Relationships among Prenatal Risk Factors, Early Life Events, and Asthma in At-Risk Children

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LIST OF ABBREVIATIONS

aOR	Adjusted Odds Ratio
CI	Confidence Interval
CRP	Pneumococcal C-polysaccharide Reactive Protein
C-section	Cesarean Section
ER	Emergency Room
F-COPES	the Family Crisis Oriented Personal Evaluation Scales
FFFS	Feetham Family Functioning Survey
FHI	Family Hardiness Index
IL-6	Interleukin-6
IQR	Interquartile Range
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
PEPS	Peer Education in Pregnancy Study
SD	Standard Deviation
SES	Socioeconomic Status
Th1	T-helper Type 1 cells
Th2	T-helper Type 2 cells
T_{Reg}	Regulatory T-cells
US	United States

SUMMARY

Childhood asthma has increased in developed countries over the past 30 years with the factors driving these increases remaining essentially unknown. Asthma and allergic responses in the child may be created by a combination of pre-, peri-, and postnatal microbiome alterations, immune responses in T-helper type 2 cells (Th2) and regulatory T-cells (T_{Reg}), and early life events.

We sought to investigate the relationships among prenatal risk factors, early life events, and subsequent asthma and wheezing. We focused on three primary exposures: antibiotics in the first year of life, prenatal antibiotic use, and systemic maternal inflammation as measured by pneumococcal C-polysaccharide reactive protein (CRP). Using data from a longitudinal cohort study of mostly Hispanic children at risk for asthma in urban Chicago, three hundred mother-child pairs were followed from the mothers' first trimester of pregnancy through the children's third year of life. The primary endpoints of the study were asthma diagnosis by a physician by the third year of life and parentally reported wheezing in the third year of life.

First, we investigated the relationship between antibiotic use during the first year of the infant's life and subsequent asthma and wheezing. The reason for antibiotic use was separated into respiratory reasons versus non-respiratory reasons. We found a significant relationship between antibiotic use within the first year of life and incident asthma and wheezing, which has been identified consistently in retrospective studies. This association held only for antibiotics used for respiratory reasons, suggesting the relationship may be explained by reverse causation or confounding by indication.

Secondly, we found that prenatal systemic antibiotic use was a significant predictor of incident asthma, and weakly associated with wheezing, in at-risk children by age three after controlling for confounders. Modification of microbial load may be occurring prenatally, affecting the maturation of the infant immune system and increasing a child's risk for developing asthma.

Thirdly, we assessed the relationship between systemic maternal inflammation, as measured by CRP levels, and subsequent asthma and wheezing in the offspring within these asthmatic or at-risk women. After risk adjustment, we found a significant relationship between prenatal continuous CRP levels and asthma by year three as well as wheezing in year three.

SUMMARY (continued)

We also found a stronger relationship between prenatal continuous CRP levels and asthma by year three within children of Mexican ethnicity compared with children of non-Mexican ethnicity.

We conclude that the relationship between early life antibiotic use and asthma may be the result of confounding by respiratory infections. In the case of prenatal antibiotic use, we suggest an association with the development of asthma within at-risk children. We found that systemic inflammation during pregnancy in at-risk mothers may reflect a prenatal environment that could increase offspring susceptibility to develop asthma and wheezing in early life. Further research is needed to explore the underlying mechanisms of the in utero environment and immune changes on the subsequent development of asthma or wheezing.

I. INTRODUCTION

A. Background

Prevalence of asthma has doubled in developed countries over the last 30 years, with the factors driving these temporal increases remaining essentially unknown (Eder, Ege, and von Mutius, 2006). Asthma disproportionately affects minorities; however, individuals of Mexican ethnicity have significantly lower prevalence of asthma than other populations within the United States (US) (Rodriguez et al., 2002; Lara et al., 2006; Ledogar et al., 2000; Freeman, Schneider, and McGarvey, 2003). Asthma prevalence in children is associated with being born in the United States and increases with duration of US residency (Iqbal et al., 2014). These differences in asthma prevalence may be due to interactions among genetic, environmental, and lifestyle factors (Choudhry et al., 2007; Rodriguez et al., 2002; Davis et al., 2006).

The "hygiene hypothesis" suggests that early life exposures from an environment with fewer microbial exposures may shift development of the immune system and promote atopic immune responses (Strachan, 2000; Lynch et al., 2014). The hygiene hypothesis may be explained through an interaction of environmental factors with innate immune mechanisms, such as T-helper type 1 (Th1), Th2, and T_{Reg} responses. At birth an infant's immune system is strongly biased toward Th2 patterns of immune response (Johnson et al., 2002). Early environmental exposures may, or may not, shift immune development toward a Th1 pattern of immune response. If immune development is not shifted toward Th1 type responses the child is at a higher risk of developing asthma and other allergic diseases (Renz et al., 2006; Macaubas et al., 2003; Johnson et al., 2002). Additionally, patients with asthma have an alteration of T_{Reg} cells that plays an important role in immune suppression during fetal development (Robinson, 2009).

In recent decades, concurrent increases in antibiotic use to treat infections in children have led to studies examining possible associations between asthma and atopic disease. Retrospective studies have shown correlations between early antibiotic use and asthma, but the findings may be due to lack of complete control for confounding factors (Heintze and Petersen, 2013). Reverse causation or confounding by indication suggests that asthma symptoms may be misdiagnosed as respiratory infections, leading to the prescription of antibiotics. Alternatively, severe or frequent respiratory infections could be an indicator of a genetic predisposition for asthma, or infections

could damage the airways and contribute to the development of asthma (Rosenthal et al., 2010). Although many studies have attempted to analyze potential causality, the evidence is inconsistent, and there have been few studies within at-risk cohorts (Ball et al., 2000; Custovic and Woodcock, 2001; von Mutius, 2004; Strachan, 2000; Foliaki et al., 2009).

Asthma research has typically focused on postnatal exposures, but there is increasing evidence indicating atopic immune responses may be initiated in utero (Devereux, Barker, and Seaton, 2002). Emerging research suggests the in utero environment plays a much larger role in the risk of childhood asthma than previously believed, with factors such as maternal smoking having a deleterious effect on the fetal immune system and on lung development (Devereux, Barker, and Seaton, 2002). The maternal microbial environment influences the immune development of the offspring, with environmental exposures potentially altering the gene expression to increase disease susceptibility in the child (West, Jenmalm, and Prescott, 2014; Jenmalm, 2011; Renz, Brandtzaeg, and Hornef, 2012).

Microbes, particularly the gastrointestinal microbiome, are important influences on the development of the immune system, influencing the risk of allergic sensitization (Holt and van den Biggelaar, 2010). It has been postulated that antibiotic use in infants influences the development of the gut microbiome, which could result in reduced or slowed maturation of immune responses in Th1 and T_{Reg} cells (Thomas and Price, 2003). Antibiotic exposure during the first year of life could increase the risk of allergic diseases through altering this microbial exposure. Additionally, maternal antibiotic use during pregnancy could potentially alter the maternal gut, vaginal, or placental microbiomes. Recent data have shown that prenatal antibiotic use may increase the offspring's risk of developing allergic disease (Lange et al., 2012; Aagaard et al., 2014). Exposure to certain placental or vaginal microbes during the delivery process could also lead to the development of subsequent asthma. Therefore, factors that could potentially modify microbial exposure prenatally and perinatally may influence the development of subsequent atopic disease (McKeever et al., 2002; West, Jenmalm, and Prescott, 2014).

Systemic inflammation during pregnancy may indicate a maternal environment that could increase propensity in the offspring to develop wheezing and asthma in early life. A greater level of maternal inflammation could be due to environmental exposures, psychological stress, or excess weight gain. C-reactive protein is an acute phase protein that is commonly used as a biomarker of systemic inflammation (Pepys and Hirschfield, 2003).

Elevations in serum CRP levels are caused by stress and infection and elevated with older age, obesity, smoking, lack of physical activity, diets high in protein, and low socioeconomic status (SES) (Picklesimer et al., 2008). Older maternal age, previous delivery, maternal obesity, alcohol consumption, infections, and lack of physical activity during pregnancy have been associated with higher levels of CRP during pregnancy (Morales et al., 2011). Several studies have also shown a correlation between elevated CRP levels in pregnancy and an increased risk of wheezing in offspring (Morales et al., 2011; Sonnenschein-van der Voort et al., 2013). Prenatal CRP levels do not pass the placenta, so elevated CRP may be a marker for an indirect effect on the developing fetus (Sonnenschein-van der Voort et al., 2013; Jaye and Waites, 1997). Elevated CRP levels during pregnancy could indicate adverse prenatal conditions that could influence the maturation of the fetal respiratory system toward an increased risk of atopy and asthma. The role of maternal CRP levels in the development of childhood wheezing and asthma remains unclear.

B. **Conceptual Framework**

Asthma is a complex chronic disease of the lungs that is the result of interactions between the microbiome, genetics, environment, and social factors. Prenatal, perinatal, and postnatal microbiome alterations, immune responses in Th1, Th2, and T_{Reg}, and early life events work together to create allergic responses and asthma in the child. Figure 1 shows a conceptual framework for the development of allergic response and allergic asthma in young children (Johnson et al., 2002). The child's family history, gender, race, and genetics shape the inherited susceptibility. The immune responses in Th1, Th2, and T_{Reg} can be influenced through many different variables and interactions: lifestyle factors, SES, the physical environment and home, allergen exposure, and other unknown or unstudied factors. An atopic response or bronchial hyperresponsiveness in the child can be exacerbated by illness, infections, or exposure to smoke, which can lead to wheezing. Subsequent illnesses or wheezing may then lead to persistent atopic asthma.

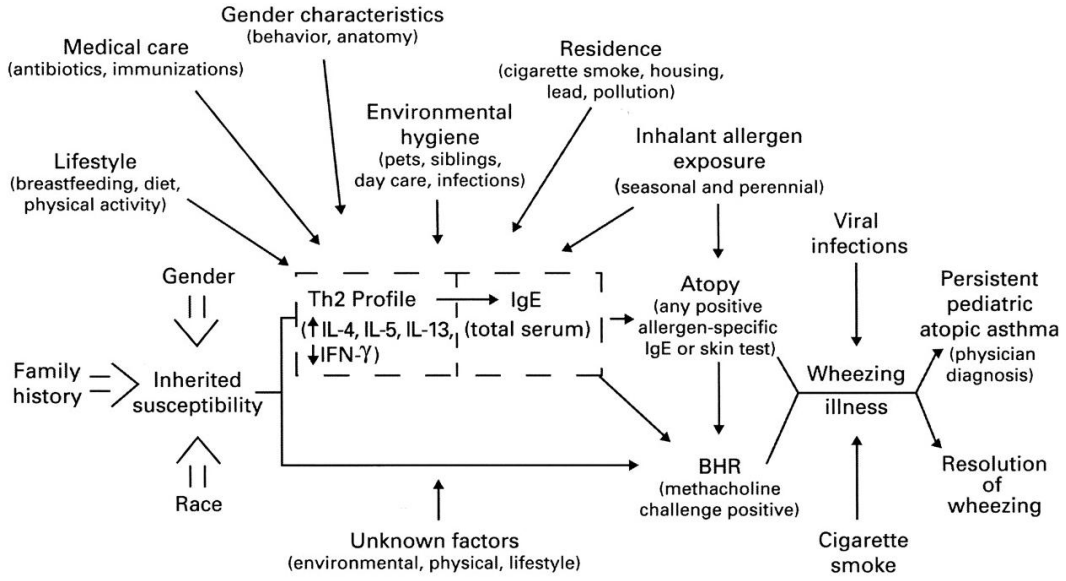


Figure 1. Diagram of factors associated with the development of asthma in children. (Johnson et al., 2002)

C. **Purpose of the Study**

The purpose of this research was to explore the relationships among prenatal risk factors, early life events, and subsequent asthma and wheezing. We focused on three primary exposures: antibiotics in the first year of life, prenatal antibiotic use, and systemic maternal inflammation as measured by CRP. The first study aimed to investigate whether antibiotic use during the first year of life increased the subsequent development of asthma or wheezing in the child. The second study aim explored the relationship between prenatal antibiotic use and the development of asthma and wheezing in the offspring. The third study aim investigated the relationship between maternal CRP levels and the subsequent development of asthma or wheezing in the offspring. As an exploratory study, psychosocial factors, specifically family functioning, hardiness, and coping styles, were investigated in association with maternal CRP levels and their combined impact on incident asthma and wheezing. Controlling for temporal confounders within a prospective study utilizing a high-risk urban cohort, these three study aims were explored:

1. To investigate the effect of antibiotic use in the first year of life on the subsequent development of asthma by year three and wheezing in year three.
2. To investigate the effect of prenatal antibiotic use on the subsequent development of asthma by year three and wheezing in year three.
3. a. To investigate the relationship between maternal CRP levels and the subsequent development of asthma by year three and wheezing in year three.

   b. To investigate the relationships among psychosocial factors, maternal CRP levels, and the subsequent development of asthma by year three and wheezing in year three.

D. **Significance of the Study**

Asthma research utilizing prospective birth cohorts has been limited. There have been very few prospective birth cohort studies examining asthma outcomes within impoverished urban residents; despite the fact these communities experience elevated asthma incidence and morbidity rates. Our study design offered pertinent temporal risk factors for childhood asthma and wheezing, and the prospectively collected measures of maternal and child confounders strengthened the study results. This unique population of predominantly Hispanic mothers was closely followed from the first trimester of pregnancy through the offspring's third year of life.

Additionally, recent research into childhood asthma has focused on findings that maternal microbe transfer to the offspring begins during pregnancy (McKeever et al., 2002; West, Jenmalm, and Prescott, 2014). Our dataset contains information on maternal health throughout each trimester of pregnancy and into the child's third year of life. In conclusion, the strengths of our study include a unique inner-city, mostly Hispanic population at risk for asthma, and prospective assessment of risk factors.

II. REVIEW OF RELATED ASTHMA LITERATURE

A. Antibiotic Use in Early Life

A dose-response relationship between antibiotic use and subsequent asthma diagnosis has been identified consistently in retrospective studies. A study of 251,817 children examined antibiotic exposure before one year of age and found an increased risk of asthma after the age of two as the number of courses of antibiotics increased (Marra et al., 2006; Marra et al., 2009). The association remained significant after adjustment for important confounders such as SES, birth factors, and infections in the first year of life. A systematic review and meta-analysis showed a significant effect in retrospective studies, as well as a small but nonsignificant effect in prospective studies (Marra et al., 2006). The question has remained as to whether these associations were the result of reverse causation or confounding by indication. Prior to antibiotic use, the presence of undiagnosed asthma may result in more frequent respiratory symptoms and diagnoses of respiratory tract infections leading to the prescription of antibiotics. Research into the positive association between antibiotics and incident asthma has been inconclusive. In a prospective cohort of 1,400 US children, early antibiotic use within the first six months of life was associated with an increased risk of asthma, even in children with no history of respiratory infections (Risnes et al., 2011). A longitudinal study of 13,116 children attempted to address the issue of reverse causation and found an association remained between antibiotics during the first year of life and asthma by age seven after accounting for potential confounders (Kozyrskyj, Ernst, and Becker, 2007). However, a longitudinal study of 4,408 children found that antibiotic use in early life was not associated with asthma, but there was more frequent antibiotic use among asthmatic children (Celedon et al., 2004). Similarly, a retrospective cohort study of 746 adults investigated the effect of antibiotic use in the first five years of life and subsequent asthma diagnoses. They found the relationship was explained by reverse causation and prescriptions were more likely to be written for the early manifestations of preexisting asthma (Cullinan et al., 2004). Yet another study of a birth cohort of 1,100 children found that the effect of antibiotic exposure before three months on the development of asthma between birth and 15 months and at four years may be due to confounding by chest infections, since infections may be difficult to distinguish from asthma at an early age (Wickens et al., 2008). Two meta-analyses concluded the association of antibiotics with childhood asthma reflected various forms of bias, with the most prominent being reverse causation (Penders, Kummeling, and Thijs, 2011; Heintze and Petersen, 2013). A recent large meta-analysis also found that early antibiotic use and

asthma in children was unproven and the result of confounding by indication (Heintze and Petersen, 2013). Respiratory infections in early childhood could contribute to the development of asthma by damaging the airways, or alternatively, severe respiratory infections could be an indicator of genetic predisposition for asthma (Rosenthal et al., 2010).

