# CONTRIBUTIONS OF PRENATAL INFECTION AND ANTIBIOTIC EXPOSURE TO OFFSPRING INFECTION AND ASTHMA RISK

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A Dissertation
Submitted to
the Temple University Graduate Board

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In Partial Fulfillment
of the Requirements for the Degree
DOCTOR OF PHILOSOPHY

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August 2020

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## ABSTRACT

Increasing evidence from both animal and human research implicates prenatal infection in the development of long-lasting disruptions in immune function of offspring, including an increased risk of infection and allergic disorders, such as asthma. Infection, specifically during early life, also has been repeatedly associated with subsequent risk of asthma in childhood. Moreover, antibiotic exposure, during both the pre- and post-natal period, has been linked with several of these immune-related health outcomes. The aim of the current study was to investigate the role of maternal infection during pregnancy in relation to the development of offspring infection and subsequent asthma risk. Further, we considered the confounding factors of pre- and postnatal antibiotic exposure in the context of offspring infection and asthma inception. Participants (N=2062) were pregnant women and their offspring prospectively enrolled in a longitudinal birth cohort study with follow-up data through childhood. Extensive health information, including data on illness episodes and antibiotic exposures, was obtained from medical records for both mothers and their offspring. Results indicated that second trimester prenatal infection was associated with an increased risk of offspring infection from birth through age five. Both offspring infection and antibiotic exposure from birth through age five were significantly associated with a diagnosis of asthma in children at age five. Offspring infection was found to mediate the relationship between mothers' second trimester infection and offspring asthma. Sensitivity analyses suggested that our results primarily were due to infections and not antibiotic use, given that findings were replicated when restricting analyses to samples of mothers and offspring without antibiotic exposure. These findings suggest fetal origins of offspring infection and asthma risk.

This dissertation is dedicated to:

My parents, Karen and Joe, for supporting me in all things
and allowing me the luxury to follow my dreams;

My brother, Chris, and my sister-in-law, Liz, for
showing me the true meaning of family;

And my partner, Jesse, for being a never-ending source of optimism,
laughter, and comfort; you have made Philadelphia feel like home.

## ACKNOWLEDGMENTS

First, I would like to express my deepest gratitude to my graduate advisor, Dr. Lauren Ellman, whose support and guidance over the last five years has been instrumental in shaping my development as a psychologist. I also would like to thank the other members of my dissertation core committee, Dr. Lauren Alloy and Dr. Thomas Olino, for their generous contributions throughout the preparation of this dissertation, as well as during the defense of my preliminary examination and dissertation proposal. I am also very grateful to my graduate faculty readers, Dr. Debbie Bangasser, Dr. Marsha Weinraub, and Dr. Mathieu Wimmer for their time and input in this endeavor.

There are several other individuals – faculty members, clinical supervisors, lab members, and "swell" cohort mates – who have been critical to my growth, happiness, and success throughout my graduate school career. In particular, I would like to thank Dr. Richard Heimberg and the wonderful women of the AACT; I have learned so very much from each and every one of you. I am also especially grateful to our lab manager, Seth Maxwell, whose help throughout this project has been nothing short of life saving. I also would like to give a special thanks to the CHDS team in Berkeley, California for their tireless efforts maintaining and growing one of the most valuable birth cohort studies in the United States; thank you for your constant support and collaboration over the last five years.

Finally, thank you to my incredibly supportive family and friends, including my dog, Charlie, who somehow convinced me that getting a puppy in graduate school was a great idea.

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# MANUSCRIPT IN JOURNAL ARTICLE FORM

## Introduction

Accumulating data (primarily from preclinical studies) provide evidence of a link between maternal infection during pregnancy and enduring alterations in immune processes of offspring. Several studies have shown that maternal immune activation (MIA) during pregnancy can induce fetal inflammatory responses, evidenced by heightened expression of pro-inflammatory cytokines in the placenta, amniotic fluids, and neonatal tissues, especially the fetal brain (reviewed in Boska, 2010; Patterson, 2009). Additionally, a small, but growing body of work has demonstrated longer-lasting immunological impairments in offspring following MIA, including disruptions in both innate and adaptive immunity (Hsiao, McBride, Chow, Mazmanian, & Patterson, 2012; Mandal et al., 2013; Ponzio et al., 2013). One study found that offspring of immune-activated mothers display systemic deficits in a number of T regulatory cells and hyperresponsiveness of $CD4^+$ T cells, components of the immune system that are critical for suppressing immune responses and preventing autoimmune diseases by maintaining immunological self-tolerance (Hsiao, 2012). Similarly, other researchers have found that, following prenatal MIA and postnatal immune stimulation, offspring exhibit accelerated development and heightened responsiveness of T helper (Th) cell subsets, factors that are central to the adaptive immune response and have been shown to mediate immunopathology in several disorders (Mandal, Marzouk, Donnelly, & Ponzio, 2010, 2011; Ponzio, Servatius, Beck, Marzouk, & Kreider, 2007). In similar experimental models, prenatal MIA has been linked to upregulation and expression of innate immune

cells in offspring (Mandal et al., 2013; Ponzio et al., 2013). Altogether, these findings have led to the suggestion that prenatal MIA can induce a pro-inflammatory phenotype in offspring, potentially leading to changes in immune function that persist throughout the offspring's postnatal life and contribute to more robust and exaggerated responses to subsequent postnatal immune challenges (Mandal et al., 2013; Ponzio et al., 2013).

Despite burgeoning evidence in animal models of fetal programming of the offspring immune system, there have been few studies to examine this hypothesis in humans. To date, the preponderance of human studies in this area have focused on the association between maternal infection during pregnancy and immune alterations in offspring in relation to risk for offspring psychopathology (Patterson, 2009, 2011; Fineberg & Ellman, 2013). Evidence that a number of psychiatric disorders may originate, at least in part, during fetal development has led some researchers to hypothesize that exposure to prenatal infection may 'prime' the fetal brain and immune system, increasing vulnerability to immune alterations and immune-mediated diseases later in life (Bale et al., 2010; Bilbo & Schwarz, 2009; Merlot, Couret, & Otten, 2008). Nevertheless, little attention has been given to the impact of prenatal infection on offspring immune development and immune-related physical health outcomes, despite established associations between a number of psychiatric disorders and alterations in immune function (Fineberg & Ellman, 2013; Kohler, Krogh, Mors, & Benros, 2016; Patterson, 2011; Raison, Capuron, & Miller, 2006).

Only two studies to our knowledge have specifically examined the relationship between maternal infection during pregnancy and risk of infection in offspring. In the first of these studies, maternal self-report at birth of vaginal infection during pregnancy

significantly predicted a measure of offspring illness susceptibility (i.e., maternal report of frequency of medical attention and symptoms of infection) during the first six months of life (Betts, Williams, Najman, Scott, & Alati, 2014). However, this study was limited by its non-specific measurement of infant illness, as well as its narrow assessment of prenatal infection, which did not include other types of commonly occurring infections during pregnancy (e.g, respiratory infections, influenza). In the second study, using cohort data obtained from national Swedish registers, the authors found an increased risk for offspring hospitalization during childhood among mothers with a hospital diagnosis of a viral or bacterial infection during pregnancy (Blomström et al., 2016). Despite methodological improvements in the assessment of infection, which included a wide range of clinically diagnosed maternal and childhood infections, questions remain as to the nature of the relationship between less severe forms of prenatal infection (i.e., those not requiring hospitalization) and risk of offspring infection. Additionally, neither study addressed the impact of timing of prenatal infections on offspring illness; this is important given research suggesting that adverse offspring outcomes may depend on timing of infectious exposure during specific windows of developmental vulnerability (Brown & Derkits, 2010; Fineberg & Ellman, 2013).

A number of other studies have examined prenatal infection in relation to other health outcomes, including allergic disorders (Algert et al., 2011; Calvani et al., 2004; Collier et al., 2013; Hughes, Jones, Wright, & Dobbs, 1999; Pesce, Marcon, Marchetti, Girardi, & de Marco, 2014; Xu, Jarvelin, & Pekkanen, 1999; Xu, Pekkanen, Jarvelin, Olsen, & Hartikainen, 1999). A recent meta-analysis of ten studies demonstrated a positive association between maternal infection and risk of offspring asthma (OR = 1.55)

and eczema (OR = 1.36; Zhu, Zhang, Qu, & Mu, 2016). However, the authors noted substantial heterogeneity among the study designs, including differences in prenatal exposures, outcome measurements, and confounding factors, limiting the interpretative power of their findings. Somewhat related to these findings is a larger and more well established body of work linking viral respiratory infections during early life to the development and exacerbation of asthma (Beigelman and Bacharier, 2016; Busse, Lemanske, & Gern, 2010). In particular, infection-related wheezing during early life as a result of respiratory syncytial virus (RSV) and human rhinovirus (HRV) has been identified as an important determinant of future asthma (Bacharier et al., 2012; Jackson et al., 2008; Kusel et al., 2007; Sigurs et al., 2005; Zomer-Kooijker et al., 2014). Additionally, research suggests a direct link between severity of RSV infection in infancy and risk for asthma in early childhood, with greater risk of asthma following episodes that required hospitalization among full-term infants (Carroll et al., 2009; Escobar, Masaquel, Li, Walsh, & Kipnis, 2013). Despite findings that both pre- and postnatal infection are likely to increase risk for offspring asthma and related allergic disorders, no studies have examined whether childhood infection mediates the relationship between prenatal infection and development of offspring asthma.

However, potentially complicating the findings presented above is the growing body of research to support the role of prenatal and childhood antibiotic exposure in the development of a variety of immune-related health outcomes. Maternal antibiotic exposure during pregnancy has been found to be associated with increased risk of the development of allergic disorders in offspring, including asthma and atopic dermatitis (Dom et al., 2010; Jedrychowski, Galas, Whyatt, & Perera, 2006; McKeever, Lewis,

Smith, & Hubbard, 2002; Rusconi et al., 2007; Stensballe, Simonsen, Jensen, Bønnelykke, & Bisgaard, 2013). Further, in a recent population-based cohort study using Danish register data, prenatal antibiotic exposure was shown to significantly increase risk of infection-related hospitalizations in offspring during childhood (Miller et al. 2018). Outside of pregnancy, antibiotic use during early life has been associated with increased susceptibility to intestinal infections (Mai et al., 2011; Wilcox, 2003; Young & Schmidt, 2004), as well as a number of other severe health outcomes, including sepsis and death (Kuppala, Meizen-Derr, Morrow, & Schibler, 2011; Madan et al., 2012; Mai et al., 2013). Further, multiple studies have shown increased risk of allergic disorders in individuals treated with antibiotics during infancy and early childhood (reviewed in Francino, 2014, 2016). A recent meta-analysis of 22 studies found a small, but significant effect (OR = 1.26) of antibiotic exposure during the first two years of life on risk of eczema (Ahmadizar et al., 2016). Similarly, a handful of prospective studies, as well as several retrospective epidemiological studies, have demonstrated associations between antibiotic exposure in early life and risk of developing asthma in childhood (Kozyrskyj, Ernst, & Becker, 2007; Marra et al., 2009; Murk, Risnes, & Bracken, 2011; Risnes, Belanger, Murk, & Bracken, 2010). Nevertheless, debate remains in the literature as to role of pre- and postnatal antibiotic exposure in the development of allergic disorders, as findings to date have been inconsistent (Kuo et al., 2013; Murk et al., 2011) and a number of other prospective studies have failed to detect an association after controlling for several confounding factors, including presence of childhood infection (Celedon, Fuhlbrigge, Rifas-Shiman, Weiss, Finkelstein, 2004; Mai, Kull, Wickman, & Bergstrom, 2010; Su, Rothers, Stern, Halonen, & Wright, 2010; Wickens et al., 2008).

In the present study, using prospectively collected longitudinal cohort data, we investigate the role of maternal infection during pregnancy in relation to the development of offspring infection and asthma. Further, we consider confounding factors, specifically pre- and post-natal exposure to antibiotics, in the context of offspring infection and asthma inception. We hypothesize that prenatal infection, independent of prenatal antibiotic exposure, will increase risk for infection and subsequent asthma risk in offspring. Additionally, we predict that offspring infection will mediate the relationship between prenatal infection and development of offspring asthma.

