCQ) (180). As part of the data collection protocol, children were administered developmental assessments in-person by a trained clinician and were given a more extensive assessment if they screened positive for possible ASD (SCQ score >11), were determined to have a previous ASD diagnosis, or were suspected to have ASD based on the study clinician's direct observation. The more extensive assessment of the child included the Autism Diagnostic Observation Schedule (ADOS)(181); additionally, their caregivers were administered the Autism Diagnostic Interview-Revised (ADI-R) (183). Final classification of ASD (yes or no) was based on the findings from these two developmental assessments regardless of previous diagnoses (188). More detail about the SEED outcome assessment is described elsewhere (26, 188).

Ascertainment of maternal hypertension and diabetes

Multiple SEED instruments included information on maternal hypertension and diabetes: 1) maternal prenatal care and delivery records; 2) 60-minute telephone interview administered to the caregiver that included questions on her health during the index pregnancy; and 3) self-administered checklists of the mother's medical history both before and during pregnancy, used specifically for diabetes. The mother was classified as having the condition if it was reported anywhere in the prenatal medical record or through maternal self-report (either telephone interview or on self-checklist). Overall, maternal prenatal care and delivery record data were available for 67.3% of study subjects, and maternal self-report data were available for 99.5%.

Restricting to the 1,711 subjects that had both medical record and self-reported data available, substantial or high agreement was found for both maternal diabetes (κ=0.85 95% CI 0.80, 0.90) and maternal hypertension (κ=0.67 95% CI 0.61, 0.73), based on Landis and Koch criteria (249). We thus decided it was appropriate to classify the mother as having the condition based on only one data source. Additionally, if the mother reported having the condition by both medical record and maternal report, or if the medical record included details establishing that criteria on a diagnostic test were met, the mother was further classified as having a 'Confirmed' condition (see below).

Using any data source, 246 mothers were classified as having diabetes during pregnancy. From these, 45 (18.3%) were classified as having diabetes only by maternal report and 15 (6.1%) only by prenatal medical record. Mothers in the diabetes group were classified as having either gestational diabetes (GDM) or pre-existing diabetes (including Type 1 and Type 2 Diabetes Mellitus). These subclassifications are mutually exclusive. In addition to reported diagnoses of GDM, results from oral glucose tolerance tests (OGTT) in the prenatal medical record were used

to classify the mother as having GDM. Clinical guidelines by Carpenter and Coustan requiring two elevated measurements in the OGTT were used (189). Four mothers (1.6%) were classified as having diabetes only by OGTT criteria. Mothers were classified as having 'Confirmed' diabetes if they met criteria on the OGTT or reported diabetes through both self-report and medical record. If only one data source specified a diabetes type but the other confirmed a non-specified type of diabetes or use of antidiabetic medications (Insulin, Glyburide, or Metformin), then the mother was also classified as having 'Confirmed' diabetes through both sources. We defined 'No Diabetes' as not having a diagnosis in the medical record and no maternal report of diabetes.

Additionally, using any data source, 386 mothers were classified as having a hypertensive disorder during pregnancy. From these, 102 (26.4%) were classified as having a hypertensive disorder only by maternal report and 47 (12.2%) only by prenatal medical record. Mothers in the hypertension group were further classified as having pre-existing chronic hypertension, pregnancy induced hypertension (PIH), or a more severe hypertensive disorder of pregnancy, including preeclampsia, eclampsia or hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome. Subclassifications were not mutually exclusive as mothers may have pre-existing chronic hypertension and then go on to develop preeclampsia, eclampsia, or HELLP syndrome in pregnancy. In addition to reported diagnoses, serial blood pressure measurements from clinic and hospital visits were used to classify a woman as having a hypertensive disorder, depending on the timing of these measurements. Clinical guidelines requiring two separate elevated measurements (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) were used to classify the mother as having pre-existing chronic hypertension (if <20 weeks gestational age), PIH (if >20 weeks gestational age), or preeclampsia (if ≥20 weeks

gestational age and accompanied by report of protein in urine or use of Magnesium) (192). Forty-one (10.6%) mothers were classified as having hypertension only by blood pressure measurement criteria. Mothers were classified as having 'Confirmed' hypertension if they met the blood pressure measurement or reported a hypertensive disorder through both self-report and medical record. We defined 'No Hypertension' as not having a diagnosis for a hypertensive disorder in the medical record and no maternal report of a hypertensive disorder.

Covariates

We conducted a thorough literature search to identify covariates that may be potential confounders in the association of these maternal conditions with ASD or broader developmental delays. For each condition, a Directed Acyclic Graph was used to identify potential confounders in order to obtain the least biased estimates (238). Data from birth certificates, prenatal medical records, and caregiver phone interview were used to define covariates. Odds ratios and 95% confidence intervals were obtained for the effect of each covariate on both the maternal condition and the outcome to confirm an association.

Confounders included maternal age at conception (<35 [referent], ≥ 35 years), maternal race/ethnicity (white non-Hispanic [referent] vs. other race/ethnicity), maternal education (high school degree or less vs. some college or more [referent]), maternal smoking during pregnancy (any vs. none [referent]), and study site (Georgia as referent). For hypertension, parity (categorized as first birth [referent], second birth, third or more) and plurality (dichotomized as singleton [referent] vs. multiple) were included as additional confounders. For both maternal conditions, other potential confounders included having the other condition (diabetes or hypertension) and pre-pregnancy body mass index (BMI), categorized using World Health Organizations levels for low or normal BMI (referent; <25 kg/m^2), overweight (≥ 25 - 30 kg/m^2),

and obese ($\geq$ 30 kg/m^2). However, because the exact onset of the maternal condition is unknown, it is possible that one maternal condition could affect or be affected by the occurrence of the other condition or by weight gain; therefore, we treated the other condition and BMI as potential confounders (added separately in the adjustment set), also as potential mediators (excluded from the adjustment set), and evaluated for effect measure modification.

Statistical analysis

We calculated distributions of maternal characteristics by child outcome and maternal condition. Multivariable logistic regression models estimated odds ratios (OR) and 95% confidence intervals (CI) for the association between maternal conditions and either ASD (vs. POP) or DD (vs. POP), adjusting for all confounders. We further adjusted for high pre-pregnancy BMI and diabetes or hypertension. In unadjusted models, we used the Breslow-Day test for homogeneity of the odds ratio with an alpha level α=0.20 for interaction. In adjusted models, we obtained stratum-specific effect estimates by BMI category for diabetes and hypertension in association with ASD and DD, and tested the assumption of a multiplicative model.

Analyses were replicated in separate samples including only those with a confirmed condition and also for subclassifications of the condition. Effect estimates were also obtained for ASD with and without intellectual disability (ID) vs. POP, as previous studies have concluded that they may be etiologically distinct (167). A Mullen Early Learning Composite Standard Score of <70 was used to identify ASD children with ID. All analyses were conducted using SAS statistical software, version 9.3 (SAS Institute Inc., Cary, NC).

C. Results

This analysis comprises a sample of 2,564 mother-child pairs enrolled in SEED. The study sample included 698 children with ASD, 887 with a non-ASD DD, and 979 from the POP group. Compared to our analytic sample, the excluded subset (n=1,062) was less likely to be non-White and less likely to have attained a higher education. Mothers of children with ASD and DD were older, less likely to be White, less educated, and more likely to smoke during pregnancy, have higher pre-pregnancy BMI, or deliver a multiple birth, compared with POP mothers (Table 4.1). They were also more likely to have diabetes or a hypertensive disorder, compared with POP mothers. Mothers with diabetes were older, less likely to be White, less educated, and more likely to smoke during pregnancy and have higher pre-pregnancy BMI. Mothers with hypertension were less educated and more likely to smoke, have higher pre-pregnancy BMI, be primiparous, and pregnant with multiples.

There were 246 mothers (9.6%) with diabetes during pregnancy; 65 (2.6%) were classified as having pre-existing diabetes and 181 (7.1%) with GDM (Table 4.1). Hypertensive disorders were present during pregnancy for 386 mothers (15.1%), with 88 (3.4%) classified as pre-existing chronic hypertension, 122 (4.8%) with PIH, and 206 (8.0%) with more severe hypertensive disorders of pregnancy such as preeclampsia, eclampsia, and HELLP syndrome (Table 1). Using the criteria for 'Confirmed' condition described above, 151 (5.9%) mothers had confirmed diabetes and 231 (9.0%) mothers had a confirmed hypertensive disorder during pregnancy.

In unadjusted analyses, we did not observe an association between maternal diabetes and ASD (OR=1.24 [0.89, 1.75]) (Table 4.2). In contrast, maternal diabetes was associated with DD (OR=1.47 [1.07, 2.00]). Adjustment for confounders attenuated the effect estimates in both

groups (ASD aOR=1.10 [0.77, 1.56]; DD aOR= 1.32 [0.96, 1.82]). Additional adjustment for BMI and/or hypertensive disorders further attenuated the effect estimates, but did not materially alter the results. In subgroup analyses, a stronger association was observed with 'Confirmed' diabetes, particularly in the DD group. Additionally, while stronger associations with ASD and DD were observed for mothers with GDM versus pre-existing diabetes, these estimates were imprecise with few mothers having pre-existing diabetes.