Interestingly, the relationship between antibiotic use and subsequent asthma within children with a family history of asthma has not been shown previously. A subgroup analysis of high-risk children within a meta-analysis found no significant association with antibiotics (pooled odds ratio (OR): 1.38; 95% confidence interval (CI): 0.63–3.03) (Marra et al., 2006). A birth cohort study of 198 high-risk children investigated the association between antibiotic use in the first year of life and the subsequent development of allergic disease at five years (Kusel et al., 2008). After adjustment, they found no relationship between antibiotic use and subsequent eczema, wheezing, asthma, or atopy. It is hypothesized that within higher-risk children with a predisposition to asthma, exposures such as antibiotics may have less of an effect on the immune response. Kozyrskyj et al. (2007) found no relationship between antibiotics and subsequent asthma within children whose mothers had asthma (Kozyrskyj, Ernst, and Becker, 2007). They hypothesized that postnatal maturation of immunity is faster in low-risk children so they could be more susceptible to the effect of antibiotics on immune development. In high-risk children, the microbiome may already be permissive toward the development of atopy and thus unaffected by antibiotics.

In a similar vein, antibiotic use has not been found to be a risk factor for asthma in urban children. It is thought that rural children are protected from asthma since the gut microbiome of infants in rural countries have been found to be more similar to the gut compositions of nonallergic infants than to allergic infants (Bjorksten et al., 2001; Bottcher et al., 2000). For that reason, antibiotics may alter the gut microbiome in rural children but they may have little effect on the gut microbiome of urban children, who have microbiomes that are already predisposed to develop allergic disease. A study of an urban birth cohort found that exposure to high levels of certain allergens and bacteria within the first year of life might be negatively associated with wheezing by their third year (Lynch et al., 2014). Additionally, animal models have shown that pregnant mice infected with cowshed microbes have pups protected against asthma (Conrad et al., 2009). The T_{Reg} cells, which down-regulate immune responses, are important in preventing allergies and asthma. Infants born on a farm have more T_{Reg} cells at birth than infants born

to nonfarmers (Prescott et al., 1999). It is hypothesized that antibiotic-related suppression of inflammatory responses may alter immune responses in early childhood (Jedrychowski et al., 2011).

Type of antibiotic may also be an important factor in the association of antibiotics with asthma development. Broad-spectrum antibiotics became more prevalent in the 1980s and may alter the microbiome more than the narrow-spectrum antibiotics used in the past (Foliaki et al., 2009; Kozyrskyj, Ernst, and Becker, 2007; Risnes et al., 2011). Kozyrskyj et al. (2007) investigated the effects of narrow-spectrum versus broad-spectrum antibiotics and found a significant association between exposure in the first year of life and childhood asthma for the broad-spectrum antibiotics but not the narrow-spectrum (Kozyrskyj, Ernst, and Becker, 2007). This could support the interpretation that early antibiotic exposure alters atopic disease risk through alterations in the microbiome.

B. **Prenatal Antibiotic Use**

Associations have been found between prenatal antibiotic use and the development of asthma and wheezing in early life (Benn et al., 2002; Rusconi et al., 2007; McKeever et al., 2002; Stensballe et al., 2013; Jedrychowski et al., 2006; Martel et al., 2009; Murk, Risnes, and Bracken, 2011; Calvani et al., 2004). There could be many explanations for this association. It could be caused by the maternal infection, inflammation from the infection, or a direct effect of broad-spectrum antibiotics on the microbiome. Data have suggested that antibiotics in utero may change the maternal or placental microbiome and increase the child's risk of developing allergic disease (Lange et al., 2012; Aagaard et al., 2014). Factors, such as antibiotics, that could modify microbial exposure prenatally may have a long-term impact on the risk of developing subsequent atopy and asthma (McKeever et al., 2002; West, Jenmalm, and Prescott, 2014; Hafkamp-de Groen et al., 2013).

Unlike the association between early-life antibiotic use and subsequent asthma, the association between prenatal antibiotic use and asthma is more likely to be a true etiologic relationship since the issue of reverse causation is naturally avoided. However, the relationship could be confounded by maternal asthma, infections, smoking, premature birth, or antibiotic use in infancy (Benn et al., 2002). A few studies have examined the association between prenatal antibiotic use and incident asthma in children. McKeever et al. (2002) utilized a birth cohort to show a relationship between prenatal antibiotics exposure and an increase in the child's risk of allergic

disease (McKeever et al., 2002). Several other cohort studies have also shown an increased risk of asthma and wheezing with prenatal and perinatal antibiotic use (Stensballe et al., 2013; Benn et al., 2002; Rusconi et al., 2007; Calvani et al., 2004). Previous research has been hesitant to conclude a possible causal relationship since the positive association between prenatal antibiotic use and asthma and wheezing may be confounded by antibiotic use in infancy since mothers who use antibiotics may be more apt to ask for antibiotics for their infants (Benn et al., 2002). Besides infant antibiotic use, the positive association between prenatal antibiotics and subsequent asthma could be confounded by maternal asthma or respiratory infection. The Copenhagen Prospective Study on Asthma in Childhood showed an increased risk of asthma associated with maternal antibiotic use and the investigators were able to replicate their findings in a subgroup of mothers using antibiotics for non-respiratory infections (Stensballe et al., 2013). They concluded the effect was not confounded by the mother's asthma or infections and could be due to a disturbed bacterial ecology that may trigger the disease process in perinatal life.

A cohort study found that antibiotic use in the second and third trimester, but not the first trimester, was associated significantly with persistent wheezing in one-year olds (Jedrychowski et al., 2006). Similarly, acetaminophen use later in pregnancy has been found to increase the risk of respiratory symptoms in the first year of life (Persky et al., 2008). It is believed that microbial changes prenatally, perinatally, and postnatally are required to optimally develop Th1 and T_{Reg} to avoid allergy (West, Jenmalm, and Prescott, 2014). Research is still necessary to determine whether this microbial variation is the cause or the effect of allergic disease. A study using murine models found that when the gastrointestinal microbiota was disrupted with antibiotics, there was a subsequent up-regulation of Th2, leading to airway allergic response (Noverr et al., 2005). Additionally, studies have found that the composition of maternal vaginal and intestinal bacteria was related to an increased risk of wheeze in infants (Benn et al., 2002; Lange et al., 2012). Contrary to the previously held belief that the womb is sterile, recent studies have shown maternal microbial transfer starts during pregnancy (West, Jenmalm, and Prescott, 2014). Aagard et al. (2014) recently published a study showing the placental microbiome was affected by maternal prenatal infections (Aagaard et al., 2014). It is unknown whether the infection itself, an inflammatory response, or antibiotic use for the infection, causes this change in the placental microbiome. Prenatal antibiotic use could modify the placental, vaginal, or maternal gut microbiome, which may increase a child's risk of developing asthma.

As the prevalence of asthma has increased over recent decades, the early infant gut composition has been found to also change (Adlerberth et al., 2006). A small study found the composition of the maternal intestinal microbiome, specifically higher total aerobes and enterococci, was related to an increased risk of wheeze by six months (Lange et al., 2012). The bacteria colonizing the gut in the first days of life are predominately from the mother and environment (Palmer et al., 2007; Penders et al., 2006). A study investigating the influences on the gut microbiome in early infancy found no association between maternal antibiotic use and the infant's microbiotic composition, but they did find a different microbiotic composition for infants who did not take antibiotics (Penders et al., 2006).

The maternal intestinal or vaginal microbiome could also influence the constitution of the infant microbiome through mode of delivery. Along the lines of the hygiene hypothesis, children born via cesarean section (C-section) may be less likely to be exposed to bacteria during childbirth and therefore be at an increased risk for developing asthma when compared to vaginally delivered children. The intestinal bacterial microbiome is an important component in immune development and with increasing rates of C-sections over the last two decades; it is possible that altered gut colonization caused by bacterial exposure during labor could interfere with the immune system (Guibas et al., 2013). Infants born through C-section have lower numbers of bacteria compared to vaginally born children (Penders et al., 2006). A study found that infants born vaginally on time at home and who were breastfed exclusively had a favorable gut microbiota with high numbers of bifidobacteria and low numbers of *Clostridium difficile* and *E coli* (Penders et al., 2006). During vaginal birth the fetus encounters microbes within the birth canal and genital tract, and some of these microbes may lead to the development of chorioamnionitis during delivery. A couple of studies have shown that maternal chorioamnionitis increased the risk of early wheezing and asthma (Kumar et al., 2008; Keski-Nisula et al., 2009). Chorioamnionitis causes a proinflammatory response that is associated with an increased risk of lung disease. Asthma could be associated with early chorioamnionitis at the time of birth through inflammatory mechanisms. A birth registry of almost 2 million children showed a 52% increased risk of asthma in children delivered by C-section when compared with vaginally delivered children (Tollanes et al., 2008). Moreover, a meta-analysis of 20 articles found a 20% increase in the risk of asthma within children who were delivered by C-section versus vaginal birth (pooled OR: 1.20; 95% CI: 1.14–1.26) (Thavagnanam et al., 2008). Another study found an association with early-onset wheezing in children whose mothers received antibiotics at delivery but did not find an association with C-section (Rusconi et al., 2007).

Most asthma and respiratory research that has investigated the microbiome has focused on the gastrointestinal microbiome with recent studies examining the vaginal and placental microbiome. It was believed healthy infant lungs were sterile, although a recent study investigated the bacterial population of the lung microbiota in mice and found the lower airways had a distinct bacterial microbiome (Barfod et al., 2013). They found the mouse lung microbiome was not similar to the gut microbiome but did overlap considerably with the vaginal microbiome. Further research is needed to determine the role of the lung microbiota in infant asthma.

C. **C - Reactive Protein Levels**

Prenatal CRP levels may have an indirect effect on the development of asthma in children. Elevations in serum CRP levels are caused by stress and infection, and are elevated with older age, obesity, smoking, lack of physical activity, diet, and low SES (Picklesimer et al., 2008). An analysis of the National Health and Nutrition Examination Survey (NHANES) data showed that adults with asthma and asthma symptoms have higher levels of CRP (Arif, Delclos, and Colmer-Hamood, 2007). This study also showed CRP levels were higher with more asthma symptoms indicating that CRP levels may be an indicator for asthma severity. The CRP levels are significantly higher in people with reduced lung function, independent of asthma (Rasmussen et al., 2009). Children with uncontrolled asthma have higher CRP levels than children with controlled asthma (CRP median interquartile range (IQR): 0.56 (0.60) versus 0.25 (0.34) mg/L, $p<.01$) (Navratil et al., 2009). However, the role of blood inflammatory biomarkers in asthmatics is not well understood or studied.

Picklesimer et al. (2008) demonstrated that normal pregnancy is an inflammatory stressor with elevated serum CRP levels (Picklesimer et al., 2008). His study of healthy pregnant women yielded a median CRP level of 4.8 mg/L (IQR: 0.63–15.7 mg/L) suggesting that previously considered extremely high CRP values of >10mg/L may be within the normal range for healthy pregnant women. These elevations in CRP have been found to remain consistently high from the earliest stages of pregnancy through to child birth (Sacks et al., 2004; Belo et al., 2005; Picklesimer et al., 2008). However, higher than usual inflammatory response in pregnant women, as measured by CRP, has also been related to adverse pregnancy outcomes including preeclampsia, neonatal sepsis (Jeon et al.,

2014), preterm delivery (Ernst et al., 2011; Lohsoonthorn, Qiu, and Williams, 2007), and intrauterine growth restriction (Ernst et al., 2011; Morales et al., 2011; Tjoa et al., 2003).

Recent research has focused on the role of the prenatal environment as an important factor in the development of the fetal immune system and in the subsequent occurrence of allergic and respiratory diseases (Devereux, Barker, and Seaton, 2002). Studies investigating the impact of increased prenatal CRP on subsequent allergic disease have shown inconsistent results. A prospective cohort study of 504 mother-child pairs showed elevated prenatal CRP levels were associated with a three-fold increased risk of wheeze and a two-fold increased risk of lower respiratory infections in children at one year of age (adjusted odds ratio) [aOR]: 2.87 [95% CI: 1.23–6.71] for wheezing and aOR: 2.37 [95% CI:1.01–5.55] for respiratory infections) (Morales et al., 2011). They also found that higher maternal CRP levels were associated with increasing maternal age, parity, pre-pregnancy obesity, and lack of physical activity. Reproductive outcomes such as birth weight, prematurity, and small for gestational age were not related to maternal CRP levels. Another prospective study of 4,984 mother-child pairs found that higher maternal CRP levels in early pregnancy were associated with a lower risk of wheezing by year two, and a higher risk of eczema (Sonnenschein-van der Voort et al., 2013). They attributed the difference in wheezing effects to the larger sample size and ability to adjust for more effect modifiers. Sonnenschein et al. (2013) analyzed CRP levels in cord-blood and found an increased risk of wheezing and lower respiratory infections in the first four years of life. They hypothesized that the timing for CRP elevations may be critical for the association with the lung and airway development. A study of 636 children found the risk of allergic sensitization by 4.5 years of age, as measured by immunoglobulin E concentrations**,** was decreased in children who had increased CRP levels at one year of age (OR: 0.48; 95% CI: 0.24–0.95) (Mustonen et al., 2013). They found no association between the CRP levels and asthma, but did find a protective effect of low-grade inflammation in the control of immunoglobulin E -mediated allergic diseases. They concluded that a poor response to inflammatory triggers, or an impaired induction of CRP, could be reflecting a poor response of the innate immune system, indicating a risk for the subsequent development of atopy.

Elevated levels of CRP do not pass the placenta, but they may indicate a process leading to a direct effect on the developing fetus (Sonnenschein-van der Voort et al., 2013). The pathways may include fetal growth restriction with smaller lungs and airways, an inflammatory fetal status, or other alterations in the infant's immune system that may affect the development of wheezing or asthma. Prenatal cytokines and immune modulators, Th1

and Th2, are thought to play important roles in controlling the maturation of the developing immune system and conditioning it for postnatal responses against allergens (Macaubas et al., 2003). Elevated prenatal CRP levels may be a marker of adverse prenatal conditions, influencing the fetal respiratory system response to microbial agents and allergen exposure.

D. **Psychosocial Factors**

Psychosocial factors may be important for promoting healthy pregnancy outcomes. It has been hypothesized that social support can contribute to higher birth weight by reducing stress (Campos et al., 2008). Maternal stress during pregnancy has been shown to contribute to poor pregnancy outcomes and infant health (Coussons-Read et al., 2012; Dunkel Schetter and Tanner, 2012). Evidence for the negative effects of maternal stress, depression, and anxiety during pregnancy on neurodevelopment is considered to occur through fetal programming (Dunkel Schetter and Tanner, 2012). The adverse programming of the fetal nervous system develops through alterations in functioning of both the maternal and fetal hypothalamic pituitary adrenal axes (Coe and Lubach, 2005; O'Connor et al., 2005). A study found an association between optimism during pregnancy and healthy infant birth weight, but this was due to the mediating effect of optimistic women having healthier prenatal behaviors (Lobel et al., 2000). A study by Catov et al. (2015) proposed anxiety during pregnancy may be moderated by resilience factors such as optimism and may be mediated by health behaviors such as tobacco use and weight gain (Figure 2) (Catov et al., 2015). Alternatively, anxiety may lead to unhealthy behaviors. Immune changes and inflammation are biological pathways through which these factors could converge, potentially leading to poor birth outcomes. Catov et al. (2015) hypothesize that high anxiety or low optimism may induce proinflammatory cytokines that could affect immune responses during pregnancy. Inflammation, as measured by CRP, may be mediating the relationship between poor psychosocial health and adverse pregnancy outcomes.