## Method

### *Participants*

Study procedures were approved by the following Institutional Review Boards: the Public Health Institute, University of California-Los Angeles (UCLA) and Temple University. Participants were drawn from the Child Health and Development Studies (CHDS), a prospective, longitudinal cohort study that enrolled pregnant women from 1959-1967 (N = 19,044 live births) in a racially and socio-economically diverse county of California. The CHDS recruited almost all pregnant women receiving obstetric care through the Kaiser Foundation Health Plan (KFHP) at its clinics in the area (for review see van den Berg et al., 1988). Participants in the current study were drawn from a subset of the original CHDS cohort, which included offspring of mothers with abstracted medical record data of all contacts (e.g., doctor's visits, hospitalizations) with Kaiser facilities from birth (n = 4188). Due to funding limitations, medical record abstraction was restricted to distinct subsets of offspring within the total CHDS population of live born children. Additional medical abstractions were conducted on CHDS children who

had medical diagnoses and developmental difficulties; however these children were excluded from analyses given the potential for introduction of bias given that these medical abstractions were not chosen at random. The excluded subset consisted of CHDS children who were identified as having 1) severe congenital anomalies (n = 718), 2) non-severe congenital anomalies (n = 702), or 3) certain selected disabilities (n = 482). Among the remaining 2286 children, an additional 54 children had no further medical record data after the initial birth hospitalization and were excluded from study analyses. As there were multiple offspring from the same mother in the sample, the present study randomly selected one member of each sibling set for removal to eliminate non-independent observations (n = 170), resulting in a final analytical sample of 2062 children (see Fig. 1 for details). See Supplementary Table 6 for comparative demographics between our analytic sample of 2062 and the remainder of CHDS live births).

### *Demographic Variables*

Detailed sociodemographic information, including maternal age, parity, education, and race, was collected at all study timepoints. Maternal education at birth of her offspring was categorized as *'Less than or equal to High School'* or *'Greater than High School'*; maternal education was treated as representative of socioeconomic status for the present study, since this variable has been strongly correlated with other measures of SES (e.g., income) in this sample (Fineberg et al., 2016) and is often used in the literature as a proxy for postnatal adversity (Schlotz and Phillips, 2009). Maternal race was categorized as *'White'* vs. *'Non-white'* with the *'Non-white'* subsample comprised of *'African-American'* (21.1%) and *'Asian'* (5.0%). Offspring sex was categorized as *'Male'*

or '*Female'*. Parity (i.e., number of previous pregnancies) was categorized as 'First Child', 'Second Child,' or 'Three or more Children.'

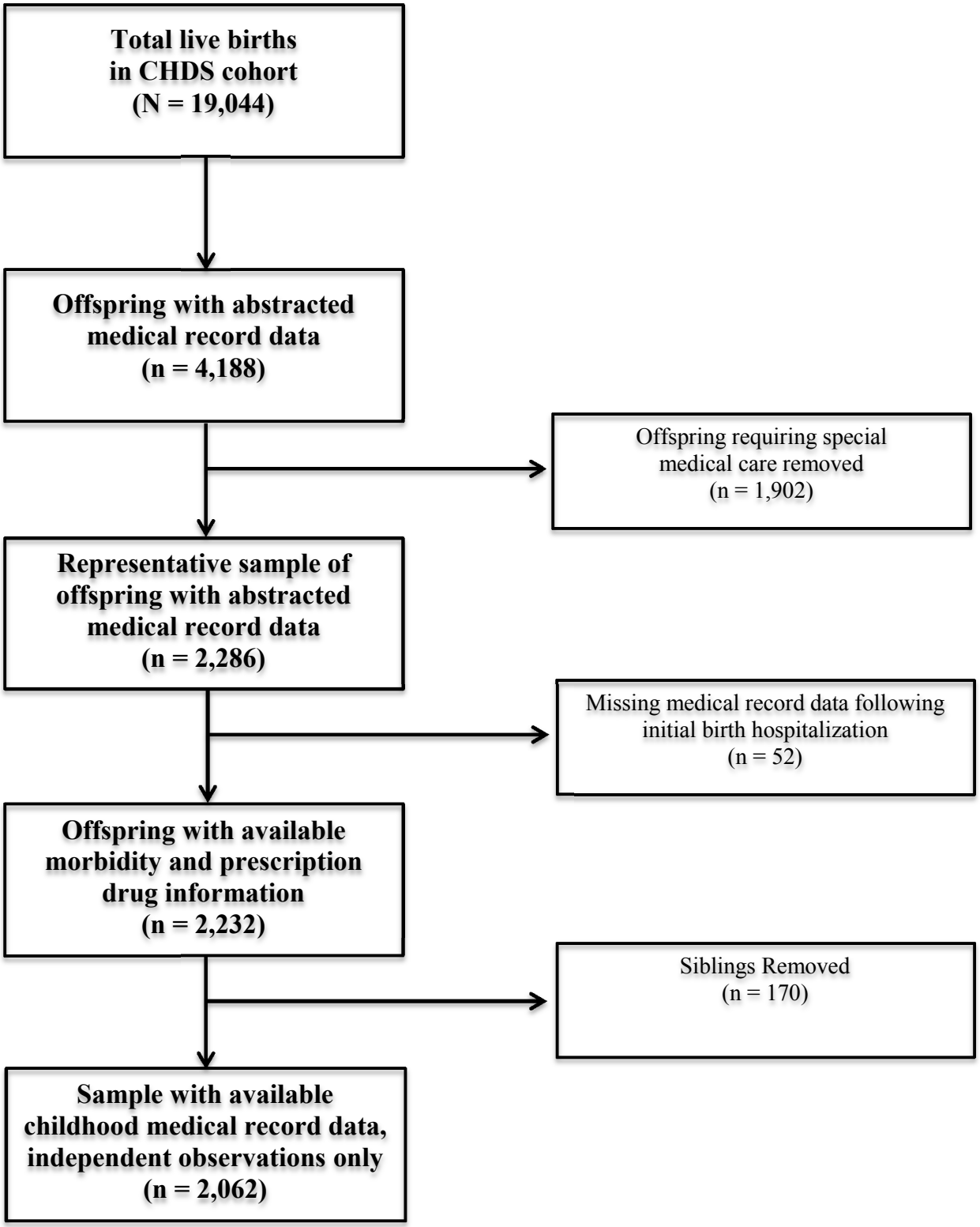


Figure 1. Flow chart of participants in current study.

## *Maternal Health Data*

### *Maternal Infection and Antibiotic Exposure during Pregnancy*

Maternal medical events were abstracted in detail from women's Kaiser medical records. Records included virtually all medical events occurring six months prior to the women's last menstrual period until after delivery, including detailed information regarding the presence and timing of a variety of maternal infections during pregnancy. All diagnoses of infection were identified according to the International Classification of Diseases, Seventh Edition (ICD-7; see Table 1). Infection variables were collapsed across categories (i.e., viral, bacterial, other) and a single dichotomous infection variable was created to indicate presence or absence of infection in each trimester, similar to previous CHDS studies (Brown *et al.* 2000; Babulas *et al.* 2006; Brown, Deicken *et al.* 2009; Brown, Vinogradov *et al.* 2009; Murphy et al., 2017; Parboosing *et al.* 2013).

Additionally, type, timing, and frequency of oral antibiotics taken in response to infection during pregnancy were recorded. Exposure to oral antibiotics was limited to broad-spectrum antibiotics because 1) they have been associated consistently with a variety of immune-related health outcomes in offspring (Francino, 2016; Vangay, Ward, Gerber, Knights, 2015), 2) they are designed to act on a wide-range of disease-causing bacteria in comparison to narrow-spectrum agents (Sarpong & Miller, 2015), and 3) at least 11 types of broad-spectrum antibiotics are known to cross the placenta and reach the fetus (Ledger & Blaser, 2013; Nahum, Uhl, & Kennedy, 2006). Tetracyclines, sulfonamides, select penicillins (e.g., Ampicillin) and select macrolides (e.g., erythromycin) were defined as broad-spectrum antibiotics, as these agents act on a wide range of bacteria (gram-positive and gram-negative) and have been classified similarly in

related studies (Borg & Camilleri, 2019; Kozyrsky et al., 2007; McKeever et al., 2002). Frequency of broad-spectrum antibiotic use by trimester was summed and antibiotic exposure was examined as a continuous dimensional variable.

*Delivery Type*

Extensive information on the events during labor and delivery, including method of delivery, also was abstracted from maternal obstetric records. Delivery type was categorized as *'vaginal'* or *'caesarian section'*.

## *Offspring Health Data*

*Offspring Infection and Antibiotic Exposure during Childhood*

Detailed health information was abstracted from medical records of offspring's visits to Kaiser pediatric clinics, specialty clinics, and hospitalizations. If medical care was received outside of Kaiser or elsewhere, permission was requested to contact the doctor or medical facilities and the obtained information was abstracted. From these records, medical data for every visit the child made, including diagnosed conditions, treatments, and prescriptions from birth to ages 9-12 was collected. Pediatric record abstraction for morbidity and prescription drug information occurred from birth (1960-1966) through 1972. Participant attrition increased gradually over subsequent study years leading to substantial missing data by middle childhood; therefore, the presence of childhood infections (identified according to ICD-8 codes, see Table 1) and/or antibiotic exposure was limited to the first five years of life. Frequency of both childhood infections and antibiotic exposures during this period were summed and examined as continuous dimensional variables. Similar to prenatal antibiotic exposure data, offspring antibiotic use was limited to treatment with broad-spectrum antibiotics.

| Table 1. Diagnoses of infections for mothers during pregnancy and offspring from birth through age five | | |
|---|---|---|
| | Prenatal (ICD-7) | Offspring (ICD-8) |
| Viral | 010, 081-082*, 085-087, 089, 092-093, 470-471, 473, 480-483, 492, 500, 518, 519* | 052-057, 065, 070, 072, 074-075, 079, 360-369, 460-466, 470-474, 480, 485, 490-492, 500, 508, 511*, 518*, 561 |
| Bacterial | 020, 030, 045*, 049*, 051, 064, 391, 400-402, 490, 605, 609, 622 | 003-005, 011, 032-034, 038, 091-092*, 098*, 380-382, 390-392*, 481-482, 535, 540-543, 580-584, 590, 595, 597, 598, 680-686 |
| Other | 039, 630 | 099*, 110-112, 124, 130*, 133 |

*Note*. * Indicates diagnosis codes that were not given to any participants in analytic sample.

*Childhood Asthma*

Abstracted medical record data also indicated whether offspring ever received a diagnosis of asthma during childhood. The presence of asthma was defined by a diagnosis of *'Respiratory Allergy'*, which encompassed the following conditions: wheezing or allergic bronchitis, asthmatic bronchitis, asthma or hay fever. Presence or absence of the diagnosis at five years of age was used in the current study.

*Breastfeeding*

Any reference to breastfeeding by mothers during a pediatric visit was noted and abstracted from medical records. Since mothers were not directly asked if and when they breastfed, the length of the breastfeeding period could not be accurately estimated. Therefore, breastfeeding was examined as a dichotomous variable, in which any maternal

report of breastfeeding indicated 'presence' of breastfeeding. Given the limitations to this method of data abstraction, breastfeeding information was only available for n=1230 women in our sample.

## Data Analysis

Bivariate relationships were examined between the following variables and the main independent and dependent variables: maternal age, maternal race, maternal education, parity, delivery type, infant sex, and breastfeeding. Variables significantly correlated with at least one predictor variable and at least one outcome variable were added as covariates in statistical models. Negative binomial regression (Hilbe, 2011) was conducted to evaluate the association between each trimester of prenatal infection and frequency of offspring infection, as well as offspring antibiotic exposure, in separate models controlling for covariates. These analyses were repeated using prenatal antibiotic exposure by trimester as the predictor variable. Negative binomial regression, which is a generalization of Poisson regression, was used in analyses as it is designed to analyze count variables and corrects for over-dispersion in the data (Beck & Tolnay, 1995; Cameron & Trivedi, 1998). Model results were reported using Incidence Rate Ratios (IRR). Separate logistic regressions also were conducted to determine whether frequency of childhood infection and frequency of childhood antibiotic exposure predicted asthma in offspring at age 5, controlling for covariates.