We observed associations between hypertensive disorders in pregnancy and ASD (OR=1.78 [1.35, 2.35]) and DD (OR=1.67 [1.28, 2.18]) in unadjusted analyses (Table 4.2). After adjustment for confounders, effect estimates were slightly attenuated for ASD, but not for DD (ASD aOR=1.67 [1.25, 2.23]; DD aOR=1.69 [1.29, 2.23]) and for both remained elevated with narrow confidence intervals above the null. Additional adjustment for BMI and/or diabetes did not notably alter effect estimates. In subgroup analyses, stronger associations were observed with a 'Confirmed' condition, particularly in the ASD group. A stronger association was also observed for pre-existing chronic hypertension and DD, but not for other sub-classifications of hypertensive disorders.

Similar associations were generally observed for maternal diabetes and ASD with ID (vs. POP) compared to ASD without ID (vs. POP) (Table 4.3). Associations with hypertensive disorders also did not vary greatly among those with ASD with or without ID, remaining significant for the broader group, but with imprecise confidence intervals for the pre-existing hypertension and PIH subclassifications due to a smaller sample.

We did not find any evidence of interaction between covariates and the maternal conditions of diabetes and hypertensive disorders in association with ASD and DD. We evaluated BMI stratum-specific estimates and did not find any departure from multiplicativity in

adjusted models (Table 4.4). When we added an interaction term between BMI category and the maternal condition, we did not find interaction at $p<0.20$. Additionally, we did not find interaction between the maternal conditions (results not shown). However, confidence intervals were very wide due to the small sample size of mothers with both conditions.

D. Discussion

We did not observe an association between diabetes in pregnancy and ASD, but there was some suggestion of an association between prenatal diabetes and DD. We observed that hypertensive disorders during pregnancy were associated with ASD in the child. Similar associations were also observed between hypertensive disorders and DD, suggesting a more general effect on neurodevelopment rather than specific to ASD processes. Further adjustment for BMI and diabetes did not notably materially these associations for hypertensive disorders. Overall, stronger associations were seen in mothers with a 'Confirmed' condition reported in both the medical record and through maternal report, or in a diagnostic test. This may reflect some exposure misclassification in mothers without confirmed conditions, or the possibility of more severe conditions in pregnancy occurring among mothers in the confirmed group. Limiting the analyses to confirmed condition status did not change the interpretation of results for hypertensive disorders, but did strengthen the association of diabetes with DD.

There are several hypotheses regarding how maternal hypertension may affect fetal neurodevelopment, including its association with inflammation and oxidative stress.(22, 120) Oxidative stress may bring about cell necrosis or changes to the epigenome, affecting DNA methylation and gene expression (120, 123). Additionally, some markers of inflammation are known to cross the blood brain barrier (22, 49). Hypertensive disorders are also associated with altered fetal nutrient delivery and intrauterine growth restriction (148). Changes in nutrient

delivery may alter fetal metabolism and how the fetus grows and develops in utero, thereby affecting brain development.

We observed stronger associations with pre-existing hypertension, particularly with DD. This may be related to the timing of the condition during pregnancy and fetal brain development. However, some of these mothers (18%) were later diagnosed with a severe hypertensive disorder in pregnancy such as preeclampsia, eclampsia, or HELLP, so this group may be composed of mothers who had a more severe condition throughout pregnancy. Further study with a larger sample of mothers with pre-existing chronic hypertension is needed to explore this association. Unlike previous studies suggesting etiologically distinct pathways between ASD with and without ID (167), we did not observe differences in the associations for ASD with ID versus ASD without ID.

Previous studies have reported an interaction with high BMI and both maternal diabetes and hypertension (12, 166, 167). Comparing BMI stratum-specific estimates, we did not observe differences in the association among mothers with pre-pregnancy BMI in the low/normal, overweight, or obese categories. Our results suggest that weight may not play a role in the association between maternal hypertension and ASD.

Our results are generally in agreement with other case-control studies (12, 43, 52, 163), but there are subtle differences that may be due to the study population and sample size, with SEED having the largest number of affected dyads over multiple sites in the U.S.. While our results were similar to some cohort studies evaluating this association (8, 67, 71, 165), they differed from others (68, 144, 166, 167). Most large cohort studies used less detailed data from administrative datasets (68, 166) and/or birth certificates (144, 166). The somewhat rare exposures limited power in smaller studies, relative to SEED (166, 167). Additionally, SEED

confirmed ASD through a standardized, direct evaluation, instead of relying on ICD-9 codes (68, 166, 167) or school records (144) that may instead be used to refer the child for further testing and not as a final diagnosis. In contrast, all children enrolled in SEED received an in-person evaluation to confirm ASD diagnosis with the gold standard instruments, the ADOS and ADI-R. Children in the DD and POP group were also screened, reducing outcome misclassification.

The SEED population-based study design is specifically tailored to identify etiologic factors contributing to ASD. Data regarding risk factors are collected in great detail, including mailed-in questionnaires, telephone interviews, birth certificates, and medical records, ensuring that pregnancy conditions are well-captured. For this study, we also had results from OGTT and clinic and hospital blood pressure measurements. These additional data allowed us to confirm the maternal condition from medical record and maternal report, reducing the likelihood of exposure misclassification.

Although there are many strengths of this study, some limitations exist. While a number of families of potentially-eligible children, did not respond to the SEED invitation letter, analyses of data from one SEED site with the most complete data available to assess non-response indicated that maternal age, education, and race-ethnicity were associated with non-response but other pregnancy and health variables were not (unpublished analysis). We adjusted our findings for all 3 factors aforementioned demographic factors.

Although diabetes and hypertension are the most common complications in pregnancy, they are heterogeneous conditions in their timing and severity, which may affect our ability to observe significant associations. Further, our statistical power was reduced for analyses that were stratified or were conducted among subclassifications of the conditions or ASD. However, SEED is one of the few studies with the detailed data necessary to carry out these analyses. While

agreement for data from caregiver interview and medical record was substantial, there may be some exposure misclassification due to poor maternal recall or incomplete data in the medical record. Additional analyses in mothers with the condition reported in both caregiver interview and medical record showed stronger effect estimates, which may be due to misclassification when maternal and medical record data were discordant. However, it may also be that more severe conditions were more easily recognized and more frequently reported. Further, while we aimed to obtain least-biased estimates by adjusting for previously identified confounders, there may still be residual confounding by some unknown or unmeasured factors, such as maternal diet or genetic predisposition.

In summary, while we did not find an association between maternal diabetes and ASD, we did observe an association with maternal hypertensive disorders and both ASD and DD, complementing what has been reported in some studies (8, 12, 43, 52, 67, 71, 163, 165). Further exploration of the mechanism by which these associations are present is necessary. A better understanding of the role of hypertension on the etiological pathways involved in neurodevelopment can potentially lead to improved interventions in high-risk mothers and earlier identification of developmental disorders in children.

Table 4.1. Distribution of maternal characteristics during pregnancy with index child born September 2003-August 2006, by child outcome classification and maternal condition in SEED (N=2,564)

	Column percent by child outcome and maternal condition					
Maternal Characteristics	**Total Population (*N*=2,564)**	**ASD (*n*=698)**	**DD (*n*=887)**	**POP (*n*=979)**	**Diabetes (*n*=246)**	**Hypertensive Disorders (*n*=386)**
Maternal Age at Conception						
< 35 years	89.2	88.7	88.4	90.2	83.7	88.9
≥ 35 years	10.6	11.0	11.4	9.6	15.5	11.1
Maternal Race/Ethnicity						
White, non-Hispanic	63.0	55.6	61.0	70.0	58.1	64.0
Other	36.5	44.1	38.0	29.6	41.5	35.2
Maternal Education						
High school degree or less	18.3	20.2	22.4	13.3	23.6	20.2
Some college or more	81.6	79.7	77.6	86.7	76.4	79.8
Maternal Smoking						
Yes	13.2	16.2	14.1	10.2	17.5	18.1
No	86.0	82.8	85.3	89.0	81.3	81.6
Pre-pregnancy Body Mass Index						
<25 kg/m^2 (Low/Normal)	1492	58.2	54.7	54.5	64	37
25-<30 kg/m^2 (High)	603	23.5	24.5	24.9	21.6	28.9
≥30 kg/m^2 (Obese)	419	16.3	18.9	17.8	13.18	32.5

Parity						
First birth	43.7	46.7	39.5	45.5	43.5	54.4
Second	35.7	34.0	37.0	35.9	36.2	29.5
Third or more	20.1	18.8	23.1	18.3	20.3	16.1
Plurality						
Singleton	93.4	91.1	92.8	95.5	91.1	88.6
Multiple	6.6	8.9	7.2	4.5	8.9	11.4
Study Site						
California	15.4	15.3	15.0	15.5	15.5	10.9
Colorado	19.4	20.2	17.7	20.2	15.9	24.6
Georgia	19.9	19.6	21.7	18.3	18.7	18.1
Maryland	14.4	15.5	13.3	14.7	14.6	15.8
North Carolina	17.7	14.8	20.5	17.6	22.8	20.7
Pennsylvania	13.3	14.6	11.8	13.7	12.6	9.8
Diabetes in pregnancy						
No diabetes	90.3	90.3	88.6	91.8	-	81.1
Any diabetes	9.6	9.7	11.3	8.0	-	18.9
Confirmed	5.9	6.2	7.8	4.0	-	11.1
Not confirmed	3.8	3.6	3.6	4.1	-	7.8
GDM	7.1	7.5	8.3	5.7	-	11.7
Pre-existing diabetes	2.6	2.3	3.0	2.4	-	7.3
Hypertension in pregnancy						