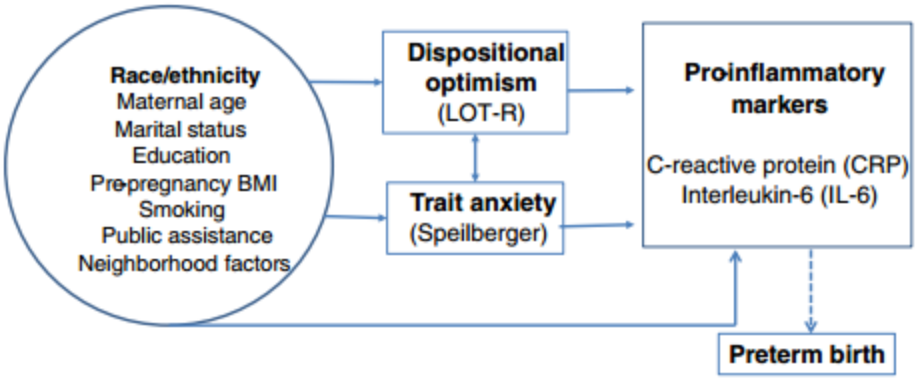


Figure 2. Conceptual model relating psychosocial factors to CRP levels during pregnancy. (Catov et al., 2015)

Psychosocial stress and low social support have been associated with elevations in CRP and interleukin-6 (IL-6), a proinflammatory cytokine (Coussons-Read, Okun, and Nettles, 2007; Pearce et al., 2010). Chronic stress and anxiety have been shown to increase the risk of preterm birth with pregnancy-related anxiety contributing to a two-fold increased risk of preterm birth (Dunkel Schetter and Tanner, 2012). An association has been found between excess stress and lower concentrations of IL-6 in Hispanic and African American women (Coussons-Read et al., 2012; Catov et al., 2015). However, an association between anxiety, optimism, and inflammatory markers has not been identified in Caucasian women (Catov et al., 2015).

Elevated CRP has been associated with excess anxiety and depression in nonpregnant adults (Bankier et al., 2008; Hickman, Khambaty, and Stewart, 2014; Howren, Lamkin, and Suls, 2009). Anxiety, stress, and depression have also been related to an increased production of CRP through effects on the nervous system pathways (Raison, Capuron, and Miller, 2006; Steptoe, Hamer, and Chida, 2007). Elevated CRP levels have been associated with excess preterm birth risk, as well as other adverse birth outcomes such as infection and hemorrhage (Pitiphat et al., 2005; Sorokin et al., 2010; Catov et al., 2007).

Elevated CRP levels are associated with stressors in children, with the co-occurrence of asthma and depression shown to substantially elevate inflammation that may persist over time (Shanahan et al., 2013; Chen and Miller, 2007). The co-occurrence of stressors with asthma may lead to further asthma-related morbidity. The causal relationship between stress and asthma is largely unknown, although inflammatory markers are also elevated with

chronic or acute stressors such as poverty, life events, or depression (Coussons-Read, Okun, and Nettles, 2007). A study of 52 women found that elevated stress and low social support during pregnancy were associated with elevated CRP levels (Coussons-Read, Okun, and Nettles, 2007). Psychosocial stress, including individual, family, and community-level stress, has been associated with asthma morbidity (Chen et al., 2013; Yonas, Lange, and Celedon, 2012). Living in an unsafe neighborhood and other related community stressors have been linked to asthma diagnosis in urban minority children (Vangeepuram et al., 2012). A prospective study of 1,420 children aged 10–16 found heightened inflammation with the coexistence of asthma and depression. The level of heighted inflammation remained elevated over the course of a year (Shanahan et al., 2013). Additionally, family functioning is likely to contribute to the family's ability to successfully manage asthma and wheezing symptoms, but there are little to no data on this topic (Clarke and Calam, 2012).

E. **Hispanic Ethnicity and Acculturation**

Given that the majority of our study population is of Mexican ethnicity, we would expect asthma rates to be lower than in the general population. Asthma disproportionately affects minorities; however, Mexicans have been found to have significantly lower prevalence of asthma than other populations within the United States (Rodriguez et al., 2002; Lara et al., 2006; Ledogar et al., 2000; Freeman, Schneider, and McGarvey, 2003), including other Hispanic subgroups. Asthma prevalence has been found to be highest in Puerto Ricans, followed by Cubans and Dominicans, and lowest among Central Americans and Mexicans (Carter-Pokras and Gergen, 1993; Koinis-Mitchell et al., 2011). The reason for this disparity has remained unclear, and it is debatable whether the protection is due to differences in early-life exposures or due to lifestyle and environmental factors that are likely to change with acculturation. The hygiene hypothesis postulates that in utero or early childhood microbial exposure protects against atopy and asthma. Lower exposures to early-life protective factors in the developed world, such as bacteria and infections, may result in a modification of T_{Reg} cells and the Th2 immune response (Douwes and Pearce, 2002; Cohet et al., 2004).

The lower prevalence of asthma within Mexicans may diminish after extended residence in the United States (Eldeirawi and Persky, 2007). One study found a two-fold increased risk of asthma in Mexican children living

in the United States during their first year of life as compared to Mexican children who had lived in Mexico during their first year of life (Eldeirawi and Persky, 2007). An analysis of NHANES data by Eldeirawi et al. (2006) illustrated that Mexico-born, as well as less-acculturated US-born Mexicans, had lower rates of asthma as compared to highly acculturated Mexicans (Eldeirawi and Persky, 2006). Holguin et al. (2005) also showed a higher asthma prevalence in US-born Mexican American adults compared to those born in Mexico, but the prevalence of asthma increased with more years residing in the United States (Holguin et al., 2005). These studies imply that factors modified by acculturation could affect the risk of asthma and wheezing and protection may diminish when confronted with new allergens, pollutants, and other factors.

Less-acculturated Latinos are among the healthiest Americans, specifically involving birth outcomes (Campos et al., 2008; Abdou, Dominguez, and Myers, 2013). This is referred to as the "Latino Paradox" where despite low SES, US Latinos are healthier than non-Latinos (Markides and Coreil, 1986; Cagney, Browning, and Wallace, 2007). It is still unclear whether the protection is due to differences in early-life exposures or due to lifestyle and environmental factors that are likely to change with acculturation. Eldeirawi et al. (2006) showed that more-acculturated and US-born Mexican American children were more likely to have asthma and wheezing independent of age, gender, insurance, and having a regular place for health care, as compared to low-acculturated and Mexico-born peers (Eldeirawi and Persky, 2006). The results also suggested that acculturation may have stronger effects than country of birth. In the United States, Puerto Ricans have the highest prevalence of asthma among all Hispanics (Lara et al., 2006). Within Puerto Ricans, birth outside the United States has the opposite effect from other ethnic groups, and island-born Puerto Ricans have higher rates of asthma than US-born Puerto Ricans (Lara et al., 2006; Cohen et al., 2007). In a study comparing island-born Puerto Ricans to US-born Puerto Ricans living in the South Bronx, researchers found that despite US-born Puerto Ricans having lower SES and more comorbidities, such as low birth weight and mothers who smoke, they had lower rates of asthma (Cohen et al., 2007). While this study did not account for acculturation, it appears there may be a basic difference between Puerto Ricans and other Latinos that still needs to be explored.

Factors that are changed with immigration and acculturation could be associated with the development of asthma and wheezing. Early-life exposures that are protective to Mexico-born children could fade away with immigration and acculturation. This protection might derive from factors such as rural location, early exposure to

infections, increased pet ownership, multiple siblings, day care attendance, and exposure to farm animals (Douwes and Pearce, 2002; Martinez and Holt, 1999; Ball et al., 2000).

Diet is one of the main factors that changes with migration and acculturation. Mexico-born adults consume less fat and more vitamins than second-generation Mexican-American adults (Guendelman and Abrams, 1995). It has been hypothesized that diets rich in antioxidants and omega-3 fatty acids may be protective towards the development of asthma and allergic disease (Devereux and Seaton, 2005). Rottem et al. (2005) concluded that migration exposes individuals to different allergens, pollutants, dietary changes, and housing conditions (Rottem, Szyper-Kravitz, and Shoenfeld, 2005). In addition to these differences, there is also a change in lifestyle. Obesity and lack of consistent physical activity, both characteristics of the US lifestyle, are predictors of asthma (Platts-Mills et al., 2005).

Higher acculturation is also associated with low birth weight, prematurity, smoking during pregnancy, and less breast-feeding with one study finding that acculturation affects low birth weight indirectly through smoking and diet (Cobas et al., 1996). Specifically, it has been shown that more acculturated Latinos have higher rates of insurance coverage and access to health care, as well as fewer barriers to care, and increased usage of preventive services (Thamer et al., 1997).

Data have shown that Latino immigrants have better mental health than US-born Latinos and Caucasians (Alegria et al., 2007; Mulvaney-Day, Alegria, and Sribney, 2007). Foreign nativity is associated with better mental health outcomes in Mexicans (Alegria et al., 2007). As immigrants become more acculturated into the American lifestyle, their mental health may decline (Alegria et al., 2007). Familialism, or commitment to and interconnectedness with family members, is a cultural value that is known to be higher in Latinos (Marin et al., 1993). A study by Campos et al. (2008) followed pregnant foreign-born and US-born Latinas, as well as European-American women living in the United States, and found that familialism was associated with less stress and higher social support (Campos et al., 2008). Latinas reported higher levels of familialism compared to European-Americans. Furthermore, the foreign-born Latinas with greater social support had higher infant birth weights. They concluded that cultural values, especially familialism, may lead to psychological benefits that positively affect mental and physical health. There is limited research into the effect of familialism on asthma or atopic disease.

III. Methodology

A. Study Sample

The Peer Education in Pregnancy Study (PEPS) was a randomized education intervention study examining the effect of community educators working with pregnant women at risk for having children with asthma on modification of factors in the home known to exacerbate the disease (Persky et al., 1999). All women in the study received general health education, advice for smoking cessation, and encouragement to breast feed. Half of the women received three home intervention visits from a community health educator to identify and decrease asthma triggers in the environment. Women were identified as being pregnant and considered eligible if they lived in the targeted area, were less than four months pregnant, did not suffer a miscarriage, and the unborn child had a family history of asthma or allergies. A total of 383 women were randomized into the trial with 351 mother-child pairs followed out to one year. The complete outline of participant flow through the study has been published elsewhere (Persky et al., 2009; Persky et al., 2008). Mothers were followed and surveyed in each trimester of pregnancy and children were followed from four weeks of age through 2009. A total of 300 mother-child pairs were followed through the child's third year of life and were included as the study cohort in our research aims.

The study was approved by the University of Illinois at Chicago Human Subjects Institutional Review Board (IRB# 1998-0655).

B. Data Collection

Initial examinations in pregnancy included a visual inspection of the home environment, health, and lifestyle of the mother and immediate family members during the mother's first trimester of pregnancy. Medical and lifestyle questionnaires were administered in English or Spanish depending on the mother's preference during 4–5 months of gestation, 7–8 months of gestation, and then when the child was 4–6 weeks, 6 months, 1 year, 1.5 years, 2 years, 2.5 years, and 3 years of age. Nurse phone follow-ups at 3 and 9 months of age were also completed to obtain interim medical and symptom histories.

Prenatal risk factors during pregnancy such as antibiotic use, infections, and smoking status were evaluated by questionnaire at enrollment in the first trimester, at 4–5 months of gestation, and at 7–8 months of gestation. Only systemic antibiotics were noted, and reason for prenatal antibiotic use was not captured. Information on type of delivery and antibiotic use during delivery was not collected as part of the study. Maternal characteristics including history of asthma, maternal age, maternal ethnicity, and body mass index were assessed at baseline. Maternal acetaminophen and ibuprofen use were also evaluated by questionnaire three times during pregnancy. Family history of asthma was determined at baseline questionnaire if a first-degree relative of the unborn child had a history of asthma.

Breast feeding was determined on the first visit after delivery (at 4–6 weeks). Other potential confounders were also collected at the first visit after delivery: medication usage, infections, child's gender, gestational age, birth weight, age when formula was introduced, child's medication usage, and infections.

Child characteristics such as exposure to smoke in the home, infections, vitamin use, and acetaminophen or ibuprofen use were evaluated five times throughout the child's first year of life and then every six months thereafter. Antibiotic use and indication for use in the child's first year of life was determined from phone interviews at three and nine months, and from three different home visits: at 4–6 weeks old, at 6 months old, and at 12 months old. Topical antibiotics and antifungal agents were not included. Home environment questionnaires, assessing exposure to factors such as pets, mice and cockroaches, were completed by peer educators at baseline and once a year.

Perceived exposure to passive smoke at home during pregnancy was determined from the nurse question "In an average week about how many hours are you exposed to other peoples' cigarette smoke at home, including by family members and visitors?" A summary dichotomous variable was created from all pregnancy nurse surveys to represent any perceived exposure to passive smoke at home at anytime during pregnancy. Similarly, active smoking in pregnancy was determined from nurse questions at the same time and a dichotomous summary variable was created as the presence of any smoking by the mother in mid-to-late pregnancy.

Development of asthma and respiratory endpoints were determined by any positive response to nurse-administered questions at each home visit and during nurse follow-up phone calls. Information was obtained about asthma diagnosed by a healthcare provider, wheezing, sleep-disturbed wheezing, frequent coughing, sleep disturbed

by coughing, emergency room (ER) visits for breathing problems, hospital admissions for breathing problems, and eczema diagnosis. Follow-up assessments after one year of age also asked about coughing without a cold, shortness of breath or chest tightness, and sneezing or runny nose without a cold.

The PEPS also collected data on psychosocial factors, using established surveys, at certain points during the study. Spanish translations were available and utilized according to the mother's preference. Acculturation was measured by the mother during the first trimester visit using the Bidimensional Acculturation Scale for Hispanics (Appendix A) (Marin et al., 1993). These 24 items examined Hispanic and non-Hispanic cultural domains including questions on language, thinking, and music. Each participant was assigned two scores: one for the average of the 12 items comprising the Hispanic domain and one for the 12 items forming the non-Hispanic domain. These scores were used on a continuous scale.

The Feetham Family Functioning Survey (FFFS) (Appendix A) assessed intra- and inter-family relationships during pregnancy and was structured in three areas: (1) relationship between family members; (2) relationship between family and subsystems, including the division of labor such as housework; and (3) relationship between family and society (Roberts and Feetham, 1982). A higher score indicated a higher discrepancy between the reported experience for each family function and the expectation for that item.

The Family Hardiness Index (FHI) (Appendix A) questionnaire translated the measure of the individual hardiness to the family unit and was assessed at the child's third year visit (McCubbin, McCubbin, and Thompson, 1986). Twenty items fit three components of the family: (1) *commitment* measured the family's sense of internal strengths, dependability, and ability to work together; (2) *challenge* measured the family's efforts to be innovative, active, and to experience new things and learn; and (3) *control* measured the family's sense of being in control of family life rather than being shaped by outside events and circumstances. A total score was also obtained by summing all 20 items. Higher scores suggest a higher family hardiness.

The Family Crisis Oriented Personal Evaluation Scales (F-COPES) (Appendix A) questionnaire was also assessed at the child's third year visit and featured 30 coping behavior items to identify problem solving behavioral strategies utilized by families in difficult problem situations (McCubbin, Larsen, and Olson, 1982). There were five subscales involved: (1) *acquiring social support* measured a family's ability to actively engage in acquiring social

support from relatives, family, friends, and neighbors; (2) *reframing* assessed the family's capability to redefine stressful events in order to make them more manageable; (3) *seeking spiritual support* measured the family's ability to acquire spiritual support; (4) *ability to mobilize* measured the family's ability to acquire and accept help; and (5) *passive appraisal* measured the family's ability to accept problematic issues minimizing reactivity (Figley, 2013). A total coping score was obtained by summing the response for each item. Higher scores suggest a higher frequency of behaviors considered to be problem-solving behaviors. FFFS, FHI, and F-COPES were not validated within Hispanic populations.

A total of 324 women gave 55cc of blood during pregnancy at six months gestation for the purpose of future research. Serum samples from these mothers were kept frozen at -70°C from the time they were obtained until the time of analysis. Funds for analyzing CRP levels within the serum came from a 2014 award from the Division of Epidemiology and Biostatistics and from the Dean's Office of the University of Illinois at Chicago's School of Public Health. High-sensitivity CRP levels were analyzed using an assay by LabCorp Clinical Trials (Cincinnati, Ohio). Ten samples were reanalyzed in a different assay run on a different day to provide an estimate of inter-assay reliability over time. Correlation between assay measurements of CRP was high (r=.96), with an intra-class correlation coefficient of 0.95, and an inter-assay coefficient of variation (CV) of 11%.