To evaluate the potential for the confounding effect of antibiotic exposure on 1) the relationship between prenatal infection and offspring infection, and 2) the relationship between offspring infection and asthma, two sensitivity analyses were performed. First, we restricted the analysis only to mothers who had not had antibiotic exposure during

pregnancy. Second, we restricted the analysis only to those offspring that had not been exposed to antibiotics during the first 24 months of life (analyses restricted to offspring without antibiotic exposure during the first five years of life resulted in a sample size too small to interpret). Additionally, multicollinearity was assessed among the infection and antibiotic study variables using variance inflation factor (VIF) and examination of the correlation matrix. Study variables with a correlation greater than 0.5 or a VIF greater than 5 were not included together as predictors in regression models.

To test whether frequency of offspring infection mediated the relationship between presence of prenatal infection and offspring asthma at age five, Preacher and Hayes SPSS indirect bootstrapping macro for mediation was used, which has been considered the most appropriate test for mediation with a single mediator (Preacher and Hayes, 2008). Significance of mediation was determined by examination of the 95% bias-corrected bootstrap confidence interval (CI). There are no p-values associated with this method, and significant mediation was declared if the CI did not include zero. All analyses were performed using IBM SPSS (v25). Statistical significance was based on $p < 0.05$ and all tests were two-tailed.

## Results

### *Bivariate Analyses*

Descriptive statistics and bivariate correlations are presented in Table 2. Prenatal infection during the first and second trimester, as well as first trimester antibiotic exposure, were found to be significantly positively associated with frequency of offspring infection and antibiotic exposure from birth through age five. Additionally, significant positive associations were observed between asthma at age five and offspring infection

and antibiotic exposure. Parity was related inversely to offspring infection, but was not related to other main study variables. Maternal education was associated significantly with offspring infection and antibiotic exposure; higher education was associated with fewer infections and antibiotics in offspring. Breastfeeding was associated significantly with offspring infection, such that mothers who breastfed had offspring with a greater number of infections; however no significant relationship was observable between breastfeeding and any other outcome or predictor variables. Maternal race was associated positively with offspring infection and antibiotic exposure, such that those identifying as white had a greater number of infections and antibiotic exposures from birth through age five. Further, race was related inversely to asthma; non-white offspring were more likely to have a diagnosis of asthma at age 5 than white offspring. Given associations with several main study variables, race and maternal education were controlled for in all analyses. Parity also was controlled for in all analyses, given the strong *a priori* rationale for its inclusion and association with at least one main study variable.

### *Offspring Infection and Antibiotic Exposure*

Adjusted and unadjusted negative binomial regression analyses are reported in Table 3. Adjusted analyses indicated that presence of prenatal infection during the second trimester was associated with higher frequencies of offspring infection from birth through age five. Inspection of multicollinearity among prenatal infection and prenatal antibiotic variables revealed no indication that predictor variables were highly correlated. Therefore, analyses were further adjusted for second trimester prenatal antibiotic exposure; findings linking prenatal infection during the second trimester to offspring infection remained significant after controlling for prenatal antibiotic exposure (IRR =

1.22; 95% CI = 1.07-1.39; $p$ = .002). First trimester infection was associated with non-significant, trend level increases in offspring infection. There were no significant associations between prenatal antibiotic exposure during any trimester and offspring infection. Prenatal infection during the first and second trimesters also was associated significantly with higher frequencies of offspring antibiotic exposure from birth through age five.

Table 2. Descriptive statistics and bivariate correlations of study variables (n = 2,062)

| Measure | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1: T1 Infection | -- | .09** | .02 | .37** | .07** | .01 | .07** | .07** | .00 | .02 | .02 | .00 | .00 | .02 | .06 | .01 |
| 2: T2 Infection | | -- | .05* | .04 | .30** | .05* | .12** | .13** | .02 | -.01 | .03 | .01 | .01 | .01 | .01 | .01 |
| 3: T3 Infection | | | -- | .00 | .04 | .32** | .03 | .04 | .00 | -.03 | .03 | .02 | .00 | .01 | .04 | .00 |
| 4: T1 Antibiotic | | | | -- | .05* | .03 | .05* | .05* | .03 | .00 | .06* | -.01 | .04 | .03 | .03 | .00 |
| 5: T2 Antibiotic | | | | | -- | .10** | .04 | .03 | -.02 | .03 | .02 | -.03 | .04 | -.02 | .04 | -.02 |
| 6: T3 Antibiotic | | | | | | -- | .03 | .04 | -.02 | -.01 | .04 | .04 | .03 | .01 | -.01 | .00 |
| 7: Child Infection | | | | | | | -- | .81** | .23** | .02 | -.11** | -.08** | .17** | .01 | .07* | -.03 |
| 8: Child Antibiotic | | | | | | | | -- | .25** | .03 | -.04 | -.07** | .16** | .01 | .01 | -.03 |
| 9: Asthma | | | | | | | | | -- | .01 | -.04 | -.02 | -.06** | -.01 | -.01 | -.04 |
| 10: Gravida Age | | | | | | | | | | -- | .44** | -.14** | -.03 | -.05* | .08** | .04 |
| 11: Parity (≥ 3) | | | | | | | | | | | -- | -.10** | .11** | .01 | -.08** | .02 |
| 12: Educ (>H.S.) | | | | | | | | | | | | -- | .03 | -.03 | -.17** | -.03 |
| 13: Race (White) | | | | | | | | | | | | | -- | -.03 | .14** | -.01 |
| 14: Del (Vaginal) | | | | | | | | | | | | | | -- | -.08** | -.01 |
| 15: Breastfed | | | | | | | | | | | | | | | -- | .03 |
| 16: Sex (Female) | | | | | | | | | | | | | | | | -- |
| **Mean** | -- | -- | -- | .05 | .05 | .06 | 8.08 | 3.62 | -- | 28.2 | 2.26 | -- | -- | -- | -- | -- |
| *(SD)* | | | | (.24) | (.24) | (.25) | (5.32) | (3.22) | | (5.90) | (2.05) | | | | | |
| **N** | 291 | 346 | 285 | 102 | 98 | 98 | 1969 | 1733 | 180 | -- | 1178 | 982 | 1516 | 1961 | 470 | 1088 |
| (%) | (14.1) | (16.8) | (13.8) | (4.9) | (4.8) | (4.8) | (95.5) | (84.0) | (8.7) | | (57.1) | (48.3) | (73.5) | (95.1) | (22.8) | (52.8) |

Note: T1 = Trimester 1; T2 = Trimester 2; T3 = Trimester 3; Antibiotic = Broad Spectrum Antibiotic Exposure; Parity (≥ 3) = Parity (Three or more children); Educ (>H.S) = Education of Gravida (Greater than High School Education); Del = Delivery Type; Race Reference Category is Non-White; Delivery Type Reference Category is Caesarian Section; Gravida Education Reference Category is Less Than or Equal to High School; Parity Reference Category is First Child; The following variables had missing data: gravida age (n = 6); parity (n=1); maternal education (n = 29); race (n=7); delivery type (n = 13); breastfed (n = 832); Probability: *$p$<.05; ** $p$<.01.

Table 3. Adjusted and unadjusted negative binomial regression models predicting frequency of offspring infection from birth through age five, and frequency of offspring antibiotic exposure from birth through age five (n = 2,062).

| | **Frequency of Offspring Infection, Adjusted** | | | **Frequency of Offspring Infection, Unadjusted** | | |
|---|---|---|---|---|---|---|
| **Exposure** | **IRR** | **95% CI** | ***p*-value** | **IRR** | **95% CI** | ***p*-value** |
| Infection | | | | | | |
| T1 Infection | 1.13 | .99-1.29 | .061† | 1.14 | 1.0-1.30 | .051† |
| T2 Infection | 1.22 | 1.08-1.38 | .001** | 1.22 | 1.08-1.38 | .001** |
| T3 Infection | 1.07 | .94-1.23 | .301 | 1.07 | .93-1.22 | .348 |
| BS Antibiotic | | | | | | |
| T1 Antibiotic | 1.10 | .92-1.32 | .296 | 1.12 | .93-1.34 | .221 |
| T2 Antibiotic | 1.12 | .93-1.36 | .245 | 1.11 | .92-1.35 | .277 |
| T3 Antibiotic | 1.08 | .89-1.32 | .418 | 1.08 | .89-1.31 | .421 |
| | **Frequency of Offspring Antibiotic Exposure, Adjusted** | | | **Frequency of Offspring Antibiotic Exposure, Unadjusted** | | |
| **Exposure** | **IRR** | **95% CI** | ***p*-value** | **IRR** | **95% CI** | ***p*-value** |
| Infection | | | | | | |
| T1 Infection | 1.17 | 1.02-1.35 | .024* | 1.19 | 1.03-1.36 | .015* |
| T2 Infection | 1.31 | 1.15-1.49 | .000** | 1.31 | 1.16-1.49 | .000** |
| T3 Infection | 1.11 | .96-1.28 | .160 | 1.10 | .96-1.27 | .165 |
| BS Antibiotic | | | | | | |
| T1 Antibiotic | 1.15 | .95-1.39 | .153 | 1.17 | .97-1.42 | .101 |
| T2 Antibiotic | 1.11 | .90-1.36 | .336 | 1.12 | .91-1.37 | .280 |
| T3 Antibiotic | 1.13 | .92-1.39 | .236 | 1.15 | .94-1.4 | .191 |

Note: Adjusted analyses controlled for maternal education, race, and parity; The following variables had missing data: maternal education (n = 29); race (n = 7); parity (n = 1); T1 = Trimester 1; T2 = Trimester 2; T3 = Trimester 3; BS = Broad Spectrum; IRR = Incident Rate Ratios; CI = Confidence Interval; †p < .10; *$p$<.05; ** $p$<.01.

### *Offspring Asthma*

Adjusted and unadjusted logistic regression analyses are presented in Table 4. Adjusted analyses indicated that higher frequencies of offspring infection from birth through age five were associated significantly with a diagnosis of offspring asthma at age five. Additionally, higher frequencies of offspring antibiotic exposure from birth through age five were found to be associated significantly with offspring asthma at age five. Given findings of multicollinearity between offspring infection and offspring antibiotic exposure ($r = 0.81$, VIF = 2.89), these variables could not be examined together in relation to offspring asthma risk.

### *Sensitivity Analyses*

The results of the sensitivity analyses are shown in Table 5. Among mothers who had no antibiotic exposure during pregnancy, there still was an association with respect to second trimester infection and offspring infection. An association that approached significance was observed between first trimester infection and offspring infection. Additionally, when restricting analyses to children without exposure to antibiotics from birth to 24 months, offspring infection during this period still was related significantly to development of asthma at age five.

Table 4. Adjusted and unadjusted logistic regression analyses for offspring infection and offspring antibiotic exposure predicting offspring asthma at age five.

| | **Offspring Asthma, Adjusted** | | | **Offspring Asthma, Unadjusted** | | |
|---|---|---|---|---|---|---|
| **Predictor** | **OR** | **95% CI** | ***p*-value** | **OR** | **95% CI** | ***p*-value** |
| Maternal Education | 1.01 | .73-1.4 | .936 | -- | -- | -- |
| Race | 2.36 | 1.65-3.39 | .000** | -- | -- | -- |
| Parity | .94 | .86-1.02 | .151 | -- | -- | -- |
| Child Infection | 1.17 | 1.13-1.20 | .000** | 1.15 | 1.12-1.18 | .000** |
| | | | | | | |
| Maternal Education | 1.01 | .73-1.40 | .953 | -- | -- | -- |
| Race | 2.41 | 1.68-3.46 | .000** | -- | -- | -- |
| Parity | .91 | .83-.10 | .042* | -- | -- | -- |
| Child Antibiotic | 1.28 | 1.22-1.33 | .000** | 1.25 | 1.20-1.30 | .000** |

Note: OR = Odds Ratio; CI = Confidence Interval; *$p$<.05; ** $p$<.01.