No hypertensive disorder	84.9	81.8	82.9	89.0	70.3	-
Any hypertensive disorder	15.1	18.1	17.1	11.0	29.7	-
Confirmed	9.0	11.6	10.3	6.0	19.5	-
Not confirmed	6.1	6.5	6.9	5.0	10.2	
Pre-existing hypertension	3.4	3.7	4.9	1.9	3.4	-
PIH	4.8	3.9	5.3	5.6	8.1	
Preeclampsia, eclampsia, HELLP syndrome	8.0	10.0	8.6	6.1	15.5	-

Table 4.2. Associations between maternal diabetes or hypertensive disorders and ASD or DD in SEED

Model	Exposed *n* (Column %)			ASD vs. POP	DD vs. POP
	ASD	DD	POP	OR (95%CI)	OR (95% CI)
Maternal Diabetes					
Diabetes unadjusted	68 (9.7)	100 (11.3)	78 (8.0)	1.24 [0.89, 1.75]	1.47 [1.07, 2.00]
Diabetes adjusted[a]	65 (9.5)	97 (11.1)	78 (8.1)	1.10 [0.77, 1.56]	1.32 [0.96, 1.82]
Diabetes adjusted[a] + BMI, HTN	64 (9.5)	94 (11.1)	78 (8.2)	0.96 [0.67, 1.39]	1.19 [0.86, 1.65]
Confirmed diabetes[a]	42 (6.3)	69 (8.2)	39 (4.2)	1.45 [0.92, 2.29]	1.86 [1.23, 2.80]
Pre-existing diabetes[a]	15 (2.4)	26 (3.3)	23 (2.5)	0.86 [0.44, 1.70]	1.13 [0.63, 2.02]
GDM[a]	50 (7.2)	56 (5.9)	72 (8.5)	1.20 [0.80, 1.80]	1.42 [0.98, 2.05]
Maternal Hypertensive Disorders					
Hypertension unadjusted	126 (18.1)	152 (17.1)	108 (11.0)	1.78 [1.35, 2.35]	1.67 [1.28, 2.18]
Hypertension adjusted[b]	125 (18.2)	150 (17.3)	107 (11.1)	1.67 [1.25, 2.23]	1.69 [1.29, 2.23]
Hypertension adjusted[b] + BMI, DM	125 (18.5)	148 (17.5)	107 (11.2)	1.56 [1.16, 2.11]	1.59 [1.20, 2.12]
Confirmed hypertension[b]	81 (12.6)	59 (6.4)	89 (11.0)	2.02 [1.40 2.91]	1.85 [1.30, 2.63]
Pre-existing hypertension[b]	26 (4.4)	43 (5.7)	19 (2.2)	1.67 [0.90, 3.11]	2.44 [1.39, 4.27]
PIH[b]	38 (6.3)	47 (6.1)	37 (4.1)	1.45 [0.90, 2.33]	1.51 [0.96, 2.37]
Preeclampsia, eclampsia, and HELLP[b]	70 (11.1)	60 (6.5)	74 (9.3)	1.61 [1.11, 2.34]	1.41 [0.98, 2.04]

a. Adjusted for maternal age, maternal race/ethnicity, maternal education, maternal smoking, and study site.
b. Adjusted for above (a.) plus plurality and parity.

Table 4.3. Associations between maternal diabetes or hypertensive disorders and ASD with (*n*=258) and without (*n*=429) intellectual disabilities (ID) in SEED

	Exposed *n* (Column %)			ASD with ID vs. POP	ASD without ID vs. POP
Model	**ASD with ID**	**ASD without ID**	**POP**	**aOR (95% CI)**	**aOR (95% CI)**
Maternal Diabetes					
Diabetes[a]	77 (18.0)	45 (17.4)	108 (11.0)	1.23 [0.82, 1.84]	1.00 [0.60, 1.66]
Pre-existing diabetes[a]	44 (10.4)	21 (8.2)	78 (8.1)	0.89 [0.41, 1.98]	0.83 [0.31, 2.21]
GDM[a]	76 (18.1)	45 (17.6)	107 (11.1)	1.38 [0.87, 2.18]	1.07 [0.60, 1.92]
Maternal Hypertensive Disorders					
Hypertension[b]	34 (8.3)	16 (6.4)	56 (5.9)	1.67 [1.19, 2.35]	1.61 [1.10, 2.38]
Pre-existing hypertension[b]	18 (5.0)	7 (3.2)	19 (2.2)	1.69 [0.84, 3.39]	1.52 [0.63, 3.69]
PIH[b]	21 (5.8)	15 (6.6)	37 (4.1)	1.33 [0.75, 2.37]	1.52 [0.81, 2.84]
Preeclampsia, eclampsia, HELLP[b]	41(10.7)	27 (11.3)	60 (6.5)	1.61 [1.04, 2.49]	1.61 [0.99, 2.62]

a. Adjusted for maternal age, race/ethnicity, maternal education, maternal smoking, and study site.
b. Adjusted for above (a.) plus plurality and parity.

Table 4.4. Stratum-specific effect estimates for maternal diabetes and hypertensive disorders for three strata of maternal pre-pregnancy BMI in SEED

Model / Strata	**ASD vs. POP aOR (95% CI)**	**p-value for interaction**	**DD vs. POP aOR (95% CI)**	**p-value for interaction**
Maternal Diabetes				
BMI: low, normal (<25 kg/m^2)[a]	0.95 [0.71, 1.27]	Reference	1.23 [0.97, 1.57]	Reference
BMI: overweight (25-<30 kg/m^2)[a]	0.98 [0.70, 1.35]	0.913	0.96 [0.71, 1.31]	0.213
BMI: obese (≥30 kg/m^2)[a]	1.11 [0.80, 1.53]	0.490	1.11 [0.81, 1.51]	0.594
Maternal Hypertensive Disorders				
BMI: low, normal (<25 kg/m^2) [b]	1.35 [1.05, 1.64]	Reference	1.34 [1.09, 1.66]	Reference
BMI: overweight (25-<30 kg/m^2)[b]	1.23 [0.93, 1.64]	0.734	1.30 [0.99, 1.70]	0.844
BMI: obese (≥30 kg/m^2)[b]	1.17 [0.90, 1.54]	0.538	1.14 [0.87,1.48]	0.324

a. Adjusted for maternal age, race/ethnicity, maternal education, maternal smoking, and study site.
b. Adjusted for above (a.) plus plurality and parity.

CHAPTER 5: NEONATAL JAUNDICE IN ASSOCIATION WITH AUTISM SPECTURM DISORDER

A. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent impairment in social interaction and communication, and restrictive and repetitive patterns of behaviors, interests, or activities (239). In 2012, the prevalence of ASD in the U.S. was estimated to be 1 in 68 children aged 8 years (1). ASD is a heterogeneous disorder, and its etiology is suspected to involve both genetic and environmental factors (2, 9). While environmental factors associated with ASD are not well understood, recent studies have implicated several exposures in the perinatal period (13, 52, 55).

Jaundice, a yellow discoloration of the skin and the sclera of the eyes, occurs in about half of newborn infants and most preterm infants, usually as a result of increased bilirubin from the breakdown of fetal hemoglobin after birth (168). While jaundice typically resolves in the first week of life (169, 170), bilirubin can cross the blood brain barrier and may have toxic effects on the developing brain (170, 250, 251). Hyperbilirubinemia in the neonatal period has been associated with intellectual disability, cerebral palsy, and brain dysfunction in children (250, 252).

Some studies have reported an association among neonatal jaundice and hyperbilirubinemia and ASD (52, 105, 174-176), while others have reported no association (10, 177). The inconsistent results may be partly due to differences in methods of data collection and classification of jaundice. Jaundice occurs more frequently among infants born preterm (14), and

preterm birth has also been consistently associated with increased risk of ASD. Yet, few studies have addressed the role of gestational age in exploring associations between jaundice and ASD. To address the limitations of previous studies, we analyzed data from the Study to Explore Early Development (SEED), a multisite case-control study with extensive data from the perinatal period, to investigate the association between neonatal jaundice and ASD.

B. Methods

Study population

The Study to Explore Early Development (SEED) is a multisite case-control study of children with ASD, children with developmental delays or disorders other than ASD (DD), and children sampled from the general population (POP) (26). Children in the ASD and DD groups were ascertained through multiple school systems, agencies, and clinical settings that evaluate or serve children with developmental challenges, based on a broad range of special education or International Classification for Disease (ICD) codes indicative of autism/ASD or other developmental challenge. State birth records were randomly sampled to identify children from the general population who were born in the same time period and catchment area as children with ASD. SEED participants were born between September 1, 2003 and August 31, 2006 and at the time of enrollment resided in one of six study catchment areas (California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania). The children were required to be 30 through 68 months of age at the time of in-person assessment and living with a knowledgeable caregiver who spoke English (or Spanish in California and Colorado) and who was at least 18 years of age and able to provide legal consent. SEED recruited 3,769 participants. For this study, we restricted analyses to the 2,561 participants who had data available on neonatal complications and had a final outcome classification of ASD, DD, or POP.