C. **Study Endpoints and Their Relationship with Cohort Characteristics**

The primary and secondary endpoints of the study are shown in Table I. The primary endpoints for each study aim were asthma diagnosis by year three and reported wheezing in the third year of life. There were 44 (15%) children with asthma by year three and 65 (22%) with wheezing in year three. Asthma was defined as ever having an asthma diagnosis by a healthcare professional by three years of age based on the self-reported answer to the question "Has a doctor ever told you that your child has asthma?" Secondary endpoints include other respiratory symptoms in the third year: exercise-induced wheezing, sleep disturbed by wheezing, wheezing without a cold, and ER visits for breathing problems. Development of the primary endpoint of wheezing in the past 12 months and the secondary endpoints of other respiratory symptoms were determined by a positive response at the 2.5 year old visit or at the third year visit based on the following questions: "Has your child's chest sounded wheezy or whistling?"; "Has your

child's chest sounded wheezy or whistling during or shortly after vigorous exercise?"; "Has your child been awakened at night by wheeze or by shortness of breath?"; "Has your child had episodes of wheezing or whistling without a cold?"; "Was your child treated in the emergency room for breathing problems (coughing, congestion, runny nose, wheezing)?"

TABLE I

THIRD YEAR OUTCOMES IN CHILDREN FROM THE PEPS COHORT (N=300)

	N (%)
Primary Outcomes	
Asthma by Year 3	44 (14.7)
Wheezing in Year 3	65 (21.7)
Secondary Outcomes in Year 3	
Eczema	58 (19.3)
Exercise Induced Wheezing	35 (11.7)
Sleep Disturbed by Wheezing	26 (8.7)
Wheezing without a Cold	27 (9.0)
Emergency Room Visits for Breathing Problems	46 (15.3)

Table II shows maternal and family history characteristics, child characteristics, and first year of life exposures by the primary endpoints of asthma by year three and wheezing in year three. In unadjusted comparisons, children with asthma and wheezing were less likely to be Mexican and more likely to be African American. Mothers who smoked in pregnancy were more likely to have children with asthma and wheezing. Prenatal antibiotic use and ibuprofen use in early pregnancy was associated with increased asthma. Maternal asthma and a family history of asthma were also associated with increased asthma and wheezing. Low birth weight was more common in children who developed wheezing. Children developed less wheezing if they were breast fed. A greater number of children wheezed if they were exposed to smoke in the home, were exposed to smoke outside of the home, or were exposed to passive smoke in their first year of life. Antibiotic use in the first year of life was associated with increased asthma and wheezing, and acetaminophen use in the first year of life was inversely associated with increased asthma. There were no associations between the endpoints and respiratory infections, ear infections, or fevers in the mother or child. All statistical analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina).

TABLE II

CHARACTERISTICS AND UNIVARIATE ANALYSIS FOR ASTHMA AND WHEEZING IN CHILDREN FROM THE PEPS COHORT (N=300)

Characteristics	**Asthma by Year Three**				**Wheezing in Year Three**			
	Yes N (%)	**No N (%)**	**OR (95% CI)**	**P-Value**	**Yes N (%)**	**No N (%)**	**OR (95% CI)**	**P-Value**
Total Number of Children	44 (14.7)	256 (85.3)			65 (21.7)	235 (78.3)		
Study Intervention	20 (45.5)	129 (50.4)	0.82 (0.43, 1.56)	0.55	29 (44.6)	120 (51.1)	0.77 (0.45, 1.34)	0.36
Maternal and Family History								
Maternal Age (Years) –Mean ± SD	25.5 ± 5.3	26.0 ± 6.0	0.99 (0.93, 1.04)	0.58	26.2 ± 6.0	25.8 ± 5.9	1.01 (0.97, 1.06)	0.62
Mexican Ethnicity	20 (45.5)	181 (70.7)	0.35 (0.18, 0.66)	<0.01	33 (50.8)	168 (71.5)	0.41 (0.23, 0.72)	<0.01
African American	5 (11.4)	10 (3.9)	3.15 (1.02, 9.72)	0.05	7 (10.8)	8 (3.4)	3.43 (1.19, 9.83)	0.02
Mother Born in United States	21 (47.7)	84 (32.8)	1.87 (0.98, 3.57)	0.06	29 (44.6)	76 (32.3)	1.69 (0.96, 2.95)	0.07
Parity—Median (Q1, Q3)	1 (0, 2)	1 (0, 2)	0.96 (0.74, 1.24)	0.75	1 (0, 2)	1 (0, 2)	0.99 (0.81, 1.24)	0.99
Vitamin Use Prior to Pregnancy	17 (38.6)	69 (27.4)	1.67 (0.86, 3.25)	0.13	22 (33.9)	64 (27.7)	1.34 (0.74, 2.41)	0.34
Any Smoking in Pregnancy	9 (20.5)	21 (8.2)	2.88 (1.22, 6.79)	0.02	14 (21.5)	16 (6.8)	3.76 (1.72, 8.19)	<0.01
Active Smoking in Early Pregnancy	9 (20.5)	21 (8.2)	2.88 (1.22, 6.79)	0.02	14 (21.5)	16 (6.8)	3.76 (1.72, 8.19)	<0.01
Active Smoking in Mid/Late Pregnancy	6 (13.6)	10 (3.9)	3.88 (1.34, 11.30)	0.01	7 (10.8)	9 (3.8)	3.03 (1.08, 8.48)	0.04
Passive Smoke Exposure in Pregnancy	27 (61.4)	163 (63.7)	0.91 (0.47, 1.75)	0.77	45 (69.2)	145 (61.7)	1.40 (0.78, 2.52)	0.27
Passive Smoke Exposure at Home in Pregnancy	18 (40.9)	81 (31.6)	1.50 (0.78, 2.88)	0.23	27 (41.5)	72 (30.6)	1.61 (0.91, 2.83)	0.10
Passive Smoke Exposure Elsewhere in Pregnancy	23 (52.3)	137 (53.5)	0.95 (0.50, 1.81)	0.88	40 (61.5)	120 (51.1)	1.53 (0.88, 2.69)	0.14
Any Antibiotic Use in Pregnancy	23 (52.3)	80 (31.5)	2.38 (1.25, 4.55)	<0.01	29 (44.6)	74 (31.8)	1.73 (0.99, 3.04)	0.06
Antibiotic Use in Early Pregnancy	10 (22.7)	33 (12.9)	1.99 (0.90, 4.40)	0.09	11 (16.9)	32 (13.6)	1.29 (0.61, 2.73)	0.50
Antibiotic Use in Mid/Late Pregnancy	18 (40.9)	57 (22.4)	2.39 (1.23, 4.67)	0.01	22 (33.9)	53 (22.8)	1.74 (0.96, 3.16)	0.07
Any Acetaminophen Use in Pregnancy	35 (79.6)	171 (67.1)	1.91 (0.88, 4.16)	0.10	50 (76.9)	156 (66.7)	1.67 (0.88, 3.15)	0.12
Acetaminophen Use in Early Pregnancy	15 (34.1)	99 (38.8)	0.82 (0.42, 1.60)	0.55	27 (41.5)	87 (37.2)	1.20 (0.69, 2.10)	0.52
Acetaminophen Use in Mid/Late Pregnancy	31 (70.5)	146 (57.0)	1.80 (0.90, 3.59)	0.10	43 (66.2)	134 (57.0)	1.47 (0.83, 2.62)	0.19
Any Ibuprofen Use in Pregnancy	10 (22.7)	33 (12.9)	1.99 (0.90, 4.40)	0.09	14 (21.5)	29 (12.3)	1.95 (0.96, 3.96)	0.06
Ibuprofen Use in Early Pregnancy	7 (15.9)	15 (5.9)	3.03 (1.16, 7.92)	0.02	8 (12.3)	14 (6.0)	2.21 (0.88, 5.51)	0.09
Ibuprofen Use in Mid/Late Pregnancy	4 (9.1)	22 (8.6)	1.06 (0.35, 3.25)	0.91	7 (10.8)	19 (8.1)	1.37 (0.55, 3.42)	0.50
Any Infections in Pregnancy	42 (95.5)	229 (89.8)	2.38 (0.55, 10.42)	0.25	61 (93.9)	210 (89.7)	1.74 (0.58, 5.22)	0.32
Infections in Early Pregnancy	31 (70.5)	158 (62.0)	1.46 (0.73, 2.93)	0.28	45 (69.2)	144 (61.5)	1.41 (0.78, 2.53)	0.26
Infections in Mid/Late Pregnancy	38 (86.4)	198 (77.7)	1.82 (0.73, 4.53)	0.20	52 (80.0)	184 (78.6)	1.09 (0.55, 2.15)	0.81

CHARACTERISTICS AND UNIVARIATE ANALYSIS FOR ASTHMA AND WHEEZING IN CHILDREN FROM THE PEPS COHORT (N=300)

	Asthma by Year Three				**Wheezing in Year Three**			
	Yes N (%)	**No N (%)**	**OR (95% CI)**	**P-Value**	**Yes N (%)**	**No N (%)**	**OR (95% CI)**	**P-Value**
Any Respiratory Infections in Pregnancy	40 (90.9)	219 (85.9)	1.64 (0.56, 4.87)	0.37	57 (87.7)	202 (86.3)	1.13 (0.49, 2.59)	0.78
Respiratory Infections in Early Pregnancy	30 (68.2)	139 (54.5)	1.79 (0.91, 3.53)	0.09	42 (64.6)	127 (54.3)	1.54 (0.87, 2.72)	0.14
Respiratory Infections in Mid/Late Pregnancy	32 (72.7)	186 (72.9)	0.99 (0.48, 2.03)	0.98	44 (67.7)	174 (74.4)	0.72 (0.40, 1.31)	0.29
Any Fevers in Pregnancy	21 (47.7)	128 (50.4)	0.90 (0.47, 1.71)	0.74	30 (46.2)	119 (51.1)	0.82 (0.47, 1.43)	0.48
Fevers in Early Pregnancy	13 (29.6)	81 (31.8)	0.90 (0.45, 1.81)	0.77	20 (30.8)	74 (31.6)	0.96 (0.53, 1.74)	0.90
Fevers in Mid/Late Pregnancy	15 (34.1)	83 (32.6)	1.07 (0.55, 2.11)	0.84	22 (33.9)	76 (32.5)	1.06 (0.59, 1.90)	0.84
Family History of Asthma	39 (88.6)	146 (57.0)	5.88 (2.24, 15.40)	<0.01	47 (72.3)	138 (58.7)	1.84 (1.01, 3.35)	0.05
Father has Asthma	8 (19.0)	45 (17.9)	1.08 (0.47, 2.49)	0.85	11 (17.2)	42 (18.3)	0.93 (0.45, 1.93)	0.84
Mother has Asthma	26 (59.1)	73 (28.5)	3.62 (1.87, 7.00)	<0.01	31 (47.7)	68 (28.9)	2.24 (1.28, 3.93)	<0.01
Mother has Asthma, Hay fever, or Eczema	36 (81.8)	160 (62.5)	2.70 (1.21, 6.05)	0.02	52 (80.0)	144 (61.3)	2.53 (1.30, 4.90)	<0.01
Child Characteristics								
First Born Child	17 (38.6)	91 (35.6)	1.14 (0.59, 2.21)	0.69	24 (36.9)	84 (35.7)	1.05 (0.60, 1.86)	0.86
Male Gender	23 (52.3)	133 (52.0)	1.01 (0.53, 1.92)	0.97	32 (49.2)	124 (52.8)	0.87 (0.50, 1.50)	0.61
Low Birth Weight <2500g	6 (13.6)	20 (7.8)	1.86 (0.70, 4.94)	0.21	10 (15.4)	16 (6.8)	2.49 (1.07, 5.79)	0.03
Breast Fed	38 (86.4)	228 (89.1)	0.78 (0.30, 2.00)	0.60	51 (78.5)	215 (91.5)	0.34 (0.16, 0.72)	<0.01
Breast Fed for at least 4 Weeks	28 (63.6)	179 (69.9)	0.75 (0.39, 1.47)	0.41	37 (56.9)	170 (72.3)	0.51 (0.29, 0.89)	0.02
Exclusively Breast Fed for 4 Weeks	7 (15.9)	39 (15.2)	1.05 (0.44, 2.53)	0.91	8 (12.3)	38 (16.2)	0.73 (0.32, 1.65)	0.45
First Year of Life Exposures								
Exposed to Smoke in Home	12 (27.3)	41 (16.0)	1.97 (0.94, 4.13)	0.07	19 (29.2)	34 (14.5)	2.44 (1.28, 4.66)	<0.01
Exposed to Smoke Outside Home	12 (27.3)	41 (16.0)	1.97 (0.94, 4.13)	0.07	18 (27.7)	35 (14.9)	2.19 (1.14, 4.20)	0.02
Exposed to Passive Smoke	18 (40.9)	70 (27.3)	1.84 (0.95, 3.56)	0.07	29 (44.6)	59 (25.1)	2.40 (1.36, 4.25)	<0.01
Vitamin Use	13 (29.6)	51 (19.9)	1.69 (0.82, 3.45)	0.15	17 (26.2)	47 (20.0)	1.42 (0.75, 2.68)	0.29
Antibiotic Use	30 (68.2)	133 (52.0)	1.98 (1.00, 3.91)	0.05	40 (61.5)	123 (52.3)	1.46 (0.83, 2.55)	0.19
Number of Antibiotics—Median (Q1, Q3)	2 (0, 3)	1 (0, 2)	1.51 (1.23, 1.84)	<0.01	1 (0, 3)	1 (0, 2)	1.28 (1.07, 1.53)	<0.01
Number of Antibiotics—Range	0 – 7	0 – 7			0 – 7	0 – 7		
3+ Courses of Antibiotics	15 (34.1)	25 (9.8)	4.78 (2.26, 10.09)	<0.01	19 (29.2)	21 (8.9)	4.21 (2.10, 8.46)	<0.01
Any Acetaminophen Use	38 (88.4)	247 (96.9)	0.25 (0.08, 0.79)	0.02	59 (92.2)	226 (96.6)	0.42 (0.13, 1.32)	0.14
Acetaminophen Use during Months 0–6	36 (81.8)	219 (85.6)	0.50 (0.19, 1.34)	0.17	14 (21.5)	43 (18.3)	1.23 (0.62, 2.41)	0.56
Acetaminophen Use during Months 7–12	37 (84.1)	242 (94.5)	0.76 (0.33, 1.76)	0.52	55 (84.6)	200 (85.1)	0.96 (0.45, 2.07)	0.92
Acetaminophen Use Early, Mid, and Late	5 (11.4)	52 (20.3)	0.31 (0.12, 0.81)	0.02	58 (89.2)	221 (94.0)	0.53 (0.20, 1.36)	0.19

CHARACTERISTICS AND UNIVARIATE ANALYSIS FOR ASTHMA AND WHEEZING IN CHILDREN FROM THE PEPS COHORT (N=300)

	Asthma by Year Three				**Wheezing in Year Three**			
	Yes N (%)	**No N (%)**	**OR (95% CI)**	**P-Value**	**Yes N (%)**	**No N (%)**	**OR (95% CI)**	**P-Value**
Any Ibuprofen Use	19 (43.2)	90 (35.2)	1.40 (0.73, 2.68)	0.31	27 (41.5)	82 (34.9)	1.33 (0.76, 2.33)	0.33
Any Infections	42 (95.5)	245 (95.7)	0.94 (0.20, 4.41)	0.94	63 (96.9)	224 (95.3)	1.55 (0.33, 7.15)	0.58
Ear Infections	20 (45.5)	92 (35.9)	1.49 (0.78, 2.83)	0.23	28 (43.1)	84 (35.7)	1.36 (0.78, 2.38)	0.28
Number of Ear Infections—Median (Q1, Q3)	0 (0, 1)	0 (0, 1)	1.20 (0.92, 1.56)	0.17	0 (0, 1)	0 (0, 1)	1.03 (0.80, 1.34)	0.80
Number of Ear Infections—Range	0 – 5	0 – 7			0 – 3	0 – 7		
Respiratory Infections	42 (95.5)	236 (92.2)	1.78 (0.40, 7.90)	0.45	63 (96.9)	215 (91.5)	2.93 (0.67, 12.9)	0.16
Any Fevers	15 (34.1)	71 (27.7)	1.35 (0.68, 2.66)	0.39	23 (35.4)	63 (26.8)	1.50 (0.83, 2.68)	0.18

D. **Aim 1 Methodology: To Investigate the Effect of Antibiotic Use in the First Year of Life on the Subsequent Development of Asthma by Year Three and Wheezing in Year Three**

Since children took anywhere from zero to seven courses of antibiotics within their first year of life, antibiotic use was analyzed as continuous. Reason for antibiotic use was separated into respiratory infections versus non-respiratory infections. If both types of infections occurred, the reason was considered respiratory. Otitis without respiratory symptoms was included as non-respiratory. The number of antibiotic courses for each indication was calculated and utilized for these separate analyses. A total of 300 mother-child pairs had antibiotic information and were followed through the child's third year of life and included in this study.