Table 5. Adjusted sensitivity analyses examining (1) first and second trimester prenatal infection among mothers with no antibiotic exposure during pregnancy, and (2) offspring infection among children with no antibiotic exposure during the first 24 months of life.

| *(1) Mothers with no prenatal antibiotic exposure (n = 1,791)* | | | | |
|---|---|---|---|---|
| | | **Offspring Infection** | | |
| | **n** | **IRR** | **95% CI** | ***p*-value** |
| T1 Infection | 189 | 1.17 | .99-1.37 | .060† |
| T2 Infection | 248 | 1.20 | 1.05-1.39 | .010* |
| (*2) Offspring with no antibiotic exposure from birth to 24 months (n=601)* | | | | |
| | | **Offspring Asthma at Age 5** | | |
| | **n** | **OR** | **95% CI** | ***p*-value** |
| Infect 0 to 24mo | 30 | 1.37 | 1.08-1.73 | .009** |

Note: Adjusted analyses controlled for maternal education, race, and parity; T1 = Trimester 1; T2 = Trimester 2; T3 = Trimester 3; Infect 0 to 24mo = Frequency of infection from birth to 24 months; IRR = Incident Rate Ratios; OR = Odds Ratio; CI = Confidence Interval; †p < .10; *$p$<.05; ** $p$<.01.

### *Mediation Analyses*

Bootstrapping analyses for indirect effects indicated that the number of offspring infections from birth through age five mediated the relationship between prenatal second trimester infection and offspring diagnosis of asthma at age five (Bias Corrected CI: 0.1502– 0.3682).

## Discussion

This is the first human study to find that prenatal exposure to infections, specifically during the second trimester, is associated with subsequent increases in diagnoses of offspring infection from birth through age five. A similar, but weaker, trend-level association also was found between first trimester infection and offspring infection; however, no association was observed with third trimester infection or prenatal antibiotic exposure. Further, frequencies of both offspring infection and antibiotic exposure from birth through age five were associated significantly with a diagnosis of asthma in children at age five. Offspring infection was found to mediate the relationship between second trimester infection and offspring diagnosis of asthma at age five. Finally, sensitivity analyses suggested that our results primarily were due to infections and not antibiotic use, given that findings were replicated when restricting analyses to samples of mothers and offspring without antibiotic exposure. Similarly, associations established in primary analyses remained after controlling for antibiotic exposure, suggesting that prenatal infection is an independent risk factor for offspring infection, as is offspring infection for risk of asthma at age five.

Our findings of an association between maternal infection during pregnancy and offspring infection are consistent with previous research (Betts et al., 2014; Blomström et

al., 2016). Nonetheless, this is the first study of its kind to demonstrate that timing of prenatal infection, specifically during mid-, and possibly early, gestation, may be important in portending risk for offspring infection. Earlier studies have lacked data as to whether there is a critical period during pregnancy for the subsequent development of offspring infection (Betts et al., 2014; Blomström et al., 2016). However, a handful of studies examining prenatal infection in relation to allergic disorders, such as asthma and eczema, have suggested that both first (Hughes et al., 1999; Pesce et al., 2014; Xu, Jarvelin, et al., 1999; Xu, Pekkanen, et al., 1999) and third (Calvani et al., 2004; Pesce et al., 2014) trimester infections may be important in the development of allergic disorders in offspring. Still, at least one study found no evidence that timing of infectious exposure during pregnancy affected risk of asthma in offspring (Algert et al., 2011). Although we did not find an association between prenatal infection and offspring asthma at age five, variability in the type and severity of maternal infections (e.g., influenza, febrile episode, urinary tract infection, respiratory tract infection, chorioamnionitis), offspring outcomes of interest (e.g., asthma, eczema, hay fever), case ascertainment of mothers and children (e.g., hospital admission, outpatient diagnosis, self-report of symptoms), and age of offspring at follow up, may help account for the discrepancy in findings. Future studies able to determine the relative importance of type and timing of prenatal infection on offspring infection and allergies are warranted.

Our results also provide support for findings linking offspring infection to increased risk of asthma. In previous prospective studies, lower respiratory tract infections during early life have been shown to be significant risk factors for childhood allergic disorders, especially asthma (Arshad, Kurukulaaratchy, Fenn, & Matthews, 2005;

Beigelman and Bacharier, 2016; Illi et al., 2001; Ramsey et al., 2007; Sigurs et al., 2005). Further, a number of studies have found no significant associations between early antibiotic exposure and allergy development after adjustment for respiratory tract infections, although this remains a matter of debate (Celedon et al., 2004; Mai et al., 2010; Wickens et al., 2008). Our study extends prior research by suggesting that the relationship between early life infection and asthma development may have prenatal determinants. Although the biological mechanisms underlying this relationship remain unclear, it is possible that maternal infection during pregnancy could lead to disruptions in the offspring's developing immune system that predispose infants to more severe (or more frequent) infections and subsequently greater asthma risk. In keeping with this hypothesis, there is evidence to suggest that children who go on to develop asthma by school age exhibit aberrant immune responses to pathogenic bacteria, characterized by increases in several inflammatory cytokines (Larsen et al., 2014). The authors posit that an abnormal immune response to pathogenic bacteria colonizing the airway of offspring during early life might lead to chronic airway inflammation and childhood asthma. These findings, together with accumulating data from animal models of long-lasting immune alterations in offspring following prenatal exposure to infection, shed light on the possible developmental origins of offspring illness (Larsen et al., 2014).

Finally, our findings do not preclude the possibility that antibiotic exposure during pregnancy and/or early childhood may be an independent risk factor for offspring illness, given our results linking childhood antibiotic exposure to the diagnosis of asthma at age five. Antibiotics repeatedly have been linked to alterations in the gut microbiota, the collection of microorganisms that populate the human intestine and play an integral

role in normal immune development (Francino, 2016; Langdon, Crook, & Dantas, 2016). Disturbances to the microbiota, especially during early life, are believed to have important long-lasting consequences on health and disease (Francino, 2014; Sprockett, Fukami, & Relman, 2018; Tanaka & Nakayama, 2017). Nevertheless, this study helps strengthen conclusions that pre- and postnatal infection may play an important role in the development of offspring infections and allergic disorders, above and beyond antibiotic use.

Among the strengths of this study is the use of prospectively collected data specifically examining maternal and offspring health outcomes with detailed assessments of offspring from birth through age five. However, several limitations to the present study should be noted. First, classification of antibiotic exposure was based on medical record data that assumed medication compliance of mothers and children. It is possible that poor drug compliance could have resulted in exposure misclassification that underestimated the effect size of antibiotic exposure. Relatedly, the preponderance of mothers diagnosed with an infection during pregnancy did not require antibiotic treatment; therefore, we may have been limited in our ability to detect a relationship between prenatal antibiotic exposure and offspring infection. A handful of studies have shown adverse health outcomes in offspring following prenatal exposure to antibiotics, including an increased risk of allergic disorders (Dom et al., 2010; Jedrychowski et al., 2006; McKeever et al., 2002; Rusconi et al., 2007; Stensballe et al., 2013) and infection-related hospitalization (Miller et al., 2018). Nevertheless, our findings may be informative in disentangling the relative contributions of prenatal infection and antibiotic exposure on offspring health, especially considering the lack of adjustment for prenatal infection in at least one of the

aforementioned studies (Miller et al., 2018) and conflicting interpretations of previous findings (Chandrakumar, Bhardwaj, & Jong, 2018; Stokholm, Sevelsted, Bønnelykke, & Bisgaard, 2014).

Second, we were unable to control for maternal history of infections or allergic disorders (i.e., indicators of a hereditary susceptibility to illness), factors that have been shown to be associated with the development of offspring asthma and infection (Blomström et al., 2016; Burke, Fesinmeyer, Reed, Hampson, & Carlsten, 2003). Data on maternal illness was limited to six months prior to pregnancy, a preconception phase (Steegers-Theunissen, Twigt, Pestinger, & Sinclair, 2013) in which maternal environmental exposures already may exert a lasting impact on offspring health (Genuis & Genuis, 2016). However, at least three studies have demonstrated associations between pre-and postnatal antibiotic use and offspring asthma risk among non-asthmatic mothers (Kozyrsky et al., 2007; Risnes et al., 2010; Stokholm et al., 2014), suggesting that heritable factors do not completely account for asthma risk. Finally, ascertainment of asthma cases was based on the ICD-8 classification system, which categorized asthma and related conditions (e.g. wheezing, hay fever) broadly as 'respiratory allergies.' Despite this, several studies, using various means of case ascertainment, support our findings linking prenatal and offspring infection to a wide range of allergic disorders.

A third limitation is the incomplete information available regarding breastfeeding in offspring. Several studies have demonstrated that breastfeeding is associated with a lower risk of immune-related diseases and may have lasting protective effects on infant health and immune function (Hosea Blewett, Cicalo, Holland, & Field, 2008). However, our results demonstrated findings to the contrary; the presence of breastfeeding was

positively associated with offspring infection, so that mothers who breastfed had offspring with a greater number of infections. Our inconsistent findings may be attributable to the method of data abstraction, which lacked detailed information as to the duration or frequency of breastfeeding.

In conclusion, the results from our study offer further evidence of the fetal origins of offspring illness, in which prenatal infection may exert a long-term impact on the immune function and health of offspring. This has important implications for prevention and intervention strategies, as episodes of infection are not uncommon and often treated in an outpatient setting (Laibl & Sheffield; Longman & Johnson, 2007). A better understanding of the relationship between prenatal infection and the development of offspring infection and allergic disorders could help inform maternal and pediatric healthcare practices.

# CHAPTER 2

## EXPANDED LITERATURE REVIEW

Abstract

A growing body of research suggests that maternal infection and inflammation during pregnancy are associated with increased risk for a number of psychiatric disorders in offspring. In both human and animal research, the most consistent and robust findings to date have been in studies linking prenatal infection/inflammation to schizophrenia-related outcomes. However, debate remains in the literature as to whether maternal immune alterations in utero confer schizophrenia-specific risk or, rather, correspond to shared phenotypes present in a number of psychiatric disorders. This has led to the suggestion that prenatal infection/inflammation may act as a neurodevelopmental disease 'primer,' broadly increasing risk for a wide-array of disorders. This review describes the current research in humans implicating prenatal infection/inflammation in offspring psychopathology. Support for the 'primer' hypothesis is discussed, including specific factors (e.g., timing, sex, stress) that may interact with prenatal infection/inflammation to further susceptibility to psychiatric disorders later in life.

# Introduction

Maternal infection during pregnancy has been implicated in a number of adverse neurodevelopmental outcomes in offspring. These findings have most consistently demonstrated a link between maternal infection during pregnancy and schizophrenia and schizophrenia-related brain alterations in offspring (reviewed in Fineberg & Ellman, 2013). Extant literature, albeit inconsistently, also points to an association between prenatal infection and a number of other psychiatric disorders, including autism spectrum disorder (ASD), bipolar disorder, depression, attention-deficit hyperactivity disorder (ADHD), and anorexia nervosa (Flinkkilä, Keski-Rahkonen, Marttunen, & Raevuori, 2016). Several mechanisms have been proposed to explain the association between maternal infection during pregnancy and abnormalities in offspring brain development (Brown & Derkits, 2010). One growing line of research has focused on immune alterations (i.e., maternal immune responses to infection), especially those related to inflammation, as possible mediators of the harmful effects of infection on the fetal brain (Fineberg & Ellman, 2013). Findings suggests that changes in a number of immune cells, particularly inflammatory proteins known as proinflammatory cytokines, during pregnancy are associated with an increased risk of schizophrenia in offspring (Brown, Hooton et al., 2004; Buka et al., 2001). For example, elevations in the levels of the proinflammatory cytokine interleukin (IL)-8 during the second/third trimesters of pregnancy have been linked to increased risk of schizophrenia and structural brain changes related to schizophrenia in offspring (e.g., increases in ventricular volume, reductions in the volume of the entorhinal cortex and posterior cingulate, Brown, Hooton et al., 2004; Ellman et al., 2010). Additional support for this hypothesis has come from

several pre-clinical studies examining maternal immune activation (MIA) during pregnancy in rodents; these studies have repeatedly established behavioral and cognitive abnormalities consistent with schizophrenia in offspring exposed to maternal infection and/or proinflammatory cytokines during pregnancy (reviewed in Meyer, 2014).