Data collection

Details on the SEED data collection protocol are provided elsewhere (26). Briefly, SEED collected detailed data on maternal reproductive health and pregnancy outcomes through a telephone interview, abstraction of prenatal, neonatal, and pediatric medical records, and linkage with birth certificates. Data on child development and behavior were obtained through telephone interviews and in-person child developmental assessment, as described below.

Outcome Assessment

We used the Social Communication Questionnaire (SCQ) (180) to screen all children for autism symptoms during the invitation phone call. Children who screened positive for possible ASD (SCQ score >11), had a previous ASD diagnosis, or were suspected to have ASD based on the study clinician's direct observation, were given a more extensive developmental assessment. The more extensive assessment included clinical observation of the child using the Autism Diagnostic Observation Schedule (ADOS)(181) and a structured interview administered to their caregivers, the Autism Diagnostic Interview-Revised (ADI-R)(183). Final ASD classification was based on the results from these two developmental assessments regardless of previous diagnoses (188).

As part of the protocol, study clinicians administered the Mullen Scales of Early Learning to all children as a general developmental assessment. We used the Mullen Scales of Early Learning Composite Standard Score to identify children with ASD and intellectual disability (ID; score <70) or ASD without ID (score ≥70). More detail about the SEED outcome assessment is available elsewhere (26, 188).

Ascertainment of neonatal jaundice

Information on neonatal jaundice was obtained from the maternal interview and the neonatal medical record. Data from pediatric medical records were also obtained for the first 28 days of life. The infant was classified as having neonatal jaundice if a diagnosis or treatment for jaundice was recorded in the medical record or through maternal report. Treatment for neonatal jaundice included phototherapy (bili light or blanket) or, in severe cases, exchange transfusion. We classified neonatal jaundice as: 1) Definite, if treatment for neonatal jaundice was reported in either the medical record or maternal interview, 2) Probable, if a diagnosis of jaundice was available in the medical record, but treatment was not reported, or 3) Possible, if neonatal jaundice was only reported through maternal interview, or 4) No neonatal jaundice, if an infant had no report of jaundice or treatment for jaundice in the medical record or maternal interview. Indicator variables were created for these mutually exclusive categories with the referent group of no neonatal jaundice.

Bilirubin measurements were collected from the neonatal medical record when available. Hyperbilirubinemia, defined as a total serum bilirubin measurement above the 95th percentile, was classified according to the Bhutani nomogram. The Bhutani nomogram is used among infants born ≥35 weeks gestation and evaluates the total serum bilirubin measurement relative to the age of the infant (in 12-hour increments). The nomogram defines 95th percentile, 75th percentile, and 40th percentile tracks for bilirubin measurements and designates the categories above as high-risk, high-intermediate risk, and low-intermediate risk zones, respectively, for significant hyperbilirubinemia (obtaining a subsequent measurement in the 95th percentile) (173, 193). We created indicator variables for these mutually exclusive categories, and defined the referent group as infants with no bilirubin measurement or with bilirubin measurements below

the 40th percentile, and no neonatal jaundice indicated in the medical record or maternal interview. The referent group includes infants that did not have a bilirubin test ordered because they had no visible jaundice. A variable encompassing any bilirubin level above the 40th percentile was also created to characterize infants with an elevated bilirubin measurement.

Covariates

Confounders of the association between neonatal jaundice and ASD or DD were identified through a thorough literature. A directed acyclic graph was used to determine a minimally sufficient set of confounders in order to obtain the least biased estimates of association (238). Data from prenatal and neonatal medical records, birth certificates, and maternal interview were used to define covariates.

Confounders included maternal age at conception (20 years, 20-34 (referent), ≥ 35 years), maternal education (high school degree or less vs. some college or more), parity (nulliparous (referent), primiparous, multiparous), diabetes in pregnancy (vs. no diabetes), infant sex (male vs. female), gestational age, infant race/ethnicity, and study site. Study sites included: Georgia (referent), California, Colorado, Maryland, North Carolina, and Pennsylvania.

Infant race/ethnicity was classified by combining maternal and paternal race as reported on the maternal interview, or the birth certificate when maternal interview data were missing. If the parents were the same race the infant was classified as White (referent), Black, Other (including Asian, Pacific Islander, or Native American). If both parents identified as Hispanic, the infant was classified as Hispanic, regardless of reported parental race. Additionally, an infant was classified as Multiracial, white (if one parent was White and the other was not) or Multiracial, other (if the parents were of different non-White races), allowing for the consideration of differences in jaundice diagnosis affected by skin tone.

Gestational age was classified according to clinical practice guidelines from the American Academy of Pediatrics (173) specifically for assessing neonatal jaundice: <35 weeks completed gestation, 35-37 weeks completed gestation, or ≥38 weeks of completed gestation.

Statistical Analyses

We calculated descriptive statistics to compare maternal and perinatal characteristics by child outcome and neonatal jaundice. Multivariable logistic regression models estimated odds ratios (OR) and 95% confidence intervals (CI) for the association between neonatal jaundice and either ASD (vs. POP) or DD (vs. POP), adjusting for all confounders (aOR) noted above.

In addition, we estimated the association between subcategories of neonatal jaundice (definite, probable, and possible) and ASD or DD. We also used logistic regression models to estimate the association between elevated bilirubin measurement levels and child outcome restricting to infants born ≥35 weeks, defining any elevated bilirubin as >40th percentile and also evaluating the association among subcategories of bilirubin elevated at the 95th, 75th, and 40th percentiles.

We tested the assumption of homogeneity of the association across strata of gestational age. We included interaction terms in the model and evaluated significance at α=0.20. We also obtained stratum-specific effect estimates from the interaction model for the association between neonatal jaundice and ASD/DD and for the association between elevated bilirubin and ASD/DD, restricting to infants ≥35 weeks gestation. Furthermore, we also assessed whether there were any differences in the association between neonatal jaundice and ASD with and without ID to evaluate the potential for these phenotypes to be etiologically distinct. We evaluated this association across strata of gestational age and among subcategories of neonatal jaundice.

We could not determine the eligibility of all families we attempted to recruit because SEED recruited participation from multiple sources and multiple sites. One site had more data available to assess the generalizability of their sample. This assessment found response to be related to maternal age, education and race-ethnicity, but unrelated to available pregnancy and health variables. Accordingly, all analyses include adjustment for maternal age, education, and race. All analyses were conducted using SAS statistical software, version 9.3 (SAS Institute Inc., Cary, NC).

C. Results

A total of 2,561 mother-child pairs enrolled in SEED were included in this analysis. The study sample was comprised of 697 children with ASD, 886 with a non-ASD DD, and 978 from the POP group. Compared to POP children, children with ASD and DD were more likely to have older and less educated mothers, and were more likely to be multiple births, born earlier, male, and non-White (Table 5.1). Children with neonatal jaundice were more likely to have been born earlier and to have had mothers who were older at conception and who had diabetes in pregnancy. A total of 1,323 children were excluded because they had missing data on neonatal jaundice or had a developmental disorder with some characteristics for ASD but not sufficient to meet diagnostic criteria. Compared to the analytic sample, children in the excluded subset were more likely to be White and less likely to have a mother who attained a higher education.

In our sample, 1,239 infants (48.4%) were classified as having neonatal jaundice according to either the maternal interview or medical records (709 (57.2%) by both sources, 235 (19%) by maternal interview only, and 295 (23.8%) by neonatal medical record only). Of these, 50.5% were classified as having definite jaundice, 30.5% with probable jaundice, and 19% with possible jaundice. There were 271 children (16.9% of 1,604 with a completed medical record)

born ≥35 weeks with any elevated bilirubin (bilirubin above the 40th percentile). Of these, bilirubin levels were above the 95th percentile for 88 (5.5%), between the 75th-95th percentile for 78 (4.9%), and between 40th-75th percentiles for 105 (6.5%). There were 143 children with a bilirubin measurement below the 40th percentile; those children along with an additional 1,088 who had no measured bilirubin or record of jaundice comprised the referent group of no hyperbilirubinemia.

The association between any neonatal jaundice and ASD and DD risk varied across strata of gestational age and an interaction term for jaundice and gestational age was significant in all strata; thus all results are presented by gestational age stratum (Table 5.2). In unadjusted analyses, we observed positive associations between neonatal jaundice and both ASD and DD risk only among children born <38 weeks gestation (Table 5.2). After adjustment, risk estimates remained elevated for children born <35 weeks gestation with ASD (aOR=3.05 [1.00, 9.25]) and DD (aOR=2.23 [0.96, 5.19]). For children born 35-37 weeks, a positive but weaker association was observed with ASD (aOR=1.83 [1.05, 3.19] yet stronger association with DD (aOR=3.34 [1.99, 5.61]). Jaundice was not associated with ASD or DD among those born ≥38 weeks.

We also further estimated the association based on three subclassifications of neonatal jaundice. Among those with definite (treated) jaundice, stronger associations were observed in the 35-37 weeks stratum with ASD (aOR=2.19 [1.26, 3.82]) and DD (aOR=3.76 [2.31, 6.12]). In practice, most infants with jaundice before 35 weeks gestation received treatment; therefore, fewer infants were classified with probable or possible jaundice and those results were unstable. We observed positive but weaker associations with probable jaundice and possible jaundice and ASD/DD in the 35-37 weeks stratum. Jaundice was not associated with ASD or DD among infants born ≥38 weeks for any subcategories.