Analyses:

1. Maternal history, child characteristics, and first year of life exposures were compared across the primary outcomes of asthma and wheezing.
    a. Frequency counts and percentages were calculated for categorical variables, and means with standard deviations (SDs) were calculated for continuous variables.
    b. Univariate logistic regression models were utilized to estimate the crude effects of confounders on the child's development of asthma or wheezing. Odds ratios and 95% CIs were calculated for each potential confounding factor and for antibiotic exposure within the first year.
    c. Multivariable logistic regression models for asthma and wheezing were constructed. Antibiotic use, and variables predictive at the univariate level ($p<.10$), as well as study intervention, were included in the multivariable logistic regression models.
2. The possibility of reverse causation between the endpoints and antibiotic use was estimated by separating out indication for antibiotic use.
    a. The number of courses of antibiotics was calculated over the first year of life separately for respiratory infections and for non-respiratory infections.
    b. Multivariable logistic regression models were used to estimate the effects of indications for antibiotic use on outcomes by the third year of life. Adjusted analyses included all variables predictive at the univariate level ($p<.10$), as well as study intervention.

Power calculations were conducted using PASS 12 (Hintze 2013). Given our sample size of 300, the power calculation was based on a preliminary analysis of our data. Testing for differences in two proportions, given β=.20 and α=.05, 162 children took antibiotics in their first year of life and 138 did not. In our cohort, 10% of children who did not take antibiotics had asthma by year three, yielding 80% power to detect a difference of 8% in the rate of asthma by age three between antibiotic use in the first year and no antibiotic use. The power calculation was based on the primary hypothesis: antibiotic use in year one is associated with increased asthma by year three.

E. **Aim 2 Methodology: To Investigate the Effect of Prenatal Antibiotic Use on the Subsequent Development of Asthma by Year Three and Wheezing in Year Three**

Antibiotic use was collected during each trimester of pregnancy. An overall variable was calculated as any antibiotic used during pregnancy. To investigate antibiotic use during trimester of pregnancy, early use was defined as antibiotics used in the first trimester and mid-to-late use was defined from 4 to 8 months gestation, since antibiotic use in the second and third trimesters of pregnancy was uncommon. A total of 298 mother-child pairs had prenatal antibiotic information and were followed through the child's third year of life and were included in this study.

Analyses:

1. Maternal characteristics during pregnancy, child characteristics through the first year, and third-year outcomes were compared by prenatal antibiotic use.
    a. Frequency counts and percentages were shown for categorical variables and compared using chi-square or Fisher's exact test.
    b. Continuous variables were presented as means with SDs and were compared using t-test.
2. Multivariable logistic regression models for asthma by year three and wheezing in year three were constructed by including prenatal antibiotic use, as well as variables predictive at the univariate level ($p<.10$), and study intervention.
3. Effect modification was evaluated using two methods.

a. Interaction terms between potential confounders and prenatal antibiotic use on asthma and wheezing were examined with $p<.20$ indicating possible effect modification.

b. Subset analyses of mothers with no history of asthma and of children who did not use antibiotics in the first year were conducted.

4. Adjusted regression models investigated the effect of antibiotics utilized during different trimesters of pregnancy on asthma and wheezing.

Given our sample size of 298, the power calculation was based on a preliminary analysis of our data. Testing for differences in two proportions, given $\beta=.20$ and $\alpha=.05$, 103 mothers took antibiotics during their pregnancy and 195 did not. In our cohort, 11% of mothers who did not take antibiotics had children who developed asthma by year three. We had 80% power to detect a difference of 9% in the rate of asthma by age three between prenatal antibiotic use and no antibiotic use with $\alpha=.05$. The power calculation was based on the primary hypothesis: prenatal antibiotic use is associated with an increase in offspring's asthma by year three. We were not powered for subset analyses.

F. **Aim 3a Methodology: To Investigate the Effect of Prenatal C–Reactive Protein Levels on the Subsequent Development of Asthma by Year Three and Wheezing in Year Three**

This aim involved the unique contribution of maternal serum samples, analyzed for CRP levels. No CRP values were under the detection limit (<.5 mg/dL). Correlation between assay measurements of CRP was high ($r=.96$), with an intra-class correlation coefficient of 0.95, and an inter-assay coefficient of variation of 11%.

Since maternal CRP levels were not normally distributed, a logarithmic-transformed CRP value was calculated. In addition to analyzing CRP as a continuous variable, two other variables were constructed. To assess a dose-response relationship, CRP values were categorized into four groups using 25th, 50th, and 75th percentile cut points. A dichotomous variable of elevated CRP was also calculated as ≥10 mg/L as CRP levels above 10 mg/L generally indicate acute infection or inflammatory processes (Ridker and Cook 2004).

A total of 244 mother-child pairs had prenatal CRP levels and were followed through the child's third year of life and were included in this study.

Analyses:

1. Geometric means of CRP levels with 95% CIs were calculated for demographics, maternal characteristics during pregnancy, child characteristics through the first year, and third-year outcomes. The P-values were derived from Mann-Whitney *U* test.
2. Maternal characteristics during pregnancy, including antibiotic use, child characteristics, and third-year outcomes were compared across the quartiles of maternal CRP levels, as well as across the dichotomous CRP variable.
    a. Frequency counts and percentages were shown for categorical variables and compared using Cochran-Armitage test for trend and chi-square or Fisher's exact test.
    b. Continuous variables were presented as means with SDs and were compared using one-way analysis of variance and Tukeys test for post-hoc comparisons, and t-tests.
3. Multivariable logistic regression models were constructed to model the adjusted association between CRP and the outcomes.
    a. All maternal characteristics and child characteristics that were significantly related to the outcomes at the univariate level ($p<.05$) or that altered the coefficient of CRP estimate by more than 10% were included in the multivariable model. The CRP was forced into the model, and all interactions were evaluated. Significant interactions at $p<.10$ were included in the final multivariable models.
    b. The same process of multivariable model building was implemented for logarithmic-transformed CRP (grand-mean centered), CRP quartiles, and for the high-risk CRP variable.

Given our sample size of 244, the power calculation was based on a preliminary analysis of our data. Testing for differences in two proportions, given $\beta=.20$ and $\alpha=.05$, 36 mothers had elevated CRP levels ($\geq$10 mg/L) and 208 did not. In our cohort, 14% of mothers who did not have elevated CRP levels had children who developed asthma by year three. We had 80% power to detect a difference of 7% in the rate of asthma by age three between mothers with high CRP versus lower CRP. The power calculation was based on the primary hypothesis: mothers with higher levels of CRP will have offspring with more asthma by year three as compared to mothers with lower CRP. We were not powered for subset analyses.

G. Aim 3b Methodology: To Assess the Relationships between Psychosocial Factors, Maternal CRP Levels, and the Subsequent Development of Asthma and Wheezing

In this exploratory aim, we investigated if increased maternal inflammation was associated with low psychosocial factors and whether this association moderated or mediated the relationship between CRP and subsequent asthma and wheezing.

Three psychosocial surveys measuring family relationships and family coping styles were evaluated: FFFS, FHI, and F-COPES.

Analyses:

1. Psychosocial measures were scored, summarized, and compared.
    a. FFFS, overall FHI and subscales, and overall F-COPES and subscales were scored then summarized using means, SDs, and ranges.
    b. Internal reliability of all surveys was measured using Cronbach's alpha.
    c. Correlations between psychosocial measures were calculated using Pearson correlation coefficients.
2. Correlations between psychosocial measures and CRP levels were calculated using Pearson and Spearman correlation coefficients.
3. FFFS, FHI, and F-COPES were analyzed with respect to year three outcomes. Differences in survey measures were compared across asthma and wheezing using t-tests.
4. Psychosocial measures were added separately to the multivariable models for asthma and wheezing from Aim 3a as covariates and as interaction terms to determine if they were confounding or modifying the relationship between systemic maternal inflammation and the outcomes.
    a. Confounding was considered present if the addition of the survey measure to the model altered the coefficient of CRP estimate by more than 10%.
    b. Effect modification was considered a possibility at $p<.10$.
5. To assess whether systemic maternal inflammation mediated the relationship between the psychosocial factors and the primary outcomes, the first steps of the approach recommended by Baron and Kenny was

implemented (Baron and Kenny 1986). Alternatively, the relationship between CRP and outcomes may be mediated by psychosocial factors.

Given our limited sample size with the psychosocial survey data, this aim was exploratory. Assuming a sample size of 200, we achieve 80% power to detect a difference of 2 points for the overall FHI score and 4 points for the overall F-COPES scores with a significance level of 0.05 using a two-sided t-test. This power calculation was based on the primary hypothesis: children with asthma reside in families with lower family coping styles as compared to children without asthma.

IV. RESULTS AND DISCUSSION

A. Aim 1 Manuscript 1. The Relationship of Early-Life Antibiotic Use with Asthma in At-Risk Children

This manuscript was published as a letter to the Editor in the Journal of Allergy and Clinical Immunology in 2014 (Lapin, Piorkowski, Ownby, Wagner-Cassanova, et al. 2014).

As the prevalence of asthma has doubled in developed countries over the last 30 years, the concurrent increase in children's antibiotic use has led to speculation of a possible causal relationship (Eder, Ege, and von Mutius, 2006). Retrospective studies focused on the hygiene hypothesis have shown correlations with early antibiotic use (Heintze and Petersen, 2013). These findings, however, may be due to reverse causation, or confounding by indication. Existing evidence is inconsistent, and there have been few prospective studies. Controlling for temporal confounders within a prospective study, we investigated the effects of antibiotic use in the first year of life with subsequent asthma diagnosis and wheezing in the third year of life within a high-risk urban cohort.

Three hundred mother-child pairs were included from the PEPs. This randomized education intervention examined the effect of educators working with pregnant women at risk for having children with asthma on the modification of factors in the home known to exacerbate the disease. From 1998 to 2004, pregnant women in urban Chicago were identified to participate in the study if the unborn child had a first-degree relative with asthma, hay fever, or eczema. Mothers were followed and surveyed in each trimester of pregnancy and children were followed from 4 weeks of age through 2009. The primary endpoints of the study are asthma diagnosis and wheezing in the third year of life. Secondary endpoints include other respiratory symptoms in the third year of life. Asthma was defined as ever having an asthma diagnosis by 3 years of age. Wheezing and other respiratory symptoms were determined by any positive response within the year prior to their third-year visit. Early antibiotic use, excluding topical antibiotics or antifungal agents, was determined at five time points during the first year of life. Reason for antibiotic use was separated into respiratory infections versus non-respiratory infections. If both types of infections occurred, the reason was considered respiratory. Otitis media without respiratory symptoms was considered non-respiratory. Unadjusted and adjusted logistic regression models were utilized and all statistical analyses were

performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina). The study was approved by the University of Illinois at Chicago Human Subjects Institutional Review Board.

Forty-four (15%) children had asthma and 65 (22%) had wheezing in the third year of life. Any smoking in pregnancy, antibiotic and ibuprofen use during pregnancy, and maternal asthma were significantly associated with an asthma diagnosis and reported wheezing. The majority of mothers in the study were of Mexican ethnicity (67%); this was protective against the development of asthma and wheezing. Having low birth weight was predictive and being breast fed was protective against wheezing, but neither was associated with the development of asthma. The prevalence of infections was high within the first year of life with 96% having infections. The majority of children (n=162, 54%) had taken antibiotics within their first year of life. After adjustment for significant predictors of asthma and wheezing, the number of courses of antibiotics remained significant for both asthma (aOR: 1.58; 95% CI: 1.27, 1.96) and wheezing (aOR: 1.29; 95% CI: 1.07, 1.55) (data not shown).

We examined the possibility of reverse causation between the endpoints and antibiotic use by separating out indication for use (Table III). Sixty-nine (23%) children took antibiotics for respiratory reasons and 124 (41%) took antibiotics for non-respiratory reasons. After adjustment for significant maternal and child characteristics, the significant relationships between asthma and other respiratory symptoms held only for antibiotics used for respiratory reasons. There was an association between antibiotics used for respiratory reasons and wheezing but it did not reach significance (aOR: 1.33, p=.08). None of the outcomes were associated with antibiotics used for non-respiratory reasons.

TABLE III

ADJUSTED[a] REGRESSION MODELS TO PREDICT THREE-YEAR OUTCOMES BY INDICATION FOR ANTIBIOTIC USE IN THE FIRST YEAR OF LIFE

	Antibiotics for Respiratory Infections in the First Year of Life		**Antibiotics for Non-Respiratory Infections in the First Year of Life**	
	OR (95% CI)	**P-Value**	**OR (95% CI)**	**P-Value**
Outcomes				
Asthma by Year 3	2.53 (1.67, 3.82)	<0.01	1.11 (0.83, 1.46)	0.49
Wheezing in Year 3	1.33 (0.97, 1.84)	0.08	1.10 (0.86, 1.41)	0.47
Other Symptoms in Year 3				
Exercise-Induced Wheezing	1.74 (1.20, 2.51)	<0.01	1.12 (0.83, 1.50)	0.46
Sleep-Disturbed Wheezing	1.74 (1.16, 2.62)	<0.01	1.06 (0.74, 1.50)	0.77
Wheezing without a Cold	1.84 (1.24, 2.73)	<0.01	1.03 (0.73, 1.44)	0.88
ER Visit for Breathing Problems	1.43 (1.02, 2.00)	0.04	0.91 (0.67, 1.24)	0.56

[a]Adjusted analyses include study intervention and significant variables (p<.10) from univariate analysis. Asthma is adjusted for Mexican ethnicity, maternal smoking, antibiotics and ibuprofen during pregnancy, maternal asthma, and exposure to smoke in the home. Wheezing is adjusted for Mexican ethnicity, maternal smoking, antibiotics and ibuprofen during pregnancy, maternal asthma, low birth weight, breast feeding, and exposure to smoke in the home.

In our prospective study we found a significant relationship between antibiotic use within the first year of life and incident asthma and wheezing in year three, which has been identified consistently in retrospective studies (Marra et al., 2006; Marra et al., 2009). The hygiene hypothesis theorizes that antibiotic-related suppression of inflammatory responses may lead to a greater impairment of immune responses in early childhood (Jedrychowski et al., 2011). However, a recent meta-analysis found that early antibiotic use and asthma in children was unproven and may be the result of confounding (Heintze and Petersen, 2013). Research into this association has resulted in mixed conclusions with one study finding that antibiotic use in early life was not associated with asthma, but rather that frequent use is more common among asthmatic children (Celedon et al., 2004). A birth cohort found the effect of antibiotic exposure on the development of asthma between birth and 4 years may be due to confounding by chest infections, when asthma may be difficult to distinguish from infection (Wickens et al., 2008). Additionally, respiratory infections in early childhood could contribute to the development of asthma by damaging the airways or, alternatively, severe respiratory infections could be an indicator of genetic predisposition for asthma (Rosenthal et al., 2010).

Our results indicate the association between antibiotic use and subsequent asthma holds only in the case of respiratory infections, adding to the previous research suggesting the relationship may be explained by reverse causation or confounding by indication. There are some limitations with our study, however. Asthma is a complex clinical disease that is difficult to assess before age 6 (Eder, Ege, and von Mutius, 2006). Given that our study included diagnoses prior to age 3, the nature of the disease progression may still be unpredictable. Since there was a high rate of infections in this cohort, there may be a covariation effect between infections and antibiotic use that we were unable to separate. Also, the use of antibiotics and type of infections noted are based on parental recall so are prone to recall bias. Another limitation is that we do not have access to the types of antibiotics used. Broad-spectrum antibiotics became more prevalent in the 1980s and may alter the microbiome more than the narrow-spectrum antibiotics used in the past (Kozyrskyj, Ernst, and Becker, 2007).