To date, research in this area has focused primarily on the relationship between maternal infection and inflammation and risk for offspring schizophrenia with more recent interest focusing on the role of MIA during pregnancy in other offspring disorders (Flinkkilä et al., 2016). Despite the research linking prenatal immune alterations to a number of adverse neurodevelopmental outcomes, there has been less attention given to the impact of prenatal MIA as it relates to offspring psychopathology other than schizophrenia. Specifically, it remains unclear whether exposure to maternal immune alterations in utero confers disease-specific risk or broader vulnerability to neurodevelopmental disorders. Current research addressing this question exists predominantly in animal models. Several preclinical studies have begun to investigate the complex interplay between MIA during pregnancy and discrete genetic and environmental factors that may contribute to increased risk for adverse effects in exposed offspring; however, little research exists in humans (Ayhan, Sawa, Ross, & Pletnikov, 2009; Giovanoli et al., 2013; Maynard, Sikich, Lieberman, & LaMantia, 2001; Meyer, 2014).

Given that maternal infection and inflammatory processes are associated with increased risk for a number of disorders, further elucidation of these processes could have a substantial impact on prevention and treatment of offspring psychopathology. Episodes of maternal illness and possible infection during pregnancy are not uncommon (Laibl &

Sheffield; Longman & Johnson, 2007). As such, prenatal infection represents a clear target for intervention and treatment. Improved understanding of MIA on offspring developmental outcomes may also lead to greater diagnostic specificity of developmental disorders, as well as better understanding of shared risk factors among disorders. Clarity regarding the role of environmental factors throughout development in offspring with prenatal exposure to MIA is also important, as these factors may uniquely contribute to the course and onset of psychopathology. The purpose of this review is to summarize the existing evidence linking maternal infection and inflammation during pregnancy to a number of developmental psychopathology outcomes in human studies. Next, we highlight the growing body of research in animal models that supports the assertion that prenatal infection and inflammation may be implicated in a wide range of psychiatric disorders. We then discuss the hypothesis that exposure to prenatal infection and inflammation during pregnancy may serve as a neurodevelopment disease "primer," broadly increasing risk for a number of psychiatric disorders in adulthood. Finally, we briefly describe factors that may interact with prenatal immune processes to further vulnerability to adverse neurodevelopmental outcomes.

**Prenatal Infection/Inflammation and Risk of Offspring Schizophrenia**

The preponderance of studies on the role of prenatal infection and inflammation in adverse developmental outcomes has been in research examining the etiology of schizophrenia. A growing body of literature from epidemiologic and clinical investigations has provided support for the association between gestational exposure to infection and inflammation and risk of offspring schizophrenia (for review, see Brown & Derkits, 2010; Ellman & Susser, 2009; Fineberg & Ellman, 2013). The earliest

epidemiological evidence originated from ecologic data that used population level exposure to influenza to study the link between infection and schizophrenia. Initial studies suggested that individuals pregnant during the second trimester of an influenza epidemic had offspring with an increased risk of schizophrenia (Mednick, Machón, Huttunen, & Bonett, 1988; O'Callaghan et al., 1991; Sham et al. 1992; Takei et al., 1996). Evidence from similarly designed studies of other infectious agents, including measles (Torrey, 1988), varicella zoster (O'Callaghan et al., 1994; Torrey, 1988), and pneumonia (O'Callaghan et al., 1994; Watson, Kucala, Tilleskjor, & Jacobs, 1984), has also suggested a relationship between prenatal infection and schizophrenia. However, a number of other research studies using population level infection data have failed to replicate these findings (Grech, Takei, & Murray, 1997; Mino, Oshima, Tsuda, & Okagami, 2000; Morgan et al., 1997; Susser, Lin, Brown, Lumey, & Erlenmeyer-Kimling, 1994; Takei, Murray, Sham, & O'Callaghan, 1995). Initially, such results led many to question the role of prenatal infection in the etiology of schizophrenia. However, the ecologic design of these studies also suggested possible misclassification of influenza exposure (e.g., over classification of "exposed" cases), potentially contributing to results biased toward the null.

Consequently, more methodically rigorous epidemiological investigations emerged based on birth cohorts in which maternal serological specimens and/or clinical diagnoses of infection were prospectively obtained during pregnancy and systematic diagnosis of offspring schizophrenia was longitudinally assessed. These serological studies have indicated consistent associations between offspring schizophrenia and elevated maternal antibodies to influenza, toxoplasma gondii (T.gondii), herpes simplex

virus type 2 (HSV-2), and rubella during pregnancy (Brown et al., 2001; Brown, Begg et al., 2004; Brown & Derkits, 2010; Brown et al., 2009; Buka et al., 2008; Mortensen et al., 2010). Likewise, large birth cohort studies using physician-based prospective diagnoses of infection have found associations between schizophrenia spectrum disorders and prenatal exposure to respiratory infections during the second trimester (Babulas et al., 2006), bacterial infections during the first and second trimesters (Sørensen, Mortensen, Reinisch, & Mednick, 2009), and genital/reproductive infections during the periconceptional period (30 days before/after the last menstrual period, Brown et al., 2000).

Despite the consistent link between maternal infections during pregnancy and risk of schizophrenia in offspring, most infections do not cross the placenta; therefore, a focus of research has turned to examinations of maternal immune responses to infection, such as inflammation (Fineberg & Ellman, 2013; Kourtis, Read, & Jamieson, 2014; Patterson, 2009; Robbins & Bakardjiev, 2012; Shi, Tu, & Patterson, 2005). As such, additional research utilizing prospectively collected prenatal sera from a large birth cohort has shown increased risk of schizophrenia in offspring of mothers with elevated levels of the proinflammatory chemokine interleukin-8 (IL-8) during the second trimester (Brown, Hooton et al., 2004). In this case-control study, maternal IL-8 levels were significantly higher in mothers of offspring with schizophrenia spectrum disorders (n = 59) compared to matched controls (n = 105); however, no differences were found between cases and controls with respect to second trimester serum levels of three other cytokines of interest: interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). In a similar case-control study using prospectively collected birth cohort data, maternal serum

samples were obtained during late pregnancy (typically at time of delivery) and analyzed for IL-1β, IL-2, IL-6, IL-8, and TNF-α (Buka et al., 2001). Results found significantly elevated levels of TNF-α among mothers of offspring diagnosed with affective and non-affective psychosis (n = 27) compared to matched controls (n = 50). No differences were found between cases and controls with respect to the other cytokines of interest, although the authors noted a trend for higher levels of maternal IL-8 in women whose offspring later developed psychosis. Despite discrepant findings between the two studies, several factors may account for the differences observed: 1) the small sample size used in Buka et al. (2001) may have been insufficiently powered to detect a significant effect of IL-8, 2) inclusion of both affective and non-affective cases in Buka et al. (2001) may have increased heterogeneity of the findings, and 3) the study by Brown, Hooton et al., 2004 suffered from many undetectable cytokine values other than IL-8, potentially obscuring key findings.

More recent evidence has also suggested a similar link between elevated levels of C-reactive protein (CRP), an established inflammatory biomarker, during pregnancy and offspring schizophrenia (Canetta, Sourander, et al., 2014). In this study, the authors utilized a national birth cohort derived from the Finnish Maternity Cohort, which consists of prospectively collected and archived maternal serum samples from early to middle pregnancy of over 1 million pregnancies. Elevated maternal CRP during pregnancy was significantly associated with an increased risk of schizophrenia in adult offspring (diagnosed via nationalized medical record). Findings indicated a 28% increase in risk for schizophrenia for every 1mg/L increase in maternal CRP (CRP values ranged from 0 to 40 mg/L with median values between 0 and 4). Despite these findings, other research has

suggested a protective effect of lifelong exposure to elevated levels of CRP on schizophrenia risk, highlighting the possibility that exposure to CRP during a specific window of time (i.e., pregnancy) may be associated with schizophrenia. Altogether, there is accumulating evidence consistent across multiple methodologies and measures of exposure data to suggest prenatal infection and inflammation likely play a role in the pathogenesis of schizophrenia. Nevertheless, there remain inconsistent findings across studies with respect to infection type, timing of exposure, and differences in which cytokine may confer risk for offspring schizophrenia, which are areas that needs to be addressed in future research.

### Prenatal Infection/Inflammation and Risk of Autism Spectrum Disorder

In humans, a small but growing body of literature provides support for the suggestion that maternal infection and inflammation during pregnancy may be implicated in risk for autism spectrum disorders (ASDs). Several studies have established a link between maternal infection during pregnancy and an increased risk of offspring autism (Atladottir et al., 2010; Atladottir, Henriksen, Schendel, & Parner, 2012; Chess, 1971; Jiang et al. 2016; Zerbo et al., 2013). One of the first studies to do this was an epidemiological study of 243 children with known prenatal exposure to rubella, which found a much higher prevalence of autism among the cohort when compared with the expectancy of autism in the general population (Chess, 1971). These findings were further supported by several case reports linking ASDs with prenatal exposure to rubella (Chess, 1977; Deykin & MacMahon, 1979; Libbey, Sweeten, McMahon, & Fujinami, 2005) and other congenital viral infections, such as cytomegalovirus (Libbey et al., 2005; Sweeten, Posey, & McDougle, 2004; Yamashita, Fujimoto, Nakajima, Isagai, &

Matsuishi, 2003). More recent methodologically rigorous research, using cohort data obtained from national Danish registers, found maternal hospitalizations for viral infections in the first trimester and bacterial infections in the second trimester were associated with diagnosis of ASDs in offspring (Atladottir et al., 2010). However, the authors observed no associations with offspring ASD when looking at all maternal infections during the total length of pregnancy, suggesting that timing and type of infection may be important factors portending risk for ASD. Importantly, this study was limited in its inclusion of only severe infections (i.e., those requiring hospital admission), which may be associated with other obstetrical complications (e.g., fetal growth restriction, hypoxia) that could have contributed to adverse neurodevelopmental outcomes in offspring (Adams Waldorf & McAdams, 2013; Clark, Harley, & Cannon, 2006). Moreover, the study was unable to investigate the effect of subclinical infections or infections treated by general practitioners that did not require hospitalization on risk for ASD in offspring. However, other research has supported a role for less severe maternal viral infections during pregnancy in offspring ASD (Deykin & MacMahon, 1973; Wilkerson, Volpe, Dean, & Titus, 2002; Zhang et al., 2010). A recent meta-analysis of 15 studies (13 case-control and 2 cohort studies) and over 40,000 cases of autism concluded that maternal infection during pregnancy was associated with a 12% increase in the odds while controlling for several confounding factors (Jiang et al., 2016). Furthermore, a more pronounced risk of autism in offspring (an increase in odds by as much as 30%) was found among women who required hospitalization for treatment of maternal infection during pregnancy (Jiang et al., 2016).

However, there is some evidence to suggest that various types of mild common infections, as well as episodes of fever during pregnancy may not be associated with ASD (Atladottir et al., 2012). One study using a population-based Danish cohort consisting of close to 100,000 children aged 8-14 years old found that maternal self-report of infections and febrile episodes (both assessed through telephone interviews prospectively collected during pregnancy) were not associated with ASD. However, findings did suggest that self-reported maternal influenza infection and prolonged febrile episodes (lasting ≥ 7 days) were associated with a 2-fold and 3-fold increased risk, respectively, of infantile autism, a subgroup within ASD in which several symptoms of impairment are present before age 3. In contrast, another study using a case-control design found that maternal self-report of fever, but not influenza, during pregnancy was associated with ASD (Zerbo et al., 2013). It is worth noting that exposure data in this study was retrospectively assessed through telephone interviews with mothers when children were ages 2-5 years old. Therefore, results may be subject to recall bias as mothers have been found to under- and over-report prenatal infections when recalling health conditions after birth (Buka, Goldstein, Seidman, & Tsuang, 2000; Voldsgaard et al., 2002).