Our results show slightly stronger patterns of association between neonatal jaundice and ASD without ID among those <38 weeks gestation (Table 5.3). However, the pattern of no associations among those ≥38 weeks remained. The association between elevated bilirubin and ASD and DD also differed by gestational age and was mostly limited to those born 35-37 weeks gestation and not those born ≥38 weeks (Table 5.4). The associations were strongest for elevated bilirubin at the 40th and 95th percentiles, however, the sample sizes in these strata are small and the confidence intervals are wide. No associations were seen at the 75th percentile of bilirubin levels.

D. Discussion

Our results show an association between neonatal jaundice and both ASD and DD among infants born between 35-37 weeks gestation. The association with both ASD and DD suggests a more general effect on neurodevelopment rather than on processes specific to ASD. Jaundice was associated with neurodevelopment among infants born <35 weeks, however, we had limited statistical power to assess associations in this smaller subgroup. We did not observe any association between neonatal jaundice and either ASD or DD among infants born ≥38 weeks gestation. Our analysis is unique in that information on neonatal jaundice was collected through medical record, maternal report, and abstracted laboratory measurements. Despite small sample sizes in some strata in our study, the large SEED population made it possible to assess for interaction between gestational age and neonatal jaundice with reasonable power compared to other studies (176-178). Our results were consistent with some previous studies (105, 174, 178), but not with others (175-177).

In our study, the magnitude of the association between jaundice and ASD/DD varied according to the jaundice definition. Among subcategories of neonatal jaundice, definite and

probable jaundice were associated with ASD and DD among infants born between 35-37 weeks. We observed weak or no association with possible jaundice, which was defined by maternal report of jaundice only. The possible jaundice group may have higher potential for misclassification because the diagnosis was not confirmed in the medical record and the condition was not severe enough to warrant treatment.

Previous studies of bilirubin levels differed in how they defined high bilirubin. This is the first study to use the Bhutani nomogram to classify bilirubin levels, a method that is recommended in current medical practice (173). The Bhutani nomogram is used among infants born ≥35 weeks gestation to evaluate bilirubin measurements relative to the age of the infant (in 12-hour increments) and tracks zones for the 95th percentile, 75th percentile, and 40th percentile measurements, designating them as high-risk, high-intermediate risk, and low-intermediate risk (respectively) for any significant hyperbilirubinemia (173, 193). While our findings suggest an underlying association with bilirubin and ASD, confidence intervals were imprecise due to small sample sizes, as fewer children had bilirubin measurements available from the medical record. However, elevated bilirubin was associated with DD among infants born between 35-37 weeks. A stronger association may be expected with higher bilirubin levels due to its potential for neurotoxicity, however we did not observe this in our study. Instead, associations were strongest for lower bilirubin levels (40th percentile) and DD among infants born between 35-37 weeks.

Previous studies show that neonatal jaundice is more common and may be more prolonged in preterm infants compared to term infants (253-255) and preterm birth is strongly associated with ASD and DD (54, 256). While we did not have the data to evaluate the length of time an infant had elevated bilirubin levels, future studies should be designed to determine if the association we observed with neonatal jaundice and ASD/DD may be due to a prolonged

exposure of bilirubin crossing the blood-brain barrier. It is also possible that infants with lower bilirubin were less likely to be treated and therefore could have had a prolonged exposure, which would explain the stronger associations we observed in the 40th percentile group compared to the higher bilirubin level groups, but we did not have data to further investigate this explanation. Larger populations and more detailed, serial data on exposure to elevated bilirubin are necessary to more precisely estimate the association and establish a causal mechanism.

SEED is the first study to account for interaction between gestational age and jaundice and to provide stratum-specific effect estimates by gestational age; prior studies restricted their analyses to term infants or adjusted for preterm birth as a confounder. Still, the health of infants with jaundice, especially preterm infants with jaundice, can be more complex than for term infants with no signs of jaundice. There may be several unknown or unmeasured factors that commonly co-occur with jaundice in preterm infants that may contribute to brain development, which include other perinatal and neonatal complications and how such complications are treated or managed. This presents a potential for uncontrolled confounding and should continue to be explored.

Inconsistencies between our results and prior studies may also be due to differences in how ASD was ascertained or defined. Most previous studies (52, 174, 177, 178) relied on ICD-9 codes or insurance databases that may reflect treatment codes that are not necessarily consistent with a final diagnosis. SEED confirmed ASD through in-person evaluation and all children had to meet criteria on the ADOS and ADI-R to obtain a final ASD classification, reducing the potential for outcome misclassification and providing some insight that jaundice was associated with development more generally, and not necessarily specifically associated with ASD.

In conclusion, we observed associations between neonatal jaundice and ASD and DD in infants born <38 weeks gestation, but not among infants born at term (≥38 weeks). This association may be due to delayed clearance of bilirubin in preterm infants, which deserves further investigation. Future studies should further explore the complexity of other neonatal conditions that accompany jaundice, and treatment profiles for jaundice, in association with ASD. While primary treatment of such conditions are a priority for neonatologist, the timely reduction of jaundice could have the added benefit of reducing subsequent neurodevelopmental effects. In addition, monitoring children with perinatal experiences that include known risk factors associated with ASD and developmental disabilities may lead to earlier diagnosis and treatment, which can improve long-term outcomes.

Table 5.1. Distribution of maternal and infant characteristics by outcome classification and jaundice type in children born September 2003-August 2006 enrolled in SEED (N=2,561)

		Column percent by child outcome and neonatal condition				
Maternal Characteristics	**Total Population**	**ASD**	**DD**	**POP**	**Jaundice Diagnosis**	**Hyperbili-rubinemia**
	(N=2,561)	**(*n*=697)**	**(*n*=886)**	**(*n*=978)**	**(*n*=1,239)**	**(*n*=88)**
Maternal Age at Conception						
<20 years	6.2	5.6	6.8	6.1	5.0	1.14
20-<35 years	83.2	83.4	81.8	84.3	83.6	85.23
≥ 35 years	10.6	11.1	11.4	9.6	11.4	13.6
Maternal Education						
High school degree or less	14.5	16.2	18.0	10.1	12.4	15.9
Any college	59.3	62.0	56.4	60.0	62.5	58.0
Graduate school	26.2	21.7	25.6	29.8	25.1	25.0
Parity						
First birth	44.0	47.1	39.7	45.6	46.6	41.5
Second	35.9	34.2	37.0	36.1	33.3	38.4
Third or more	20.15	18.8	23.3	18.3	20.2	23.9
Plurality						
Singleton	94.0	91.8	93.2	96.1	90.4	95.5
Multiple	6.1	8.2	6.8	3.9	9.6	4.6
Maternal Diabetes						
Yes	9.6	9.8	8.0	11.3	11.4	14.8
Gestational Age						
<35 weeks gestation	8.7	8.8	12.3	5.3	14.9	0
35-37 weeks gestation	16.0	17.1	19.5	12.1	21.2	28.4
≥38 weeks gestation	75.3	74.2	68.2	82.6	63.9	71.6
Infant Race/Ethnicity						
White, non-Hispanic	60.7	53.8	59.6	66.7	62.7	59.1
Black, non-Hispanic	16.5	20.7	16.9	13.2	14.3	13.6
Hispanic	7.2	7.3	8.8	5.6	6.1	10.2
Asian	3.9	6.0	3.6	2.6	4.0	3.4
Multiracial, white	6.1	6.2	5.5	6.5	6.5	6.8

Multiracial, other	5.6	6.0	5.4	5.5	6.4	6.8
Child Sex						
Male	64.5	82.1	63.0	53.4	64.5	62.5
Study Site						
California	15.4	15.2	15.0	15.8	12.4	14.8
Colorado	19.5	20.2	17.8	20.4	22.1	22.7
Georgia	19.8	19.7	21.7	18.1	17.0	10.2
Maryland	14.4	15.5	13.3	14.5	15.4	12.5
North Carolina	17.8	14.8	20.5	17.6	21.6	20.5
Pennsylvania	13.2	14.6	11.6	13.7	11.5	19.3

Table 5.2. Stratum-specific estimates by gestational age for the association between neonatal jaundice and ASD or DD in SEED

	Jaundice N			Unadjusted OR (95% CI)		Adjusted OR (95% CI)	
	ASD	**DD**	**POP**	**ASD vs. POP**	**DD vs. POP**	**ASD vs. POP**	**DD vs. POP**
Any neonatal jaundice:							
<35 weeks gestation	54	93	38	1.69 (1.02, 2.70)*	1.46 (0.98, 2.19)	3.05 (1.00, 9.25)	2.23 (0.96, 5.19)
35-37 weeks gestation	72	130	60	1.22 (0.94, 1.57)*	1.71 (1.33, 2.19)	1.83 (1.05, 3.19)	3.34 (1.99, 5.61)
≥38 weeks gestation	213	248	331	1.00 (0.90, 1.12)*	1.00 (0.90, 1.12)	0.97 (0.76, 1.24)	1.07 (0.85, 1.33)
Definite jaundice:							
<35 weeks gestation	49	89	36	1.65 (1.00, 2.73)*	1.47 (0.98, 2.21)	1.66 (0.99, 2.79)	3.80 (2.41, 6.00)
35-37 weeks gestation	41	75	25	1.42 (1.04, 1.95)*	2.01 (1.49, 2.72)	2.19 (1.26, 3.82)	3.76 (2.31, 6.12)
≥38 weeks gestation	84	108	119	1.05 (0.90, 1.23)*	1.10 (0.95, 1.28)	1.01 (0.73, 1.42)	1.28 (0.95, 1.73)
Probable jaundice:							
<35 weeks gestation	4	3	1	-	-	-	-
35-37 weeks gestation	24	35	21	1.19 (0.84, 1.69)*	1.50 (1.07, 2.10)	1.95 (1.01, 3.80)	2.49 (1.39, 4.47)
≥38 weeks gestation	66	90	134	0.88 (0.75, 1.04)*	0.95 (0.82, 1.10)	0.83 (0.58, 1.18)	0.98 (0.72, 1.34)
Possible jaundice:							
<35 weeks gestation	1	1	1	-	-	-	-
35-37 weeks gestation	7	20	14	0.79 (0.48, 1.29)*	1.39 (0.94, 2.06)	0.60 (0.22, 1.63)	2.00 (0.97, 4.11)
≥38 weeks gestation	63	50	78	1.13 (0.94, 1.35)*	0.93 (0.77, 1.12)	1.22 (0.83, 1.79)	0.87 (0.59, 1.28)

Adjusted for maternal age, maternal education, maternal diabetes, parity, plurality, infant sex, infant race/ethnicity, and study site.