Despite the limitations inherent in this research, we conclude the associations found between antibiotic use in young children and subsequent development of asthma and wheezing may be due to confounding by respiratory infections.

B. **Aim 2 Manuscript 2. The Relationship between Prenatal Antibiotic Use and Asthma in At-Risk Children**

This manuscript was published in the Annals of Allergy, Asthma and Immunology in 2014 (Lapin, Piorkowski, Ownby, Freels, et al. 2014).

Prevalence of asthma has doubled in developed countries over the last 30 years (Eder, Ege, and von Mutius, 2006). The factors causally driving these temporal increases remain essentially unknown, with poor and minority children in the United States suffering a disproportionately higher burden of asthma morbidity (Lara et al., 2006). Concurrent increases in antibiotic use to treat infections in children have led to speculation of a causal relationship. Retrospective studies have shown strong correlations between early antibiotic use and asthma, but the findings suggest the possibility of reverse causation or confounding by indication (Heintze and Petersen, 2013; Rosenthal et al., 2010). Conversely, prenatal antibiotics have been found to be associated with the development of asthma and wheezing in early life (Benn et al., 2002; Rusconi et al., 2007; McKeever et al., 2002; Stensballe et al.,

2013; Jedrychowski et al., 2006; Martel et al., 2009; Murk, Risnes, and Bracken, 2011). The hygiene hypothesis suggests that birth into an environment with fewer microbial exposures may alter development of the immune system leading to a greater risk of atopy (Renz et al., 2006). Data have suggested that antibiotics in utero may change the maternal or placental microbiome and increase the child's risk of developing allergic disease (Lange et al., 2012; Aagaard et al., 2014). Factors that modify microbial exposure prenatally and perinatally may have a long-term impact on the risk of developing subsequent atopic disease (McKeever et al., 2002; West, Jenmalm, and Prescott, 2014; Hafkamp-de Groen et al., 2013). Research utilizing prospective birth cohorts has been limited, especially among impoverished urban residents.

Controlling for maternal and child confounders within a prospective study, we investigated the effects of prenatal antibiotic use with the subsequent development of asthma by year three and wheezing in year three within a high-risk urban cohort. Since this relationship may be confounded by maternal asthma or by antibiotic use in the child, we investigated the associations within subsets of mothers without asthma and within children who did not use antibiotics. We also investigated the impact of antibiotics during different trimesters of pregnancy.

1. **Methods**

The PEPS is a randomized education intervention examining the effect of community educators working with pregnant women at risk for having children with asthma on modification of factors in the home known to exacerbate the disease. From 1998 to 2004, at-risk families living in disadvantaged areas of urban Chicago were identified to participate in the study if the unborn child had a first-degree relative with asthma, hay fever, or eczema. Mothers were followed and surveyed in each trimester of pregnancy and soon after delivery, and 301 children were followed from 4 weeks of age through age three years. The intervention did not address antibiotic utilization. All women in the study received general health education. Half of the women also received a series of home visits from a community health educator to identify and decrease in home asthma triggers. The complete outline of participant flow through the study has been published elsewhere (Persky et al., 2009; Persky et al., 2008).

Systemic antibiotic use was collected on a total of 298 mother-child pairs from the PEPS study who were followed through the child's third year of life. The primary endpoints of the study are asthma diagnosis by year three and reported wheezing in the third year of life. Asthma was defined as ever having an asthma diagnosis by a physician by 3 years of age based on the self-reported answer to the question "Has a doctor ever told you that your

child has asthma?" Secondary endpoints include eczema, as well as other respiratory symptoms in the third year: exercise-induced wheezing, sleep disturbed by wheezing, wheezing without a cold, and ER visits for breathing problems. Development of the primary endpoint of wheezing and the secondary endpoints of eczema and other respiratory symptoms were determined by a positive response within the year prior to their third-year visit based on the following questions: "Has your child's chest sounded wheezy or whistling?"; "Has a doctor ever told you that your child has eczema?"; "Has your child's chest sounded wheezy or whistling during or shortly after vigorous exercise?"; "Has your child been awakened at night by wheeze or by shortness of breath?"; "Has your child had episodes of wheezing or whistling without a cold?"; "Was your child treated in the emergency room for breathing problems (coughing, congestion, runny nose, wheezing)?". Prenatal risk factors during pregnancy such as antibiotic use, infections, and smoking status were evaluated by questionnaire at enrollment in the first trimester, at 4–5 months of gestation, and at 7–8 months of gestation. Other potential confounders including history of asthma, maternal age, maternal ethnicity, and acetaminophen and ibuprofen use were evaluated by questionnaire during pregnancy and five times throughout the child's first year of life. Information on reason for antibiotic use in the child was separated into respiratory infections versus non-respiratory infections. If both infections occurred, the reason was considered respiratory. Reason for prenatal antibiotic use was not captured, but only systemic antibiotics were noted. Antibiotic use during each trimester of pregnancy was investigated as early use in the first trimester or mid-to-late use in the second to third trimester. Information on type of delivery and antibiotic use during delivery was not available in this cohort.

The study was approved by the University of Illinois at Chicago's Institutional Review Board. All enrolled participants provided written informed consent. Maternal characteristics during pregnancy, child characteristics through the first year, and third-year outcomes were compared across prenatal antibiotic use. Frequency counts and percentages were shown for categorical variables and compared using chi-square or Fisher's exact test. Continuous variables were presented as means with SDs and were compared using t-test. Multivariable logistic regression models for asthma and wheezing were constructed by including all variables predictive at the univariate level ($p<.10$), as well as study intervention. Effect modification was evaluated using interaction terms (with $p<.20$ considered potential interaction) between probable confounders and prenatal antibiotic use, as well as with subset analyses within mothers with no history of asthma and within children who did not use antibiotics in the first year. Prenatal antibiotics use was split into any use throughout the pregnancy, first-trimester use, or second-to-third

trimester use and three separate adjusted regression models investigated the effect of antibiotics utilized during these different trimesters of pregnancy on asthma and wheezing. Table footnotes list variables included in adjustment. Statistical analyses were performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina).

2. **Results**

Maternal characteristics, child characteristics, and first year of life exposures by prenatal antibiotic use are presented in Table IV. One hundred and three (35%) mothers used antibiotics during their pregnancy. The majority of mothers in the study were of Mexican ancestry (68%) and the average age was 26. There were no differences between antibiotic use by maternal age, Mexican ancestry, smoking status, acetaminophen or ibuprofen use, or infections or fevers. Mothers who took antibiotics during pregnancy were more likely to also take vitamins (37% versus 24%, p=.02). Prenatal antibiotic use was also significantly higher within mothers with asthma (44% versus 27%, p<.01). Child characteristics, including low birth weight and prematurity, did not differ by prenatal antibiotic use. There were also no differences in first-year exposures of antibiotic or acetaminophen use within children born to mothers who took antibiotics. First-year ibuprofen use as well as respiratory infections were higher in children whose mothers took antibiotics (44% versus 32%, p=.052 and 96% versus 91%, p=.09, respectively).

TABLE IV

MATERNAL AND CHILD CHARACTERISTICS BY PRENATAL ANTIBIOTIC USE

	Total N (%)	**Prenatal Antibiotic Use N (%)**	**No Antibiotic Use N (%)**	**P-Value**
Total Number	298	103 (34.6)	195 (65.4)	
Study Intervention	148 (49.7)	51 (49.5)	97 (49.7)	0.97
Maternal Characteristics during Pregnancy				
Maternal Age (Years)—Mean (SD)	25.9 (5.9)	26.0 (6.3)	25.8 (5.7)	0.78
Mexican Ancestry	201 (67.5)	66 (64.1)	135 (69.2)	0.37
Any Smoking	30 (10.1)	13 (12.6)	17 (8.7)	0.29
Any Acetaminophen	204 (68.7)	74 (71.8)	130 (67.0)	0.39
Any Ibuprofen	42 (14.1)	12 (11.7)	30 (15.4)	0.38
Any Infections	270 (90.6)	94 (91.3)	176 (90.3)	0.78
Any Respiratory Infections	258 (86.6)	87 (84.5)	171 (87.7)	0.44
Any Fevers	148 (49.8)	58 (56.3)	90 (46.4)	0.10
Vitamin Use	84 (28.6)	38 (36.9)	46 (24.1)	0.020
Mother has Asthma	98 (32.9)	45 (43.7)	53 (27.2)	0.004
Child Characteristics				
First Born Child	108 (36.2)	39 (37.9)	69 (35.4)	0.67
Male Gender	155 (52.0)	58 (56.3)	97 (49.7)	0.28
Low Birth Weight <2500g	25 (8.4)	10 (9.7)	15 (7.7)	0.55
Premature Birth <36 weeks	14 (4.7)	4 (3.9)	10 (5.1)	0.63
Breast Fed	264 (88.6)	92 (89.3)	172 (88.2)	0.77
First Year of Life Exposures				
Exposed to Cigarette Smoke in the Home	53 (17.8)	22 (21.4)	31 (15.9)	0.24
Any Antibiotics	161 (54.0)	61 (59.2)	100 (51.3)	0.19
Any Acetaminophen	284 (95.6)	96 (94.1)	188 (96.9)	0.25
Any Ibuprofen	108 (36.2)	45 (43.7)	63 (32.3)	0.052
Any Infections	285 (95.6)	101 (98.1)	184 (94.4)	0.14
Ear Infections	111 (37.3)	41 (39.8)	70 (35.9)	0.51
Respiratory Infections	276 (92.6)	99 (96.1)	177 (90.8)	0.09
Any Fevers	86 (28.9)	35 (34.0)	51 (26.2)	0.16

Third year outcomes varied by antibiotic use during pregnancy (Table V). Asthma diagnosed by year three occurred in 23 (22%) children born to mothers who took antibiotics versus 21 (11%) children born to mothers who did not take antibiotics (p<.01). Wheezing occurred in 28% of children whose mothers took antibiotics versus 19% with no antibiotics (p=.054). Eczema in year 3, as well as all respiratory symptoms in year 3, was increased with prenatal antibiotic use but only sleep disturbed by wheezing and wheezing without a cold reached conventional statistical significance.

TABLE V

CHILD OUTCOMES BY PRENATAL ANTIBIOTIC USE[a]

	Total N (%)	**Prenatal Antibiotic Use N (%)**	**No Antibiotic Use N (%)**	**P-Value**
Outcomes				
Asthma by Year 3	44 (14.8)	23 (22.3)	21 (10.8)	0.008
Wheezing in Year 3	65 (21.8)	29 (28.2)	36 (18.5)	0.054
Other Symptoms in Year 3				
Exercise-Induced Wheezing	35 (11.7)	17 (16.5)	18 (9.2)	0.06
Sleep Disturbed by Wheezing	26 (8.7)	14 (13.6)	12 (6.2)	0.03
Wheezing without a Cold	27 (9.1)	14 (13.6)	13 (6.7)	0.048
ER Visit for Breathing Problems	46 (15.4)	21 (20.4)	25 (12.8)	0.09
Eczema	58 (19.5)	23 (22.3)	35 (18.0)	0.36

[a]Chi-square tests compare third year outcomes by prenatal antibiotic use.

In a multivariable regression model, independent predictors of asthma by year three included any antibiotic use during pregnancy (aOR: 3.12; 95% CI: 1.44, 6.77), maternal asthma, and child antibiotic use for respiratory reasons in the first year of life (Table VI). Mexican ancestry was significantly protective against asthma diagnosis.

TABLE VI

MULTIVARIABLE REGRESSION MODEL TO PREDICT ASTHMA[a] BY YEAR THREE

	OR (95% CI)	**P-Value**
Study Intervention	0.96 (0.44, 2.09)	0.92
Maternal Mexican Ancestry	0.37 (0.17, 0.82)	0.014
Any Smoking in Pregnancy	2.34 (0.86, 6.35)	0.10
Any Antibiotics in Pregnancy	3.12 (1.44, 6.77)	0.004
Any Ibuprofen in Pregnancy	1.37 (0.52, 3.58)	0.53
Mother has Asthma	2.66 (1.22, 5.84)	0.015
Child Exposed to Smoke in the Home	0.79 (0.30, 2.07)	0.63
Child took Antibiotics for Respiratory Infections	2.53 (1.67, 3.82)	<0.001

[a]N=295, 42 with asthma. Model adjusted for study intervention as well as significant variables (p<.10) from univariate analysis.

The variables related to wheezing during the third year differed slightly from those related to asthma (Table VII). Low birth weight and smoking during pregnancy were significant independent predictors of wheezing (p=.048 and p=.018, respectively). Antibiotic use during pregnancy was associated with an increased odds (aOR: 1.76; 95% CI: 0.94, 3.28) of wheezing that approached significance, as did lack of breast feeding, and antibiotic use for respiratory reasons in the first year of life.

TABLE VII

MULTIVARIABLE REGRESSION MODEL TO PREDICT WHEEZING[a] IN YEAR THREE

	OR (95% CI)	**P-Value**
Study Intervention	0.75 (0.40, 1.39)	0.36
Maternal Mexican Ancestry	0.61 (0.31, 1.19)	0.14
Any Smoking in Pregnancy	2.83 (1.20, 6.70)	0.018
Any Antibiotics in Pregnancy	1.76 (0.94, 3.28)	0.08
Any Ibuprofen in Pregnancy	1.80 (0.81, 4.03)	0.15
Mother has Asthma	1.43 (0.75, 2.71)	0.28
Child Low Birth Weight <2500g	2.56 (1.01, 6.50)	0.048
Child Breast Fed	0.45 (0.19, 1.09)	0.08
Child Exposed to Smoke in the Home	1.56 (0.73, 3.34)	0.27
Child takes Antibiotics for Respiratory Infections	1.33 (0.97, 1.84)	0.08

[a]N=295, 64 with wheezing. Model adjusted for study intervention as well as significant variables (p<.10) from univariate analysis.

There were no significant interactions with prenatal antibiotic use in either multivariable model. The significant association between prenatal antibiotic use and asthma by year three remained intact within a subset analysis of mothers with no history of asthma (aOR: 5.75; 95% CI: 1.78, 18.60, p<.01) and within a subset of children who did not use antibiotics in the first year (aOR: 3.62; 95% CI: 1.09, 12.05, p=.04) (Table VIII). However, the association with wheezing was eliminated within these subsets.

TABLE VIII

ADJUSTED REGRESSION MODELS[a] FOR SUBSET ANALYSES OF PRENATAL ANTIBIOTIC USE AS A PREDICTOR OF ASTHMA AND WHEEZING

	Asthma by Year 3		**Wheezing in Year 3**	
	OR (95% CI)	**P-Value**	**OR (95% CI)**	**P-Value**
No Maternal Asthma (n=200)	5.75 (1.78, 18.60)	0.004	1.50 (0.65, 3.46)	0.34
No First Year Antibiotics (n=137)	3.62 (1.09, 12.05)	0.036	1.61 (0.60, 4.31)	0.35

[a]Adjusted analysis includes study intervention as well as significant variables (p<.10) from univariate analysis. Asthma is adjusted for study intervention, Mexican ancestry, smoking during pregnancy, ibuprofen during pregnancy, mother having asthma, exposure to smoke in the home in the first year of life, and child antibiotic use for respiratory reasons. Wheezing is adjusted for study intervention, Mexican ancestry, smoking during pregnancy, ibuprofen during pregnancy, mother having asthma, low birth weight, any breast feeding, exposure to smoke in the home in the first year of life, and child antibiotic use for respiratory reasons.

Forty-three mothers took antibiotics during the first trimester, early in pregnancy, whereas 75 mothers took antibiotics in the second to third trimester. Analyses of the effects of the timing of prenatal antibiotic use on asthma and wheezing showed the relationship remained consistent for antibiotic use in the second-to-third trimester of pregnancy (Table IX). These outcomes were not significantly associated with antibiotic use in the first trimester of pregnancy.