In line with infection studies, findings from Brown and colleagues (2014), using the same Finnish Maternity Cohort described above, demonstrated a significant association between increasing CRP levels (measured continuously) and offspring childhood autism. When CRP was measured categorically, the authors found there was a greater than 40% increase in risk of childhood autism for maternal CRP levels in the highest quintile compared to the lowest. Three other studies have linked elevated levels

of select maternal cytokines and chemokines during pregnancy to ASD among offspring (Abdallah et al., 2012; Abdallah et al., 2013; Goines et al., 2011), although results have been somewhat tempered by a lack of adjustment for multiple comparisons, even in instances of 16 (Abdallah et al., 2013) or 17 comparisons (Goines et al., 2011). Furthermore, findings have been inconsistent as to which maternal biomarkers are implicated in offspring ASD with results suggesting possible involvement of interferon-y (IFN-γ), IL-4, IL-5, IL-10, TNF-α , and TNF-β. In summary, research to date supports an association between maternal infection and inflammation during pregnancy and ASD in offspring; however, questions remain as to the impact of infection severity on ASD outcomes, as well as the precise role of inflammation in portending risk for the disorder.

**Prenatal Infection/Inflammation and Risk of Major Depressive Disorder (MDD)**

There is also some evidence to suggest that maternal infections during pregnancy may be implicated in risk for offspring depression. Specifically, two studies have found associations between being pregnant during influenza epidemics and unipolar depression in adult offspring (Cannon et al. 1996; Machón et al., 1997). The first of these studies, utilizing an Irish cohort, found that maternal self-report of influenza at any time during pregnancy was associated with a 1.59 increased risk of a depressive disorder among adult offspring (Cannon et al., 1996). The second ecologic study, using a Finnish cohort, also found a significant increase in the proportion of psychiatric hospital admissions for unipolar MDD among individuals whose mothers were pregnant during the second trimester of an influenza epidemic (Machón et al., 1997). However, these studies were complicated by use of hospital admission data and/or familial report of offspring health to determine depression diagnoses. The study by Machón and colleagues (1997) was further

limited by use of nonspecific diagnostic codes that did not differentiate between subtypes (with or without psychotic features) of unipolar depression. As such, findings from both studies may be representative of more severe mood disorders that lack generalizability to more moderate diagnostic profiles of depression. However, current research using data from the Child Health and Development Studies (CHDS), a longitudinal, population-based birth cohort from Northern California, found that presence of infection during the second trimester of pregnancy was associated with increased depressive symptoms in adolescent offspring; moreover, this association was found to me moderated by prenatal reports of maternal stress during the same time period (Murphy et al., 2017). Nevertheless, a number of other studies have identified no associations between fetal exposure to maternal influenza and offspring depression (Brown et al., 1995; Mino et al., 2000; Morgan et al., 1997; Pang, Syed, Fine, & Jones, 2009; Takei et al., 1993). Research findings utilizing a cohort of 3076 individuals born to mothers who were clinically diagnosed with various viral infections (e.g., influenza, rubella, mumps, measles, varicella, herpes zoster, hepatitis, cytomegalovirus) in the United Kingdom between 1946 and 1980 found no associations between individual viral infections and offspring depression (Pang et al., 2009). Still, given the dearth of literature examining the role of prenatal infections in the etiology of depression, as well as the complete absence of studies investigating prenatal inflammation in depression risk, further research is necessary.

### Prenatal Infection/Inflammation and Risk of Bipolar Disorder

Several studies have sought to examine the relationship between prenatal infection and the development of offspring bipolar disorder, although findings to date are

largely mixed. A case-control study by Parboosing, Bao, Shen, Schaefer, & Brown (2013), found a significant association between clinical diagnosis with maternal influenza anytime during gestation and offspring bipolar disorder among participants in the CHDS cohort. Results showed a nearly fourfold increased risk of bipolar disorder (diagnosed via structured clinical interview and encompassing diagnoses of bipolar I, II, and not otherwise specified) in adult offspring. In separate analyses, the authors found a similar association between prenatal exposure to influenza and risk for bipolar disorder with psychotic features (Parboosing et al., 2013). These results were shortly followed by another study examining the association between serologically confirmed influenza infection during pregnancy and bipolar disorder among members of the same birth cohort. The authors found that maternal influenza exposure was related to a fivefold increased risk of bipolar disorder with, but not without, psychotic features (Canetta, Bao, et al., 2014). Although the divergent findings between the two studies may be related to differences in ascertainment of influenza status, Canetta, Bao et al. (2014) reported high concordance (>75%) between clinical assessment and serological measure of influenza within their sample. Moreover, in exploratory analyses, using a composite measure that combined both diagnostic approaches, the authors confirmed an association between influenza exposure and bipolar disorder with psychotic features, but not without.

However, a number of other studies have identified null or protective effects of prenatal exposure to maternal infection and bipolar disorder outcomes in offspring (Brown, Susser, Lin, & Gorman, 1995; Cannon et al., 1996; Machón, Mednick, & Huttunen, 1997; Mino, Oshima, & Okagami, 2000; Mortensen, Pedersen, Melbye, Mors, & Ewald, 2003; Stober, Kocher, Franzek, & Beckmann, 1997; Takei, O'callaghan, Sham,

Glover, & Murray, 1993). Four of these studies utilized an ecological design in which timing of influenza exposure was based on pregnancy during known influenza epidemics with data obtained retrospectively via national databases (Brown et al., 1997; Machón et al., 1997; Mino et al., 2000; Takei et al., 1993). Machón et al. (1997) and Brown et al. (1995) both examined population-level exposure to the 1957 A2 influenza epidemic in Finland and Holland, respectively. In the Finnish population, Machón and authors (1997) found no association between the rate of hospital diagnoses for bipolar disorder and prenatal exposure to influenza during the second trimester. In the Dutch population, however, Brown et al. (1997) reported a significant decrease in the risk for affective psychosis in females (no association was found for males) who were exposed to influenza during specific months of the first and third trimesters. A third study examined the relationship between influenza epidemics and individuals born between 1938 and 1965 in England and Wales who later developed affective psychosis (Takei et al., 1993). The authors found that females, but not males, exposed to influenza during the 5$^{th}$ month of gestation were less likely to later develop affective psychosis. However, analyses revealed that this relationship was driven by diagnoses of psychotic depression in females and not bipolar disorder with psychotic features. A similar effect of sex was demonstrated in a fourth ecological study by Mino et al. (2000), in which second-trimester and third-trimester exposure to the 1965 and 1957-58 A2 influenza epidemics, respectively, in Japan was associated with decreases in inpatient hospitalizations for mood disorders (bipolar disorder and depressive disorder) in females only. Importantly, when diagnoses were analyzed separately, no independent associations with influenza exposure were discovered, although this may be due, in part, to insufficient statistical power.

A few other studies using different methodologies have similarly found no support for an association between maternal infection during pregnancy and increased risk of offspring bipolar disorder. In one such example, an Irish cohort study identified no association between maternal self-report of influenza anytime during pregnancy during the 1957 A2 influenza epidemic and bipolar disorder in offspring (Cannon et al., 1996). However, this study has been criticized for its sample size, which was too small to detect a positive association. A more recent Danish cohort study of 2.1 million individuals found no relationship between rates of influenza during pregnancy in Denmark from 1950-1983 and offspring psychiatric hospitalizations or outpatient care for bipolar disorder (Mortensen et al., 2007). A third study using maternal interviews of cases and controls also failed to identify an association between individuals diagnosed with manic-depressive disorder (via inpatient medical records and interviews) and individual-specific maternal infections during pregnancy, such as influenza, appendicitis, and pyelonephritis (Stober et al., 1997).

Despite data suggesting no link between prenatal exposure to infection and risk of bipolar disorder, only a handful of studies have examined prenatal infections other than influenza and most that have failed to provide serological confirmation of infection status. Aside from Stober et al. (1997), Parboosing and colleagues (2013) examined the relationship between infections of the upper and lower respiratory tract (excluding influenza) during pregnancy and offspring bipolar disorder in exploratory analyses; however, no association was found. Two other studies have examined neonatal seropositivity for select infectious agents, including Toxoplasma gondii, herpes simplex virus type 1 (HSV-1) and 2 (HSV-2), and cytomegalovirus and risk of bipolar disorder

(Mortensen et al., 2007; Mortensen et al., 2011). This research observed no relationship between any of the neonatal markers of prenatal infection and bipolar disorder, although sample sizes were modest. Furthermore, only one study to date has examined prenatal inflammation in relation to bipolar disorder outcomes in offspring. A recent case-control study utilizing data derived from the Finnish Maternity Cohort examined CRP prospectively assayed in maternal sera primarily during the first trimester and observed no association with risk of bipolar disorder in offspring (Chudal et al., 2017). Interestingly, findings from this study were in contrast to previous studies utilizing the same birth cohort (discussed above), which established links between elevated maternal CRP levels during pregnancy and both childhood autism (Brown et al., 2014) and schizophrenia in offspring (Canetta, Sourander, et al., 2014). However, the authors noted the study was limited by a smaller sample size and reductions in power compared to earlier studies, in addition to restrictions in the time frame for follow-up of bipolar cases, which precluded cases with later ages of onset. Taken together, there is some, albeit sparse, evidence implicating prenatal infection in risk for offspring bipolar disorder. Nevertheless, large gaps remain in the literature, which will be important to address in future studies.

### Prenatal Infection and Risk of Other Psychiatric Outcomes in Offspring

Maternal infection during pregnancy has also been associated with increased risk for other psychiatric disorders in offspring, including anorexia nervosa and attention-deficit hyperactivity disorder (ADHD), although the research in these areas is sparse (Favaro et al., 2011; Mann & McDermott, 2011; Simanek & Meier, 2015). An ecological study by Favaro et al. (2011) using a sample of 27,682 women living in Italy between

1970-1984 found that exposure to peaks of chickenpox and rubella during the 6th month of pregnancy was associated with an increased risk of anorexia nervosa in offspring (OR = 1.5 and OR = 1.6, respectively). However, no association was found between prenatal measles or influenza exposure and anorexia nervosa risk. Another study examining the relationship between prenatal maternal infection and childhood ADHD found an increased risk for ADHD in offspring whose mothers were diagnosed with a prenatal genitourinary infection anytime during pregnancy or preeclampsia (OR = 1.3 and OR = 1.2, respectively). In this sample of 84,721 children born from 1996-2002 in South Carolina, exposure data was obtained from Medicaid billing information and based on diagnoses made by physicians anytime during pregnancy. Cases of ADHD were also identified on the basis of diagnoses made in the child's Medicaid file.

Altogether, the findings in humans highlight that maternal infection and inflammation during pregnancy have been linked to risk for several disorders; however, substantial more research is needed to determine the relationship between prenatal immune processes and psychiatric outcomes in offspring. Only a few studies have examined inflammation during pregnancy (as opposed to infection) and risk for offspring psychopathology. Moreover, the majority of studies of prenatal infection have relied on clinical diagnosis or population-level data to determine infection status (Flinkkilä et al., 2016). Replication of these findings using serological confirmation of infection status is needed to strengthen conclusions regarding the role of prenatal infection and inflammation in the etiology of multiple disorders.

**Animal Models of Maternal Infection and Inflammation during Pregnancy**

Given compelling epidemiological findings in humans, several translational models have been established to examine the consequences of prenatal exposure to infection and inflammation on brain and behavioral development in rodents and non-human primates (Meyer, 2014; Willette et al., 2011). These models differ based on the type of agent used to induce MIA during pregnancy, as different immune challenges have been shown to prompt distinct, but overlapping, immune responses (Meyer, Yee, & Feldon, 2007). Current models include exposure to human influenza virus, the viral-like immune activating agent polyriboinosinic-polyribocytidilic acid (poly[I:C]), the bacterial endotoxin lipopolysaccharide (LPS), and/or specific proinflammatory cytokines (Meyer, 2014; Meyer et al., 2009). A variety of sophisticated paradigms (e.g., behavioral, cognitive, pharmacological) have also been developed to identify and characterize schizophrenia-like symptoms within experimental MIA models (Barch et al., 2009). Implementation of these rodent models has provided substantial evidence for the causal effects of prenatal exposure to infectious and/or immune activating agents in the development of schizophrenia-related phenotypes in adult and juvenile offspring (reviewed in (Kneeland & Fatemi, 2013; Meyer, 2014; Meyer & Feldon, 2010)).