Interaction term for gestational age and jaundice was significant at $\alpha<0.20$ in all strata.

Table 5.3. Stratum-specific estimates by gestational age for the association between neonatal jaundice and ASD with or without ID SEED

	Jaundice N			ASD with ID	ASD without ID
	ASD with ID	**ASD without ID**	**POP**	**aOR (95% CI)**	**aOR (95% CI)**
Any neonatal jaundice:					
<35 weeks gestation	28	24	38	1.97 (0.59, 6.60)	8.17 (0.95, 70.28)
35-37 weeks gestation	45	26	59	1.96 (1.03, 3.71)	1.80 (0.84, 3.88)
≥38 weeks gestation	130	79	331	0.99 (0.74, 1.32)	0.95 (0.67, 1.33)
Definite jaundice:					
<35 weeks gestation	25	22	36	1.30 (0.69, 2.45)	2.47 (1.29, 4.74)
35-37 weeks gestation	23	17	25	2.05 (1.08, 3.92)	2.77 (1.37, 5.60)
≥38 weeks gestation	51	32	119	1.01 (0.68, 1.51)	1.10 (0.69, 1.75)

Adjusted for maternal age, maternal education, maternal diabetes, parity, plurality, infant sex, infant race/ethnicity, and study site.

Interaction term for gestational age and jaundice was significant at $\alpha<0.20$ in all strata.

Table 5.4. *Stratum-specific estimates by gestational age for the association between elevated bilirubin levels and ASD or DD in SEED*

	Jaundice N			Unadjusted OR (95% CI)		Adjusted OR (95% CI)	
	ASD	**DD**	**POP**	**ASD vs. POP**	**DD vs. POP**	**ASD vs. POP**	**DD vs. POP**
Any elevated bilirubin:							
35-37 weeks gestation	28	39	19	1.29 (0.91, 1.81)	1.57 (1.13, 2.19)*	1.94 (0.94, 4.01)*	2.15 (1.12, 4.14)*
≥38 weeks gestation	55	52	78	1.05 (0.87, 1.27)	0.93 (0.77, 1.12)*	1.06 (0.71, 1.58)*	0.90 (0.62, 1.33)*
95th Percentile:							
35-37 weeks gestation	8	12	5	1.34 (0.75, 2.41)	1.70 (0.98, 2.96)	2.39 (0.66, 8.70)	2.41 (0.78, 9.61)
≥38 weeks gestation	17	26	20	1.15 (0.83, 1.61)	1.30 (0.96, 1.75)	1.14 (0.56, 2.32)	1.68 (0.91, 3.10)
75th Percentile:							
35-37 weeks gestation	8	11	8	1.06 (0.63, 1.79)	1.29 (0.79, 2.10)*	1.09 (0.37, 3.21)	1.43 (0.53, 3.84)
≥38 weeks gestation	16	11	24	1.02 (0.74, 1.41)	0.77 (0.54, 1.11)*	1.15 (0.57, 2.34)	0.73 (0.35, 1.55)
40th Percentile:							
35-37 weeks gestation	12	16	6	1.50 (0.89, 2.52)*	1.79 (1.08, 2.97)*	2.99 (0.97, 9.22)*	2.92 (1.05, 8.17)*
≥38 weeks gestation	22	15	34	1.01 (0.76, 1.33)*	0.76 (0.55, 1.03)*	0.94 (0.52, 1.71)*	0.56 (0.30, 1.05)*

Adjusted for maternal age, maternal education, maternal diabetes, parity, plurality, infant sex, infant race/ethnicity, and study site.

*Interaction term for gestational age and jaundice was significant at $\alpha<0.20$

CHAPTER 6: DISCUSSSION

A. Summary

In the past two decades the prevalence of ASD has increased and more attention has focused on discovering etiological pathways associated with ASD. Despite evidence that environmental exposures during the prenatal and neonatal period play a role in the development of ASD, few independent risk factors have been identified. This study examines the associations between the most common prenatal and neonatal complications and ASD.

We use data from the Study to Explore Early Development, a large multi-site case-control study designed to identify etiological risk factors for ASD. Information regarding prenatal and neonatal risk factors is collected in SEED from medical records, caregiver phone interviews, questionnaires, and birth certificates. All children are screened and clinically evaluated to confirm ASD or a secondary outcome group classified with non-ASD developmental delay. These outcome groups are compared to children ascertained from the population matched on age and location through birth records.

This work had two specific aims: 1) to examine the associations between prenatal complications, specifically maternal diabetes and hypertension, and ASD, and 2) to examine the association between neonatal complications, specifically neonatal jaundice, and ASD. Comparing these associations to DD vs. POP allows us to determine if these associations are specific to ASD. While some studies have shown a positive association between these complications and ASD, others have shown no association, and their role in the development of ASD is still unknown. However, few studies had large sample sizes to examine these

complications and most did not model these conditions independently. Our analysis addresses limitations from previous studies and tries to identify possible etiological mechanisms for ASD for further study.

B. Prenatal complications and ASD

In our first aim, we examined the association between maternal diabetes and hypertension and ASD. Diagnoses of any diabetes or hypertensive disorder during pregnancy were identified from prenatal medical records, caregiver phone interview, and mailed-in questionnaires (for diabetes). In addition, serial blood pressure measurements from clinic and hospital records were available for hypertension as well as results from diagnostic tests for gestational diabetes.

Our analysis did not show an association between diabetes in pregnancy and ASD, but did show an association with maternal hypertensive disorders and ASD. Additionally, maternal diabetes and hypertensive disorders were both associated with DD. While we did observe an association with diabetes and hypertension and neurodevelopmental disorders, only hypertension was associated with ASD. We did not observe notable differences in the associations for ASD with ID versus ASD without ID, contrary to previous studies (167).

There are several possible biological mechanisms to explain how diabetes and hypertensive disorders in pregnancy may affect fetal neurodevelopment. A plausible explanation is through inflammation and oxidative stress that occurs with chronic hypertension (22, 120). Inflammation can lead to changes in DNA methylation and gene expression (Rose et al., 2012) and some inflammatory markers are also known to cross the blood brain barrier possibly altering neural cell connections (22, 49). Oxidative stress is also associated with cell death and differentiation and can also affect DNA methylation and gene expression (120, 123).

Diabetes and hypertension can also affect neurodevelopment through over-nutrition and neonatal hyperglycemia or under-nutrition and intrauterine growth restriction, respectively. Diabetes is associated with over-nutrition of the fetus often resulting in an infant that is macrosomic or large for gestational age (257). Larger infants are more likely to suffer birth complications and trauma, such as head injury and hypoxia, which can affect future brain development (258, 259). Infants from diabetic mothers are also at higher risk of having neonatal hyperglycemia, which has been associated with brain injury (260) and changes in brain development (179). Hypertension is associated with under-nutrition and intrauterine growth restriction (148), and often results in infants that are born preterm or small for gestation age (261). Several studies have established a connection between infants small for gestational age and changes in neurodevelopment (262, 263). However, it is still undetermined if there is a biological interaction with hypertension and growth restriction that increases the risk for ASD. This can be investigated in the future using SEED data that will become available with the larger sample size provided by additional SEED phase 2 and 3 data.

We observed stronger associations with pre-existing hypertension, particularly with DD. This may be related to the timing of the condition during pregnancy and fetal brain development. However, associations were also observed with preeclampsia, eclampsia, and HELLP, which occur later in pregnancy. These groups were not mutually exclusive and 18% of mothers with preexisting hypertension went on to have preeclampsia. The stronger association with pre-existing hypertension may also be attributed to prolonged and severe conditions having a more harmful effect. In our study, it was difficult to assess the severity of a hypertensive disorder. One indicator was preterm delivery, with which we observed stronger effect estimates with hypertension and ASD (data not shown). However, because the recommendation for mothers

with sustained hypertension is to deliver early, this variable may instead remove mothers with a misclassified exposure. Future investigations should assess if there is a joint effect of having a severe condition during a critical time window in which a hypertensive mother is at higher risk of having a child with ASD or DD.