TABLE IX

THREE ADJUSTED[a] REGRESSION MODELS FOR ASTHMA AND WHEEZING BY TRIMESTER OF PRENATAL ANTIBIOTIC USE

	Asthma by Year 3		**Wheezing in Year 3**	
	OR (95% CI)	**P-Value**	**OR (95% CI)**	**P-Value**
Model 1: Any Antibiotics in Pregnancy	3.12 (1.44, 6.77)	<0.01	1.76 (0.94, 3.28)	0.08
Model 2: Antibiotics in the First Trimester	2.23 (0.90, 5.49)	0.08	1.32 (0.58, 3.02)	0.50
Model 3: Antibiotics in the Second to Third Trimester	3.33 (1.52, 7.27)	<0.01	1.77 (0.92, 3.39)	0.09

[a]Adjusted analysis includes study intervention as well as significant variables (p<.10) from univariate analysis. Asthma is adjusted for study intervention, Mexican ancestry, smoking during pregnancy, ibuprofen during pregnancy, mother having asthma, exposure to smoke in the home in the first year of life, and child antibiotic use for respiratory reasons. Wheezing is adjusted for study intervention, Mexican ancestry, smoking during pregnancy, ibuprofen during pregnancy, mother having asthma, low birth weight, any breast feeding, exposure to smoke in the home in the first year of life, and child antibiotic use for respiratory reasons.

3. **Discussion**

Our study found that prenatal systemic antibiotic use was a significant predictor of incident asthma, and weakly associated with wheezing, in at-risk children by age three after controlling for confounders. Similarly, McKeever et al. (2002) utilized a birth cohort to show a relationship between prenatal antibiotics exposure and an increase in the child's risk of allergic disease (McKeever et al., 2002). Several other studies have also shown an increased risk of asthma and wheezing with prenatal and perinatal antibiotic use (Benn et al., 2002; Rusconi et al., 2007; Stensballe et al., 2013; Calvani et al., 2004; Martel et al., 2009). To our knowledge, only one

cohort study did not find an association between prenatal antibiotic exposure and wheeze or atopic sensitization, but they did find an association between eczema and prenatal antibiotic use (Dom et al., 2010).

The association between prenatal antibiotic use and subsequent asthma suggests an etiologic relationship since the issue of reverse causality is naturally avoided. However, the relationship could be confounded by maternal asthma, infections, or smoking, premature birth, or antibiotic use in infancy as mothers who use antibiotics may be more apt to ask for antibiotics for their infants (Benn et al., 2002). Our study attempted to identify and minimize the sources of confounding through model adjustment, effect modification, and subgroup analyses. In our cohort we found no correlation between infant antibiotic use and maternal antibiotic use. We showed there was no effect modification by maternal asthma, respiratory infections, or smoking within multivariable models of asthma and wheezing. In our subset analyses within mothers without asthma and within infants who did not take antibiotics, prenatal antibiotics remained a significant predictor of asthma. The association with wheezing, however, was reduced. Due to the large number of maternal and child infections, we could not look at a subset of mothers or children without infections. The Copenhagen Prospective Study on Asthma in Childhood found an association between prenatal antibiotic use and asthma within a cohort of children born to mothers with asthma (Stensballe et al., 2013). These investigators were able to replicate the findings in a subgroup of mothers using antibiotics for non-respiratory infections. They concluded the effect was not confounded by the mother's asthma or infections and could be due to a disturbed bacterial ecology which may trigger the disease process in perinatal life. Data on specific infections for which antibiotics were used in the PEPS were not precise enough to rule out the possibility of confounding by infection.

Antibiotics taken in the second-to-third trimester of pregnancy, but not in the first trimester, were significant predictors of asthma in our study. These results are in agreement with a cohort study that also found that antibiotic use in the second and third trimester only were associated significantly with persistent wheezing in one year olds (Jedrychowski et al., 2006). Additionally, studies have found that the composition of maternal, vaginal, and intestinal bacteria was related to an increased risk of wheeze in infants (Lange et al., 2012; Benn et al., 2002). Contrary to the previously held belief that the womb is sterile, recent studies have shown that maternal microbial transfer starts during pregnancy (West, Jenmalm, and Prescott, 2014). Aagard et al. (2014) recently published a study demonstrating the placental microbiome is affected by maternal antenatal infections (Aagaard et al., 2014). It

is unknown whether the infection itself, an inflammatory response, or antibiotic use for the infection, causes this placental microbiome change. Prenatal antibiotic use could modify the placental, vaginal, or maternal gut microbiome which may increase a child's risk of developing asthma.

Our study design offered many pertinent risk factors for childhood asthma and wheezing, and the prospectively collected measures of maternal and child confounders strengthened the study results. This unique population of disadvantaged urban, mostly Hispanic, mothers was closely followed from the first trimester of pregnancy through the child's third year of life. There are some limitations with this study, however. Given the highly selected population, the generalizability of the study results are limited to disadvantaged children at-risk for allergy and asthma. Asthma is a complex clinical disease that is difficult to critically assess before age 6 (Eder, Ege, and von Mutius, 2006). Our study included diagnoses prior to age 3 when the nature of the disease progression may still be unpredictable. Since there was a high rate of infections in the mothers and children, there may be a covariation effect between infections and antibiotic use that we were unable to separate. Also, the use of antibiotics and type of infections are based on parental recall and are prone to recall bias. Nurses administered the surveys multiple times throughout the pregnancy and five times within the child's first year to ensure recall was limited to the prior few months. Another limitation is that we do not have access to the number of days antibiotics were taken or the reason antibiotics were taken by the mothers. Nor do we know the types of antibiotics used in either the children or the pregnant mothers. Broad-spectrum antibiotics became more prevalent in the 1980s and may alter microbiomes more than the narrower-spectrum antibiotics used in the past (Kozyrskyj, Ernst, and Becker, 2007). The study findings may be limited to certain types of antibiotics, which should be investigated further. Finally, we do not have information on confounders such as chorioamnionitis during pregnancy, or information on childbirth such as C-section, which may be associated with prophylactic use of antibiotics.

Our study suggests an association between prenatal antibiotic use and the development of asthma in at-risk children. Modification of microbial load may be occurring prenatally, affecting the maturation of the infant immune system and increasing a child's risk for developing asthma. While the relationship with prenatal antibiotics does not hold for wheezing in our study, there may be a trend that could be further delineated within a larger cohort study.

C. **Aim 3a Manuscript 3. The Relationship between Prenatal CRP and Subsequent Asthma and Wheezing in At-Risk Offspring**

This manuscript will be submitted for publication. At the time of this thesis, it had not yet been submitted.

Asthma research has focused on postnatal exposures, but there is recent evidence to indicate atopic immune responses may be initiated in utero (Devereux, Barker, and Seaton, 2002). Emerging research suggests the in utero environment plays a much larger role in the risk of childhood asthma than previously believed, with factors such as maternal smoking relating to a deleterious effect on the fetal immune system (Devereux, Barker, and Seaton, 2002). The maternal microbial environment influences, or programs, the immune development of the offspring, with these environmental exposures potentially altering the gene expression to increase disease susceptibility in the offspring (West, Jenmalm, and Prescott, 2014; Jenmalm, 2011; Renz, Brandtzaeg, and Hornef, 2012).

Systemic inflammation during pregnancy may indicate a maternal environment that could increase propensity in the offspring to develop wheezing and asthma in early life. C-reactive protein is an acute phase protein that is commonly used as a biomarker of systemic inflammation (Pepys and Hirschfield, 2003). Higher maternal age, previous delivery, maternal obesity, alcohol consumption, and lack of physical activity during pregnancy have been associated with higher levels of CRP during pregnancy (Morales et al., 2011). Several studies have also shown a correlation between elevated CRP levels in pregnancy and an increased risk of wheezing in offspring (Morales et al., 2011; Sonnenschein-van der Voort et al., 2013). High levels of prenatal CRP do not pass the placenta, so elevated CRP may be a marker for an indirect effect on the developing fetus (Sonnenschein-van der Voort et al., 2013; Jaye and Waites, 1997). Elevated CRP levels during pregnancy could indicate adverse prenatal conditions, influencing the maturation of the fetal respiratory system toward an increased risk of atopy and asthma. The roles of maternal CRP levels in the development of childhood wheezing and asthma remain unclear.

Research utilizing prospective birth cohorts has been limited, particularly among impoverished urban residents. The purpose of our study is to investigate the role of prenatal systemic maternal inflammation, as primarily reflected by CRP levels, and its association with asthma and wheezing in the offspring. Controlling for confounders within a prospective cohort study, we investigated the association between prenatal CRP levels and the

subsequent development of asthma by year three and wheezing in year three within a high-risk, urban, mostly Mexican, cohort.

1. **Methods**

The PEPS is a randomized educational intervention study examining the effect of modification of the home environment on pregnant women at risk for having children with asthma or the subsequent development of allergic disease. From 1998 to 2004, at-risk families living in disadvantaged areas of urban Chicago were recruited if the unborn child had a first-degree relative with asthma, hay fever, or eczema (Persky et al., 1999). Mothers were followed and surveyed in each trimester of pregnancy and 301 children were followed from 4 weeks of age through age three years. The complete outline of participant flow through the study has been published elsewhere (Persky et al., 2009; Persky et al., 2008). Of these 301 mothers, 244 agreed to provide a blood sample during the second-to-third trimester of pregnancy (mean ± SD, 29.5 ± 3.7 (range 15–39 weeks) weeks of gestation). The serum was separated by centrifugation and frozen at -70°C prior to analyses. High-sensitivity CRP levels were determined using a high-sensitivity assay by LabCorp Clinical Trials (Cincinnati, Ohio). Ten samples were reanalyzed in a different assay run on a different day to provide an estimate of inter-assay reliability over time. Correlation between assay measurements of CRP was high (r=.96), with an intra-class correlation coefficient of 0.95, and an inter-assay coefficient of variation of 11%.

Prenatal risk factors during pregnancy such as antibiotic use, infections, and smoking status were evaluated by questionnaire at enrollment in the first trimester, at 4–5 months of gestation, and at 7–8 months of gestation. Other potential confounders including history of asthma, maternal age, pregnancy body mass index, maternal ethnicity, and acetaminophen use were evaluated by questionnaire during pregnancy and five times throughout the child's first year of life. The primary outcomes for this study were any asthma diagnosis by a health care provider before year three, and any wheezing in the third year. Asthma was defined as ever having an asthma diagnosis by a healthcare professional by three years of age. Wheezing was determined by any positive response by the mother to any of the wheezing diagnosis and symptoms survey questions administered within the year prior to their third-year visit. The full list of questions used to determine a positive response has been previously published (Persky et al., 2008).

The study was approved by the University of Illinois at Chicago's Institutional Review Board. The CRP levels were not normally distributed and were evaluated using a natural logarithmic-transformed variable. In addition, CRP levels were categorized into quartiles as well as a high-risk dichotomous variable. On the basis of previous inflammation studies, the dichotomous variable was calculated using a cut-off of 10 mg/L as CRP levels above 10 mg/L generally indicate acute infection or inflammatory processes (Ridker and Cook, 2004).

Geometric means of CRP levels with 95% CIs during pregnancy are presented for demographics, maternal characteristics during pregnancy, child characteristics through the first year, and third-year outcomes. The P-values were derived from Mann-Whitney *U* test. Characteristics and outcomes were also compared by CRP quartiles and by elevated CRP (<10 versus ≥10 mg/L). Categorical variables were compared across quartiles using Cochran-Armitage test for trend and were compared by elevated CRP using chi-square test or Fisher's exact test (for small cell sizes). Continuous variables were compared using analysis of variance, t-test, or Mann-Whitney *U* test. Multivariable logistic regression models were constructed to model the adjusted association between natural logarithmic-transformed CRP (grand-mean centered) and the outcomes. All maternal characteristics and child characteristics that were significantly related to the outcomes at the univariate level ($p<.05$) or that altered the coefficient of CRP estimate by more than 10% were included in the multivariable model. The CRP was forced into the model, and all interactions were evaluated. Significant interactions at $p<.10$ were included in the final multivariable models. The same process of multivariable model building was implemented for CRP quartiles and for the high-risk CRP variable. All statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

2. **Results**

There were 244 mother-child pairs included in the study analysis with an average maternal age of 25.7 ± 5.9 years. Median prenatal CRP levels were 4.9 mg/L (IQR: 3.2–7.7). The range of CRP values was 0.6 to 34.1 with no values under the detection limit. The study population was 66% Mexican with 34% maternal asthma (Table X). Among the non-Mexican mothers, 13 (5%) were Black, 6 (2%) were White, and 65 (27%) were other Hispanic ethnicities. Average log-transformed CRP levels were significantly higher for maternal age ≥25 years ($p=.003$). Prenatal CRP levels did not differ by Mexican ethnicity, maternal asthma, maternal smoking during pregnancy, or infections during pregnancy. If the child was the first born, the CRP levels were lower but this

association did not reach statistical significance and there were no differences between CRP and gestational age or low birth weight. There was no association between prenatal CRP levels and infections, antibiotic use, acetaminophen use, or smoke exposure in the child's first year of age. Children with an asthma diagnosis by the third year of life or wheezing in the third year of life had mothers with higher levels of CRP but these differences were not statistically significant.

TABLE X

GEOMETRIC MEANS WITH 95% CONFIDENCE INTERVALS FOR CRP LEVELS BY MATERNAL AND CHILD CHARACTERISTICS (N=244)

Maternal Characteristics	**N**	**Geometric Mean (95% CI)**	**P-Value**
Maternal Age			0.003
<25	115	4.18 (3.57–4.88)	
≥25	129	5.40 (4.84–6.01)	
Pregnancy BMI ≥30			0.117
No	96	4.24 (3.62, 4.98)	
Yes	45	5.20 (4.05, 6.67)	
Mexican Ethnicity			0.867
No	84	4.71 (3.90, 5.68)	
Yes	160	4.82 (4.34, 5.36)	
Any Smoking in Pregnancy			0.227
No	219	4.89 (4.44, 5.39)	
Yes	25	3.91 (2.74, 5.58)	
Any Antibiotics in Pregnancy			0.252
No	155	4.99 (4.46–5.57)	
Yes	87	4.33 (3.65–5.13)	
Any Acetaminophen in Pregnancy			0.117
No	75	4.33 (3.66, 5.13)	
Yes	168	5.00 (4.46, 5.60)	
Any Infections in Pregnancy			0.808
No	23	4.94 (3.76, 6.49)	
Yes	220	4.76 (4.30, 5.27)	
Any Respiratory Infections in Pregnancy			0.454
No	31	5.20 (4.04, 6.70)	
Yes	212	4.72 (4.26, 5.22)	
Mother has Asthma			0.736
No	161	4.84 (4.34, 5.39)	
Yes	83	4.67 (3.89, 5.61)	
Child Characteristics			
First Born Child			0.091
No	158	5.03 (4.49, 5.64)	
Yes	86	4.36 (3.70–5.13)	
Gender of Baby			0.741
Girl	114	4.89 (4.28, 5.59)	
Boy	130	4.69 (4.11, 5.35)	
Low Gestational Age (<36 weeks)			0.547
No	235	4.82 (4.38, 5.30)	
Yes	9	3.96 (2.02, 7.77)	
Low Birth Weight (<2500 g)			0.718
No	224	4.77 (4.32, 5.27)	
Yes	20	4.90 (1.88, 3.64)	

GEOMETRIC MEANS WITH 95% CONFIDENCE INTERVALS FOR CRP LEVELS BY MATERNAL AND CHILD CHARACTERISTICS (N=244)

	N	Geometric Mean (95% CI)	P-Value
Baby Exposed to Any Smoke during Year 1			0.155
No	169	5.01 (4.52, 5.55)	
Yes	75	4.31 (3.53, 5.27)	
Antibiotic Use for Respiratory Infections during Year 1			0.136
No	185	5.06 (4.58, 5.59)	
Yes	59	4.01 (3.20, 5.04)	
Outcomes			
Asthma Diagnosis by Third Year of Life			0.439
No	203	4.77 (4.33, 5.25)	
Yes	41	4.87 (3.59, 6.60)	
Wheezing in the Third Year of Life			0.339
No	188	4.75 (4.29, 5.26)	
Yes	56	4.88 (3.87, 6.16)	

In a multivariable model for asthma by year three, Mexican ethnicity and increased gestational age were protective against asthma, while maternal asthma was predictive of child asthma (Table XI). Prenatal CRP levels alone were predictive of asthma by year three (OR: 3.22, 95% CI: 1.36, 7.63), and there was a significant interaction between CRP and Mexican ethnicity (p=.006).