While a complete review of this literature is beyond the scope of this paper, countless preclinical studies have found structural, neurochemical, and behavioral abnormalities in adult rodents following gestational exposure to MIA (reviewed in (Kneeland & Fatemi, 2013; Meyer 2014; Meyer & Feldon, 2010; Nawa & Takei, 2006)). At the structural level, experimental mouse models of prenatal exposure to human influenza have found numerous morphological abnormalities in adult rodents, including reduced cortical thickness and hippocampal volume, impaired corpus callosum

development, changes in neuronal cell densities and numbers, and reduced brain volume (Fatemi et al., 1999; Fatemi et al., 2008; Fatemi et al., 2009; Kneeland & Fatemi, 2013). In line with this research, experimental data using prenatal treatment with exogenous IL-6 led to hippocampal neurodegeneration in rats (Samuelsson, Jennische, Hansson, & Holmang, 2006). Rodent models using poly[I:C] and LPS, immune activating agents that mimic specific innate immune responses to virulent viral or bacterial pathogens, respectively, have demonstrated similar aberrations in neuronal structure and function, as well as deficits in dendritic spine density, levels of synaptic proteins, synaptic transmission, and long-term plasticity (reviewed in (Nawa & Takei, 2006; Meyer et al., 2007; Meyer & Feldon, 2009a)). Various pre- and postsynaptic alterations in multiple neurotransmitter systems have also been observed, including changes in the central dopamine, gamiobutyric (GABA), and glutamate systems (Meyer & Feldon, 2010).

At the behavioral level, studies in rats and mice have provided robust evidence for the emergence of a number of long-lasting behavioral abnormalities in adult offspring following gestational exposure to MIA (reviewed in (Meyer, 2014; Meyer & Feldon, 2010)). This includes deficits in sensorimotor gating (the ability of the brain to filter irrelevant stimuli), social interactions, exploratory behavior, enhanced sensitivity to psychostimulant drugs, and stereotypy. Importantly, many of these behavioral deficits can be improved by treatment with antipsychotic drugs (Meyer, 2014; Shi, Fatemi, Sidwell, & Patterson, 2003). Furthermore, offspring exposed to MIA during gestation display cognitive impairments, including difficulties in working memory, cognitive flexibility, and selective attention (Meyer & Feldon, 2010).

The various aberrations observed in rodents prenatally exposed to infection and inflammation have been related to similar abnormalities seen in humans diagnosed with schizophrenia. For example, individuals with schizophrenia exhibit a range of symptoms that overlap with behavioral deficits in rodents, including disturbances in executive function, working memory impairment, ability to sustain attention, reduced social behavior, stereotypic behavior, and impaired sensorimotor gating (reviewed in (Meyer & Feldon, 2010; Meyer, Feldon, & Fatemi, 2009)). Likewise, many of the structural and functional abnormalities observed in rodent models have been related to similar changes throughout the brain of human subjects with schizophrenia (Ellman et al., 2010; Meyer & Feldon, 2010). More recently, translational models of MIA have been extended to non-human primates, strengthening support for the relevance of preclinical findings to humans. A study using rhesus monkeys infected with influenza virus during pregnancy found reduced gray matter volume throughout the cortex and decreased white matter in the parietal lobe of offspring at one year-old (Short et al., 2010). A second study of rhesus monkeys using a modified LPS model of MIA demonstrated numerous behavioral abnormalities in prenatally exposed offspring by two years of age, including increased repetitive behaviors, altered communication, and atypical social interactions (Bauman et al., 2014). Further investigation of the offspring monkeys as juveniles using eye tracking methods revealed deficits on several measures of social attention (Machado, Whitaker, Smith, Patterson & Bauman, 2015). Overall, there is large body of compelling work in animal models documenting extensive brain and behavioral abnormalities following prenatal MIA that may be representative of a variety of clinical outcomes in humans.

**Prenatal Infection and Inflammation as a Neurodevelopmental Disease Primer**

Taken together, an increasing number of preclinical studies offer compelling evidence for the role of infection and inflammation during pregnancy in offspring schizophrenia. However, uncertainty remains as to whether the impairments produced in animal models of MIA underlie phenotypes exclusive to schizophrenia. The numerous neurobehavioral abnormalities elicited by prenatal exposure to MIA are widespread and fundamental to a range of neuropsychological functions (Meyer & Feldon, 2010). Many of dysfunctions relevant to the phenotypic characterization of schizophrenia symptoms also correspond to phenotypes present in a spectrum of psychiatric disorders (Estes & McAllister, 2016; Knuesel et al., 2014). In fact, increasing evidence suggests that a number of the behavioral and neurochemical alterations seen in animal models of MIA are consistent with other disorders, such as autism (Meyer, Feldon, & Dammann, 2011) and depression (Ronovsky, Berger, Molz, Berger, & Pollak, 2016).

Converging evidence from the animal literature, with support from findings in humans, has led to the suggestion that MIA during pregnancy may act as a neurodevelopmental disease 'primer' that is likely relevant for a number of psychiatric disorders (Ellman, Murphy, & Maxwell, 2018; Meyer, 2014; Patterson, 2009). This concept suggests that the adverse effects induced by prenatal immune responses may 'prime' the developing fetal brain, broadly increasing risk for later development of psychiatric disorders (Patterson, 2009). Important to this hypothesis is the likelihood that fetal alterations due to maternal infection and inflammation during pregnancy interact with additional genetic and environmental factors (both in utero and throughout the offspring's development), to increase susceptibility to psychiatric illness (Ellman et al., 2018; Fineberg & Ellman, 2013). Further speculation suggests that the timing of genetic

x environment interactions and/or environment x environment interactions during particular developmental windows might then determine the specificity of subsequent disorders or symptoms (Ellman et al., 2018; Ellman & Susser, 2009; Meyer & Feldon, 2010; Mittal, Ellman, & Cannon, 2008; Patterson, 2009).

Mounting evidence suggests that timing of immune insults during pregnancy is differentially associated with increased risk for specific neuropsychiatric disorders or shared phenotypes between disorders, although this remains a matter of debate (Ellman et al., 2018). Initial findings from ecological data in human supports associations between second trimester infection and risk of offspring schizophrenia, but more recent prospective epidemiological studies have challenged this view making it less clear whether infection during midgestation confers the greatest risk for schizophrenia in offspring (Brown & Derkits, 2010; Ellman et al., 2018 Ellman & Susser, 2009). For example, a number of studies have provided support for a link between offspring schizophrenia and prenatal infection earlier in gestation (i.e., in the first trimester, Babulas et al., 2006; Brown et al., 2001; Brown, Begg et al. 2004; Sørensen et al., 2009), as well as later in gestation (i.e., at time of delivery, Buka et al., 2001; Ellman et al., 2009). Some researchers also contend that neurodevelopmental outcomes in offspring may depend on the type and timing of infectious exposure during specific windows of developmental vulnerability (Brown & Derkits, 2010; Ellman et al., 2018; Ellman, Yolken, Buka, Torrey, & Cannon, 2009; Fineberg & Ellman, 2013). Research in rodent models of MIA has provided support for the claim that prenatal exposure to infection and inflammation during distinct periods of fetal development may lead to dissociable patterns of pathological symptoms in adult offspring (Meyer et al., 2006; Meyer et al.,

2007; Meyer, Nyffeler, Yee, Knuesel, Feldon, 2008). In particular, experimental findings from the prenatal Poly[I:C] model in mice suggest that prenatal exposure to MIA in early/middle gestation (gestation day 9) and late gestation (gestation day 17) correspond to two windows with differing vulnerabilities to immune insults (Meyer et al., 2007). In early/middle gestational exposure (a rough equivalent to the middle/end of the first trimester in humans), prenatal MIA led to reduced exploratory behavior, impaired selective associative learning, and disrupted sensorimotor gating (Meyer et al., 2007). On the other hand, exposure to prenatal MIA in late gestation (a rough equivalent to the middle/end of the second trimester in humans), while resulting in some overlapping symptoms, was distinguished by emergence of preservative behaviors, greater impairments in cognitive flexibility, and unique fetal cytokines responses (i.e., stimulation of IL-10 and TNF-α in the fetal brain, Meyer et al., 2006; Meyer et al., 2007; Meyer et al., 2008). Such differences have led some researchers to suggest that prenatal exposure to infection and inflammation earlier in gestation may exert a greater influence on phenotypes relevant to schizophrenia compared to exposure to infection and inflammation exposure later in gestation (Meyer et al., 2007).

Animal models have also begun to investigate other factors that may interact with MIA to confer increased risk for abnormalities in offspring development, including sex and postnatal environmental factors, such as stress and substance use. Preliminary evidence from a preclinical study in mice suggests that combined exposure to prenatal MIA and postnatal stress in offspring is associated with behavioral and neurochemical abnormalities analogous to symptoms present in neurodevelopmental disorders, such as schizophrenia (Giovanoli et al., 2013). Researchers found that offspring who were born

to poly[I:C] exposed mothers and who were subjected to unpredictable stressors throughout peripubertal developmental displayed deficits in sensorimotor gating, hypersensitivity to psychotomimetic drugs, changes in hippocampal microglial cells, and elevated levels of specific inflammatory cytokines, compared to offspring exposed to only one of the two insults throughout development. Similarly, another preclinical study in rats using a prenatal poly[I:C] model found that gestational exposure to MIA together with cannabinoid exposure in offspring during adolescence led to significant changes in gene expression in the entorhinal cortex (Hollins, Zavitsanou, Walker, & Cairns, 2016). Importantly, such disruptions in transcription are believed to underlie functional deficits, including impairments in learning and memory, relevant to schizophrenia and other neuropsychiatric disorders. In humans, there is also some preliminary evidence to support preclinical findings of stress-immune interactions in the development of offspring psychopathology (Debost et al., 2017; Murphy et al., 2017). For example, a prospective study of the CHDS cohort observed higher depressive symptoms in adolescent offspring exposed to both prenatal infection and maternal reports of prenatal stress during the second trimester compared to offspring exposed to either stress or infection alone (Murphy et al., 2017). In another prospective cohort study using Danish register data, the authors found that combined exposure to prenatal infection and peripubertal psychological trauma was associated with a significantly higher risk of developing schizophrenia in males, than exposure to either insult alone (Debost et al., 2017). Regarding such sex differences, only a few studies have addressed the long-term effects of prenatal MIA on neurodevelopment in both sexes (Rana, Aavani, & Pittman, 2012). This is largely due to lack of power in human studies and male bias in preclinical studies,

as cyclical fluctuations of female offspring reproductive hormones present issues of interpretation (Beery & Zucker, 2011). Of the studies that have examined sex-dependent effects on offspring neurodevelopmental outcomes in prenatal MIA models, few have found marked differences between the sexes on brain or behavior outcomes (Meyer & Feldon, 2010). However, there have been at least some findings suggesting the effects of prenatal MIA on offspring are strongly sex dependent (Meyer et al., 2008; Schwendener, Meyer, & Feldon, 2009).

## Remarks

Overall, research from epidemiological and clinical studies has shown repeated associations between maternal infection and inflammation and increased risk for offspring schizophrenia with increasing, albeit inconsistent, evidence suggesting links between prenatal MIA and several other neurodevelopmental outcomes. Preclinical studies in rodents have provided some support for these findings, while offering promising hypotheses as to the translation of in utero insults into the development of psychiatric disorders Despite these advances, several gaps remain in the literature regarding the role of maternal infection and inflammation in the development of offspring psychopathology. Much still needs to be learned regarding the mechanism by which MIA operates to increase risk for alterations in fetal neurodevelopment and how these alterations are translated across development to produce various psychiatric disorders.