We observed stronger associations in mothers with a 'Confirmed' condition reported in both the medical record and through maternal report, or in a diagnostic test. Weaker associations for confirmed conditions may reflect exposure misclassification or the possibility that confirmed cases represented more severe conditions in pregnancy. While limiting the analyses to confirmed condition status strengthened effect estimates, this did not change the interpretation of results for hypertensive disorders. However, it did strengthen the association between diabetes and DD.

Adjusting for BMI and diabetes did not materially alter the effect estimates for hypertensive disorders. Previous studies have reported an interaction with high BMI and both maternal diabetes and hypertension (12, 166, 167). Comparing BMI stratum-specific estimates, we did not observe differences in the association among mothers with pre-pregnancy BMI in the low/normal, overweight, or obese categories. Our results suggest that weight status may not play a role in the association between maternal hypertension and ASD.

Overall, our results mostly agree with other case-control studies (12, 43, 52, 163), but there are subtle differences that may be due to the study population and sample size, with SEED having the largest number of affected dyads over multiple sites in the United States. While our results were similar to some cohort studies evaluating this association (8, 67, 71, 165), they differed from others (68, 144, 166, 167). Conducting a cohort study with a rare exposure and rare outcome is difficult however, and most of these studies relied on registries or large administrative data sets that were followed over time.

In conclusion, we observed observations with hypertensive disorders and both ASD and DD. We also observed weaker associations with diabetes and DD. We hypothesize that this may be due alterations in fetal nutrition and development during pregnancy, which deserves further investigation.

Public health implications

Our study provides additional support that hypertension may be associated with developmental disorders, including ASD. This association may be present due to the effects of hypertension on the fetus, such as growth restriction and increased likelihood of preterm birth. Further studies are necessary to understand the biological mechanism through which this association operates. Measures can be implemented to better manage hypertensive disorders in pregnancy in order to mitigate environments that may place the infant at higher risk for developing ASD. While some hypertensive disorders are not well-managed or preventable, identifying hypertensive disorders as a risk factor for ASD can possibly result in earlier screening and better monitoring for developmental disorders. Several studies have shown the benefits of early diagnosis on child developmental outcomes (264, 265), and these results may help identify children at risk for developmental disorders earlier.

C. Neonatal complications and ASD

We also examined the association between neonatal jaundice and ASD. Infants with neonatal jaundice were identified from medical records and caregiver phone interviews. In addition, bilirubin measurements were available in the medical record and the Bhutani nomogram was used to classify infants at high risk for hyperbilirubinemia.

Our analysis shows an association with neonatal jaundice and ASD and DD in infants born between 35-37 weeks gestation. The association with both ASD and DD suggests that there

may be a more general effect on neurodevelopment rather than on processes specific to ASD. There may also be an association in infants born <35 weeks, however, we had limited statistical power to assess this due to small sample size. We did not observe any association with neonatal jaundice and ASD or DD in infants born ≥38 weeks gestation. Among subcategories of neonatal jaundice, definite and probable jaundice were associated with ASD and DD in the 35-37 weeks stratum. Strong associations were not observed with possible jaundice. This may be due possible misclassification of the exposure among infants with possible jaundice, who were not treated and did not receive a diagnosis in the medical record. Or it may be due to infants with possible jaundice having a less severe or more transient form of jaundice. Infants with definite or probable jaundice may have had jaundice for a longer period of time and may have also had a longer hospital stay and other complications.

The association between jaundice and ASD among infants born <38 weeks gestation, but not ≥38 weeks, may be attributed to neonatal jaundice being more common and persisting for a longer period of time in preterm infants (253-255) and because preterm birth is shown to be strongly associated with ASD and DD (54, 256). Gestational age may also heavily determine whether an infant is diagnosed with jaundice and treated. Preterm infants may be more susceptible to bilirubin-induced neurologic dysfunction (BIND) at lower bilirubin levels and thus are treated more conservatively (266).

Still, the health of infants with jaundice, especially preterm infants with jaundice, can be more complex than for term infants with no signs of jaundice There may be several unknown or unmeasured factors that commonly co-occur with jaundice in preterm infants that may be also contribute to brain development, such as other perinatal and neonatal complications and how they are treated or managed. Bilirubin clearance after birth requires a healthy and mature liver

and gastrointestinal system, and this process may be slower in preterm infants and those with other complications. Additionally, difficulty in feeding, that is more common in infants that later develop ASD, can cause neonatal jaundice and is often observed when infants have several complications. This presents a potential for uncontrolled confounding and should continue to be explored.

We observed differences when comparing effect estimates for definite jaundice and ASD with ID to ASD without ID, however this difference was not observed with broader neonatal jaundice. Definite jaundice, defined as infants with jaundice that received treatment, was more strongly associated with ASD without ID, particularly in infants born <35 weeks gestation. This may signal a distinct etiological pathway for ASD without ID involving treatment for jaundice in preterm infants. However, sample sizes were small in the probable and possible jaundice groups and a pattern could not be established to distinguish differences in ASD with ID versus without ID. Due to the wide confidence intervals in the probable and possible jaundice groups, it is possible that patterns may emerge with larger sample sizes and may differ compared to definite jaundice.

Our study is unique in our ability to also assess the association between high bilirubin levels and ASD/DD. While our findings with neonatal jaundice suggest an underlying association with bilirubin and ASD, confidence intervals were imprecise due to our small sample sizes. However, we did observe more precise associations with elevated bilirubin and DD in the 35-37 weeks stratum. Since we suspect high bilirubin levels as a biological mechanism for the association between jaundice and ASD/DD, we expected to see stronger associations among those with bilirubin levels in the 95th percentile. However, we noticed the strongest association with lower bilirubin levels (40th to 75th percentile) and DD infants in the 35-37 weeks stratum.

It is possible that infants with lower bilirubin were less likely to be treated and therefore could have had a prolonged exposure, but we did not have data to support an investigation of this explanation. Larger populations and more detailed data on exposure to elevated bilirubin are necessary to more precisely estimate the association and establish a causal role. We did not have the data to evaluate the length of time an infant had elevated bilirubin levels, but future studies can be designed to determine if the association we observed with neonatal jaundice and ASD/DD may be due to a prolonged exposure of bilirubin crossing the blood-brain barrier.

Our results were consistent with some previous studies (105, 174, 178), but not with others (175-177). Differences in these results may be in large part due to differences in how neonatal jaundice was defined, how ASD was ascertained, which variables were adjusted for as confounders, or how gestational age was included in the analysis. Previous studies that included bilirubin levels also differed in how they defined high bilirubin. This is the first study to use the Bhutani nomogram to classify bilirubin levels according to time of measurement, a method that is recommended in current medical practice (173). In evaluating the association between jaundice and ASD/DD, the magnitude of the association largely relied on jaundice subclassification. Stronger associations were observed with definite and probable jaundice, which relied on a report of treatment or a diagnosis in the medical record, respectively. We observed weak or no associations with possible jaundice, which was defined as having jaundice only by maternal report. Additionally, this is the first study to account for interaction between gestational age and jaundice and to provide stratum-specific effect estimates by gestational age. Whereas other studies restricted their analyses to term infants or adjusted for preterm birth as a confounder.

In conclusion, we observed associations between neonatal jaundice and ASD and DD in infants born between 35-37 weeks gestation, but not among infants born at term (≥38 weeks). This association may be due to delayed clearance of bilirubin in preterm infants affecting brain development. However, there may be other unknown variables confounding this association. It is possible that jaundice may be one of several factors that together are associated with ASD and DD. Previous studies have shown associations with several perinatal complications and comorbidities and ASD, it is possible that these may all work jointly in altering brain development.

Public health implications

Our study indicates that neonatal jaundice may be associated with ASD and DD among infants born <38 weeks gestation. While the AAP recommends incorporating bilirubin measurement into newborn routine care, this practice is variably followed. Results from this study provide additional support for this recommendation. Obtaining high bilirubin levels necessitates immediate treatment, which may be delayed if bilirubin is not measured because jaundice is not visible or because the infant is born preterm with other complications. Earlier screening can therefore reduce exposure to dangerous levels of bilirubin.. Bilirubin levels can also be measured transcutaneously if jaundice is suspected following screening, without the need for a blood draw, which can be difficult to obtain for preterm infants. Identifying preterm infants that had high levels of bilirubin for a prolonged period can possibly result in earlier screening and better monitoring for developmental disorders. Several studies have shown the benefits of early diagnosis on child developmental outcomes (264, 265), and these results may help identify children with developmental disorders earlier.

D. Strengths & limitations

The case-control study design of SEED has many advantages and disadvantages. While we are limited in making causal inferences or inferences about risk, the case-control study design allows us to evaluate the association between a rare exposure and rare outcome, such as the case in this analysis. Strengths and limitations from this study are discussed below.

Selection bias

SEED is a population-based study where potential controls were identified by randomly sampling birth records of children born in the specified date range to mothers that reside in the catchment area. However, not all who were invited to participate in the study were enrolled or completed the necessary data collection steps. In fact, 64% of ASD and DD families and 68% of POP families that were invited never responded (26). Of those that were contacted, 43% of ASD and DD families and 25% of POP families consented to participate (26). Our analysis also excluded a subset of the study sample that had an incomplete maternal interview or medical record abstraction. It is possible for bias to be introduced if the association between our exposures and ASD differs among participants and non-responders.

It is possible that the population comparison group may not be representative of the true population. If this is true, then the assumption of exchangeability may not hold. In which case, we cannot assume that the case and control populations are drawn from the same target population. We observed differences between the case groups and the control group that may be due to study participation. For example, the control group was more likely to be White, younger, more likely to be educated, and less likely to smoke. While we adjusted for many of these variables in our analysis, if any of these variables serve as a collider in a confounding path, then controlling for that variable may induce bias.

Information bias

Our study had different sources of data to ascertain prenatal and neonatal complications. These included a caregiver phone interview, abstracted medical records, mailed-in questionnaires, and diagnostic results from clinical and laboratory measurements from the medical record. While having different sources of data for one exposure may be beneficial, there may be discrepancies across data sources or missing data. Potential exposure misclassification may be present with each data source.

While data from medical records may be more valid since it does not really on retrieving retrospective memories as maternal recall, present information abstracted from the medical records may be incomplete. For example, in many cases the abstraction form did not have a field to enter 'No' for not having a diagnosis, and medical records often record only conditions that exist rather than note the absence of a condition. It was therefore not always possible to distinguish not having a condition versus an incomplete medical record. Even if abstraction of the available medical record was complete, the data available to the study depended on the hospital and clinic sending a complete copy of the records to the study for abstraction. It was difficult to determine the completeness of the medical records obtained, but it is possible that important information regarding variables in these analyses could not have been sent. One such piece of information was clinical and laboratory measurements. In clinical practice most mothers have blood pressure measurements taken at every visit, most mothers have an OGTT after the 24^{th} week, and most newborns have a bilirubin measurement before being discharged. However, complete data on these measurements were not observed in our study.

The caregiver interview was conducted at the time of enrollment and contained questions regarding the mother's pregnancy and the neonatal period. The child was required to be 30 to 68

months at enrollment and is therefore possible mothers had poor recall due to long period of time. Factors that can affect recall can include maternal education, parity, and plurality, which all showed an association to our exposure and outcome groups. Because we defined exposures in our analyses as any report of the condition, either through maternal interview or medical record, it is possible that poor recall can lead to exposure misclassification that impacts study results. It is possible that this misclassification is differential by outcome status resulting in recall bias, where mothers of affected children may be more likely to search their memories and remember earlier events (267).

In our first aim, we evaluated associations among 'Confirmed' conditions, or conditions that were reported in both the medical record and through maternal interview. We found much stronger associations among mothers with these 'Confirmed' conditions, which may be indicative of exposure misclassification due to poor maternal recall or incomplete data in the medical record.

To evaluate the agreement between data sources we calculated Cohen's kappa (κ) with 95% confidence intervals (CI). The strength of agreement was interpreted as: <0 none, 0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 as substantial, 0.81–1 nearly perfect. We compared differences in agreement by outcome status, study site, maternal education, race, ethnicity, language spoken, parity, and events of the child's birth. We also conducted a two-stage logistic regression analysis that includes an offset term to adjust for the probability of agreement due to chance for identifying covariates that are predictive of agreement. This method allows parameters to be interpreted as the true odds ratio for agreement divided by the true odds ratio under chance agreement. Valid standard errors were obtained using the jackknife variance estimator. These results showed substantial agreement for maternal diabetes and maternal

hypertension, but fair agreement for neonatal jaundice. The lower agreement for neonatal jaundice may be due to mild jaundice not receiving a formal diagnosis and delayed timing of the bilirubin laboratory test. It is possible that any disagreement could have been due to potential exposure misclassification, which can bias our results toward the null. To address this issue, we created 'Confirmed' categories for maternal diabetes and hypertension, which required report of the condition from two sources, and for neonatal jaundice we created 'Definite' (received treatment), 'Probable' (diagnosis in medical record), and 'Possible' (only maternal report available) categories.

Mean elapsed time between data sources was 4.8 years (SD=0.68). Medical records and maternal data substantially agreed on maternal diabetes and maternal hypertension, but agreement was fair for neonatal jaundice. For maternal diabetes and hypertension, agreement varied by outcome status, study site, number of children, language spoken, race/ethnicity, and if the pregnancy resulted in a preterm delivery (for diabetes). No difference in agreement for maternal diabetes and hypertension was seen by maternal education level. No factors significantly predicted agreement.

For neonatal jaundice, agreement varied by the same factors as with maternal diabetes and hypertension, as well as method of delivery, and breastfeeding status during the neonatal period. No difference in agreement for neonatal jaundice was seen by maternal education level, preterm delivery, or NICU admission. Maternal awareness of neonatal jaundice may vary by severity or duration of the condition, especially if jaundice resolves without medical care. Positive maternal report may identify visible jaundice when bilirubin measurements do not meet the diagnostic criteria. Negative maternal report may identify jaundice not visible, but where bilirubin levels meet diagnostic criteria. Such disagreement may be reflected in our analysis,

where agreement was lower among infants in the third quartile of hyperbilirubin, the group most likely representing this situation. Only this category was significantly less likely to predict beyond chance agreement. The substantial agreement for perinatal conditions may reassure the use of only one data source evaluating the association between perinatal risk factors and childhood outcomes.

Sample size

The case-control study design increases statistical power to detect associations with a rare outcome. In our study, we were able to detect associations with prenatal and neonatal complications and ASD with fairly precise confidence intervals. While we had sufficient sample size for main effects, some associations among sub-classifications of the exposures and outcome show imprecise results. However, some of these associations were strong enough to reach statistical significance. Yet, it is possible that some spurious associations may be observed due to small sample size. Further studies should be conducted in larger sample sizes to confirm associations between subclassifications of maternal diabetes and hypertension, and ASD. The association between neonatal jaundice and ASD should also be evaluated in a study sample with a larger preterm population.

Strengths

The SEED population-based study design is specifically tailored to identify etiologic factors contributing to ASD. SEED is also a multi-site study and generalizable to the U.S. population, with sites in different regions of the U.S., two of which recruited Spanish-speaking participants. The study has two comparison groups to distinguish if etiological factors are specific to ASD or broader neurodevelopment. The case-control study design of SEED is ideal for investigating rare outcomes such as ASD and DD. The large SEED population made it

possible to assess for interaction and provided stratum specific estimates for the association between jaundice and ASD.

Defining conditions from either the medical record or maternal report could have lead to exposure misclassification, but it also reduced the possibility of halving false negatives for the exposure. In addition, we found good agreement between data sources to confirm their validity. Data from the medical record was abstracted from clinical notes and measurements, not just ICD9 codes that could be used to refer a patient for testing. For the first aim, we were also able to address validity by creating a subclassification for a 'Confirmed' condition where medical record and maternal report were in agreement and removing those without a confirmed condition from the dataset. For the second aim, we created subclassifications based on if a case was considered 'Definite' (treated), 'Possible' (medical record diagnosis), or 'Probable' (only by maternal report). These subclassifications require a greater level of detail on exposure status that is not available in other studies.

One of the greatest advantages of SEED is the in-person assessment conducted to confirm ASD using the ADOS and ADI-R, the gold standard instruments for ASD diagnosis. Clinical evaluation reduces outcome misclassification compared to other studies that use school records, ICD-9 codes, or administrative datasets to classify a child as having ASD or DD. Children in the DD and POP group were also screened, reducing outcome misclassification by evaluating undiagnosed children.

E. Future directions

Results from this study show an association between prenatal and neonatal conditions and ASD and DD. Further studies are needed to determine whether these associations are causal, or if other covariates involved are influencing these observations. One main area for future study will

be to determine if there is a difference in association with complications that are chronic versus acute. In specific aim 1, we observed a stronger association with preexisting/chronic hypertension. And in specific aim 2, we observed a stronger association with bilirubin levels that were elevated, but not enough to warrant treatment. Both of these findings suggest that a chronic condition may have a stronger effect on neurodevelopment. However, our study was limited in determining how long a condition was present. Future studies involving more clinical measurements over time, such as serial blood pressures and transcutaneous bilirubin, can evaluate the effect of a prolonged exposure. These studies can also help establish sensitive time windows for environmental exposures associated with ASD.

Preterm birth was an important variable to consider in our studies. In specific aim 1, it is strongly associated with maternal hypertensive disorders. And in specific aim 2, we observed an interaction between preterm birth and neonatal jaundice. It is difficult to determine in these cases whether the causal pathway operates through more severe complications or preterm birth, or a combination of the two. Further studies can be conducted in preterm populations to identify biological mechanisms by which environmental exposures are associated with ASD.

And finally, future studies should evaluate the feasibility and benefits of conducting early screening for ASD based on results from this study. Targeting early intervention and developmental service programs to children that are born to hypertensive mothers, born preterm, or with confirmed neonatal jaundice could result in early identification of ASD or DD. However, studies are needed to confirm this.

F. Conclusions

The observations reported here provide further evidence that prenatal and neonatal complications may have a role in the development of ASD and DD. These observations also

support the role of preterm birth in conjunction with these complications, and merit further study on these potential risk factors.

Continued work to identify specific risk factors for ASD is necessary, many other less common prenatal and neonatal complications may also be associated with ASD. Together, these studies can help elucidate the etiology of ASD. Identification of modifiable risk factors can help reduce the prevalence of ASD, or can increase early diagnosis and early treatment.

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