Although the precise biological mechanisms underlying the relationship between maternal inflammation during pregnancy and offspring psychopathology remain unclear, several plausible hypotheses have been offered (Meyer & Feldon, 2009b, 2010; Patterson, 2011). One possibility is that proinflammatory cytokines act directly on the

developing fetal brain. Thus far, evidence from experimental data in rodents indicates prenatal treatment with IL-6, but no other pro-inflammatory cytokines, is sufficient to induce long-lasting brain and behavioral abnormalities in adults (Samuelsson et al., 2006; Smith et al., 2007). Several animal studies have also demonstrated elevated levels of IL-6 mRNA and protein in the fetal brain following MIA with poly[I:C] or LPS (Boksa, 2010; Ghiani et al., 2011). Additionally, in humans, IL-6 is one of the only cytokines that is known to cross the placenta (Zaretsky, Alexander, Byrd, & Bawdon, 2004). Taken together, these studies suggest a key role for IL-6 in mediating the effects of MIA on fetal brain development; however, it remains to be determined if IL-6 (or other signals) is directly responsible for evoking the cytokine responses in the fetal brain, or by what precise process maternal inflammation contributes to structural and functional changes in the fetal brain. A second hypothesis proposes proinflammatory cytokines may act indirectly on the maternal placenta to alter fetal development (Hsiao & Patterson, 2011; Mandal, Marzouk, Donnelly, & Ponzio, 2011; Patterson, 2009). Several animal studies have demonstrated cytokine expression in the placenta as a result of prenatal immune activation (Boksa, 2010). Elevation in levels of inflammatory cytokines in the placenta has been implicated in placental dysfunction. For example, MIA with poly[I:C] has been found to induce endocrine changes in the placenta via IL-6 that could alter the transfer of cells, nutrients, oxygen, growth factors, and maternal antibodies, all of which could strongly impact fetal development (Patterson, 2008; Hsia & Patterson, 2011).

A third possibility is that prenatal inflammation triggers a cascade of development sequelae, in which maternal inflammation during pregnancy is one of several incremental factors contributing to risk for later psychopathology (Ellman et al., 2018). There is some

evidence in humans to support the possibility that prenatal infection may lead to subtle developmental difficulties that alter the offspring's interactions with the environment and indirectly contribute to onset of mental health problems (Ellman et al., 2009; Ellman et al., 2018). However, this theory remains largely untested in the literature, and future research is necessary to examine how prenatal inflammation may interact with genetic, contextual (e.g., peer influences, familial context), and other postnatal environmental risk factors across development to influence risk for psychiatric disorders. Lastly, important to not ignore, is the possibility that maternal inflammation during pregnancy increases risk for clinical phenotypes shared across multiple disorders through mechanisms that are still largely unknown.

While animal models offer promising leads regarding the mechanistic actions of MIA in adverse offspring outcomes, evidence is lacking regarding the cumulative and/or interactive impact of pre- and postnatal environmental factors on alterations in neurodevelopment and vulnerability to psychiatric disorders. Future research is needed to address these gaps in the literature. Consideration of protective factors that may buffer against psychiatric risk associated with exposure to MIA during pregnancy is also necessary, especially as not all offspring exposed to prenatal infection or inflammation in utero later develop psychiatric disorders. Continued pursuit of these issues is worthwhile to inform prevention and treatment efforts, particularly those related to maternal healthcare practices that could lead to more effective intervention during pregnancy, as well as contribute to improvements in overall offspring health. Furthermore, elucidating developmental mechanisms related to MIA may help increase diagnostic specificity among disorders with similar risk factors and early developmental trajectories.

# CHAPTER 3

# EXPANDED ANALYSES

Supplementary Tables

Table 6. *Comparative Demographic Characteristics for Analytic Sample and CHDS Not in Analytic Sample (Total n = 19,044)*

| Demographics | Analytic Sample n = 2062 | CHDS Children Not in Analytic Sample n = 16,982 | *p*-value |
|---|---|---|---|
| Offspring Sex, *n* (%) | | | <0.0001 |
| Male | 974 (47.2) | 8769 (51.6) | |
| Female | 1088 (52.8) | 8213 (48.4) | |
| Maternal Race, *n* (%) | | | <0.0001 |
| White | 1516 (73.8) | 11538 (69.1) | |
| Non-white | 539 (26.2) | 5167 (30.9) | |
| Maternal Education, *n* (%) | | | 0.43 |
| Less than or equal to H.S. | 1051 (51.7) | 7227 (50.8) | |
| Greater than H.S. | 982 (48.3) | 6996 (49.2) | |
| Parity, *n* (%) | | | <0.0001 |
| First child | 390 (18.9) | 5077 (27.7) | |
| Second child | 493 (23.9) | 4482 (24.5) | |
| Third or more child | 1178 (57.1) | 8745 (47.8) | |
| Maternal Age, *M (SD)* | 28.2 (5.90) | 26.9 (6.13) | <0.0001 |

*Note.* For the present study sample, the following variables had missing data: maternal race (n = 7); maternal education (n = 29); parity (n = 1); maternal age (n = 6).

For CHDS children not in the present study sample, the following variables had missing data: maternal race (n = 277); maternal education (n = 2759); parity (n = 740); maternal age (n = 170).

Table 7. *Bivariate associations between prenatal infection/inflammation and offspring inflammatory states*

| Prenatal Infection/ Inflammation | Child Infection 24 months | | Child Infection 5 year | | Child Asthma 5 year | | Child Eczema 5 year | | Child BMI 5 year | | Child BMI 9-11 year | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *p* | *F* | *p* | *F* ($X^2$) | *p* | *F* ($X^2$) | *p* | *F* | *p* | *F* | *p* |
| T1 Infection[a] | 11.58 | <0.01** | 10.76 | <0.01** | 0.02 | 0.89 | 3.34 | 0.07 | 0.13 | 0.72 | 0.15 | 0.70 |
| T2 Infection[a] | 18.77 | <0.01** | 30.61 | <0.01** | 0.63 | 0.43 | 1.85 | 0.17 | 0.07 | 0.80 | 3.09 | 0.08 |
| T3 Infection[a] | 4.06 | 0.04* | 2.38 | 0.12 | 0.04 | 0.84 | 2.78 | 0.10 | 0.30 | 0.59 | 0.01 | 0.99 |
| T1 IL-6[b] | 0.91 | 0.34 | 0.75 | 0.39 | 0.54 | 0.47 | 1.87 | 0.17 | 0.66 | 0.42 | 0.06 | 0.81 |
| T2 IL-6[b] | 0.17 | 0.68 | 0.62 | 0.43 | 0.13 | 0.72 | 0.01 | 0.92 | 0.97 | 0.33 | 1.16 | 0.28 |
| T1 IL-8[b] | 0.06 | .80 | 0.03 | 0.86 | 0.11 | 0.74 | 3.63 | 0.06 | 1.46 | 0.23 | 3.58 | 0.06 |
| T2 IL-8[c] | 0.23 | 0.63 | 2.35 | 0.13 | 0.01 | 0.95 | 0.02 | 0.90 | 0.02 | 0.89 | 1.59 | 0.21 |
| T1 IL-1ra[d] | 5.36 | 0.02* | 0.22 | 0.64 | 0.45 | 0.50 | 0.01 | 0.94 | 0.01 | 0.93 | 0.21 | 0.65 |
| T2 IL-1ra[e] | 0.38 | 0.54 | 1.34 | 0.25 | 0.01 | 0.98 | 0.41 | 0.52 | 0.69 | 0.41 | 4.45 | 0.04* |
| T1 sTNF-rII[d] | 2.24 | 0.14 | 1.40 | 0.24 | 0.16 | 0.69 | 0.01 | 0.94 | 0.01 | 0.99 | 0.30 | 0.59 |
| T2 sTNF-rII[e] | 0.33 | 0.57 | 0.51 | 0.48 | 0.30 | 0.58 | 0.31 | 0.58 | 0.05 | 0.83 | 0.63 | 0.43 |

*Note*. T1 = Trimester 1; T2 = Trimester 2; T3 = Trimester 3; All cytokine values were log-transformed; Child Infection 24 months = frequency of childhood infection from birth to age 24 months; Child Infection 5 year = frequency of childhood infection from birth through age 5; Child Asthma 5 year = diagnosis of asthma at age 5; Child Eczema 5year = diagnosis of eczema at age 5; Child BMI 5 year = BMI at age 5; Child BMI 9-11 year = BMI at age 9-11; [a]$F(2,062)$; [b]$F(455)$; [c]$F(453)$; [d]$F(425)$; [e]$F(432)$; *significant at $p < .05$; **significant at $p < .01$.

Table 8. *Bivariate associations between offspring inflammatory states and childhood/adolescent mood outcomes*

| Offspring Inflammatory States | Childhood Internalizing Symptoms[a] | | Childhood Externalizing Symptoms[b] | | Adolescent Depression Symptoms[c] | |
|---|---|---|---|---|---|---|
| | *F* | *p* | *F* | *p* | *F* | *p* |
| Child Infection_5yr | 0.61 | 0.44 | 1.04 | 0.31 | 0.14 | 0.71 |
| Child Asthma_5yr | 0.75 | 0.39 | 2.88 | 0.09 | 0.15 | 0.70 |
| Child Eczema_5yr | 1.00 | 0.32 | 3.10 | 0.08 | 2.24 | 0.14 |
| Child BMI_5yr | 0.92 | 0.34 | 3.03 | 0.08 | 0.85 | 0.36 |
| Child BMI_9to11yr | 1.78 | 0.18 | 3.54 | 0.06 | 2.69 | 0.10 |

*Note*. Child Infection_5yr = frequency of childhood infection from birth through age 5; Child Asthma_5yr = diagnosis of asthma at age 5; Child Eczema_5yr = diagnosis of eczema at age 5; Child BMI_5yr = BMI at age 5; Child BMI_9to11yr = BMI at age 9-11; [a]$F(1,339)$; [b]$F(1,341)$; [c]$F(1,600)$; *significant at $p < .05$; **significant at $p < .01$.

Table 9. *Bivariate associations between antibiotic exposure during pregnancy/early postnatal development and offspring outcome variables*

| Antibiotic Exposure | Childhood Internalizing Symptoms[a] | | Childhood Externalizing Symptoms[b] | | Adolescent Depression Symptoms[c] | | BMI at age 5[d] | | BMI at age 9[e] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *p* | *F* | *p* | *F* | *p* | *F* | *p* | *F* | *p* |
| T1 Antibiotic | 1.70 | 0.19 | 0.68 | 0.41 | 0.21 | 0.64 | 1.26 | 0.26 | 1.17 | 0.28 |
| T2 Antibiotic | 1.41 | 0.24 | 0.94 | 0.33 | 0.35 | 0.56 | 1.68 | 0.20 | 0.36 | 0.55 |
| T3 Antibiotic | 0.04 | 0.85 | 1.04 | 0.31 | 0.88 | 0.35 | 0.17 | 0.68 | 0.01 | 0.90 |
| Child Antibiotic_24mo | 1.10 | 0.30 | 0.07 | 0.80 | 1.45 | 0.23 | 0.00 | 0.99 | 0.74 | 0.39 |
| Child Antibiotic_5yr | 0.94 | 0.33 | 0.52 | 0.47 | 0.48 | 0.49 | 0.43 | 0.51 | 0.39 | 0.53 |

*Note*. T1 Antibiotic = Frequency of first trimester broad-spectrum antibiotic exposure; T2 Antibiotic = Frequency of second trimester broad-spectrum antibiotic exposure; T3 Antibiotic = Frequency of third trimester broad-spectrum antibiotic exposure; Child Antibotic_24mo = Frequency of antibiotic exposure from birth to 24 months; Child Antibiotic_5yr = Frequency of antibiotic exposure from birth through age 5; [a]$F(1,339)$; [b]$F(1,341)$; [c]$F(1,600)$; ; [d]$F(1,360)$; [e]$F(1,622)$; *significant at $p < .05$; **significant at $p < .01$.

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