TABLE XI

MULTIVARIABLE[a] MODEL FOR ASTHMA BY YEAR THREE USING LOG-TRANSFORMED CRP LEVELS (N=242)

	OR (95% CI)	P-Value
Prenatal Log-Transformed CRP	3.22 (1.36, 7.63)	0.008
Non-Mexican Ethnicity	2.32 (1.04, 5.20)	0.041
Interaction: Mexican by CRP	0.23 (0.08, 0.66)	0.006
Antibiotics during Pregnancy	2.02 (0.95, 4.31)	0.069
Smoking during Pregnancy	1.95 (0.71, 5.39)	0.198
Maternal Asthma	3.05 (1.43, 6.50)	0.004
Gestational Age	0.81 (0.67, 0.98)	0.030

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficient of CRP by more than 10%. Interaction terms were included if significant at p<.10.

Figure 3 shows CRP levels in children with and without asthma by Mexican ethnicity. The CRP values were significantly higher in Mexican mothers with children who developed asthma by year three compared to Mexican mothers who had children without asthma (p=.02). There was no difference between CRP levels and asthma in non-Mexicans (p=.22).

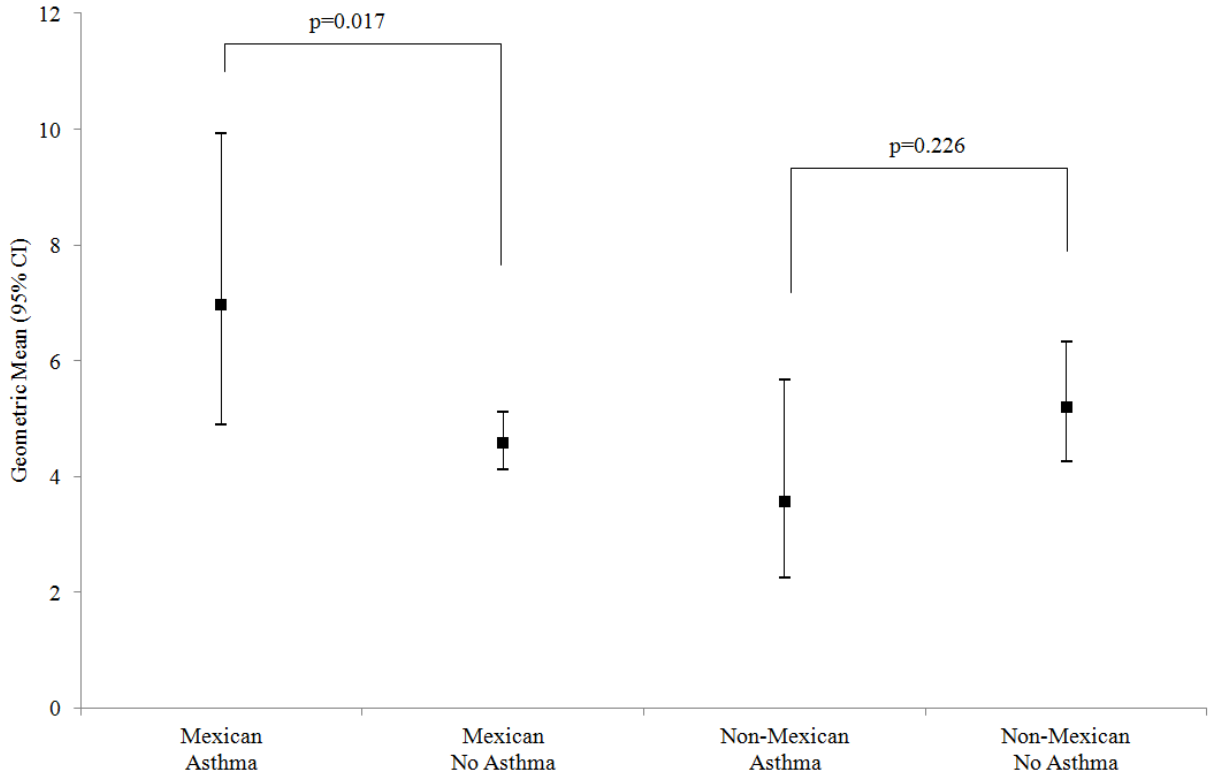


Figure 3. Prenatal CRP levels by Mexican ethnicity and asthma by year three[a]

[a] Figure 3 presents unadjusted geometric means with 95% CIs. For Mexican mothers, means are significantly higher for children with asthma by year 3 (n=19) versus no asthma (n=141) (p=.017). For non-Mexican mothers, there is no significant difference between children with asthma by year 3 (n=22) versus no asthma (n=62) (p=.226). The p-values were derived from Mann-Whitney *U* tests.

Similarly, Mexican ethnicity and gestational age were protective of wheezing in year three while maternal smoking during pregnancy and child exposure to smoke in the first year were predictive of wheezing (Table XII). Continuous CRP was also predictive of wheezing (OR: 2.17, 95% CI: 1.11, 4.26), as was an interaction between CRP and baby exposure to smoke in year one. Within babies exposed to any smoke in year one, there was no significant difference in prenatal CRP levels between children with wheezing in year three versus no wheezing (p=.26) (Figure 4). Within babies not exposed to smoke, CRP levels were significantly higher for children with wheezing in year three versus no wheezing (p=.04).

TABLE XII

MULTIVARIABLE[a] MODEL FOR WHEEZING IN YEAR THREE USING LOG-TRANSFORMED CRP LEVELS (N=244)

	OR (95% CI)	**P-Value**
Prenatal Log-Transformed CRP	2.17 (1.11, 4.26)	0.023
Mexican Ethnicity	0.47 (0.24, 0.93)	0.029
Smoking during Pregnancy	3.91 (1.53, 9.99)	0.004
Gestational Age	0.79 (0.66, 0.95)	0.011
Baby Exposed to Smoke in Year 1	2.41 (1.20, 4.83)	0.013
Interaction: Baby Exposed to Smoke in Year 1 by CRP	0.38 (0.15, 0.92)	0.032

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficient of CRP by more than 10%. Interaction terms were included if significant at p<.10.

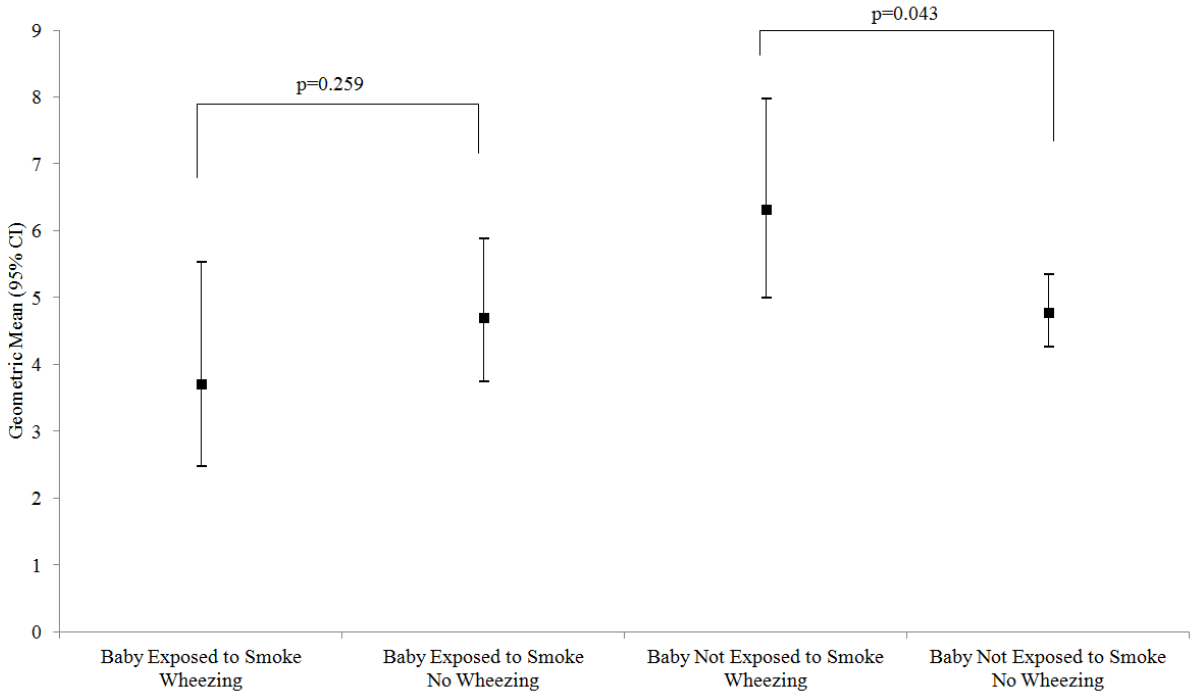
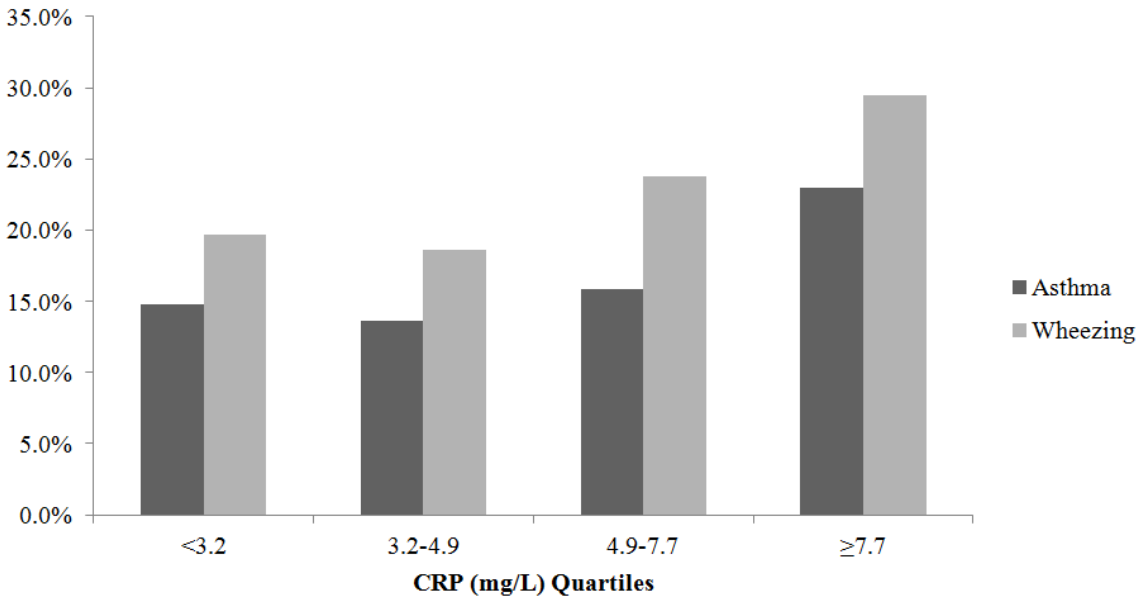


Figure 4. Prenatal CRP levels by baby smoke exposure in year one and wheezing in year three.[a]

[a] Figure 2 presents unadjusted geometric means with 95% CIs. Within babies exposed to any smoke in year 1, there is no significant difference in means between children with wheezing in year 3 (n=27) versus no wheezing (n=48) (p=.259). Within babies not exposed to smoke, means are significantly higher for children with wheezing in year 3 (n=29) versus no wheezing (n=140) (p=.043). The p-values were derived from Mann-Whitney *U* tests.

Results were similar when analyzing CRP level across quartiles (data not shown). The CRP levels were significantly higher with older maternal age, and levels of CRP were also higher with increased pregnancy body mass index and acetaminophen use during pregnancy, but these relationships did not reach statistical significance. Asthma by year three increased in a dose-response manner from 15% in the first quartile of CRP to 23% in the fourth quartile while wheezing in year three similarly increased from 20% in the first quartile to 30% in the fourth quartile, but these increases did not reach statistical significance (Figure 5).



	Quartile 1 N (%)	Quartile 2 N (%)	Quartile 3 N (%)	Quartile 4 N (%)	P-Value for Trend
Asthma	9 (14.8)	8 (13.6)	10 (15.9)	14 (23.0)	0.211
Wheezing	12 (19.7)	11 (18.6)	15 (23.8)	18 (29.5)	0.151

Figure 5. Asthma by year three and wheezing in year three by quartiles of prenatal CRP (mg/L).

After adjustment for Mexican ethnicity, smoking during pregnancy, maternal asthma, and gestational age, a dose-response relationship was observed between quartile of CRP and asthma by year three (Table XIII). Although not statistically significant, the highest quartile of CRP was weakly associated with asthma (OR: 2.64, 95% CI: 0.94, 7.40) when compared to the lowest quartile.

TABLE XIII

MULTIVARIABLE[a] MODEL FOR ASTHMA BY YEAR THREE USING QUARTILES OF CRP (N=244)

	OR (95% CI)	**P-Value**
CRP Quartile 1 (<3.2 mg/L)	Reference	
CRP Quartile 2 (3.2–4.9 mg/L)	1.70 (0.54, 5.33)	0.362
CRP Quartile 3 (4.9–7.7 mg/L)	1.74 (0.59, 5.12)	0.314
CRP Quartile 4 (≥7.7 mg/L)	2.64 (0.94, 7.40)	0.064
Mexican Ethnicity	0.53 (0.25, 1.11)	0.094
Smoking during Pregnancy	2.59 (0.98, 6.88)	0.056
Maternal Asthma	3.45 (1.65, 7.21)	0.001
Gestational Age	0.81 (0.68, 0.97)	0.023

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficients of CRP quartile by more than 10%.

In a multivariable model adjusted for Mexican ethnicity, smoking during pregnancy, maternal asthma, gestational age, and baby exposed to smoke, a similar dose-response relationship was apparent between CRP quartile and wheezing in year three (Table XIV). The highest quartile of CRP was significantly predictive of wheezing when compared to the lowest quartile (OR: 2.92, 95% CI: 1.10–7.71).

TABLE XIV

MULTIVARIABLE[a] MODEL FOR WHEEZING IN YEAR THREE USING QUARTILES OF CRP (N=244)

	OR (95% CI)	**P-Value**
CRP Quartile 1 (<3.2 mg/L)	Reference	
CRP Quartile 2 (3.2–4.9 mg/L)	1.80 (0.63, 5.11)	0.270
CRP Quartile 3 (4.9–7.7 mg/L)	2.34 (0.86, 6.36)	0.096
CRP Quartile 4 (≥7.7 mg/L)	2.92 (1.10, 7.71)	0.031
Mexican Ethnicity	0.52 (0.26, 1.03)	0.060
Smoking during Pregnancy	3.84 (1.50, 9.84)	0.005
Maternal Asthma	1.68 (0.85, 3.32)	0.139
Gestational Age	0.78 (0.65, 0.93)	0.007
Baby Exposed to Smoke in Year 1	2.32 (1.16, 4.64)	0.018

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficients of CRP quartile by more than 10%.

Thirty-six (15%) mothers had CRP levels ≥10 mg/L. There were no significant differences between maternal characteristics or child characteristics through the first year of life when compared between low and high levels of CRP (data not shown). In mothers with elevated CRP levels ≥10 mg/L, 11 children developed asthma by year three and 12 children developed wheezing in year three. After adjustment, elevated CRP levels were a significant risk factor for asthma by year three (OR: 3.40, 95% CI: 1.39, 8.33) (Table XV). Elevated CRP levels ≥10 mg/L were associated with wheezing in year three although this association did not reach statistical significance (OR: 2.03, 95% CI: 0.88, 4.69) (Table XVI).

TABLE XV

MULTIVARIABLE[a] MODEL FOR ASTHMA BY YEAR THREE USING ELEVATED CRP LEVELS (≥10 mg/L) (N=242)

	OR (95% CI)	**P-Value**
CRP ≥10 mg/L	3.40 (1.39, 8.33)	0.007
Mexican Ethnicity	0.50 (0.24, 1.04)	0.064
Antibiotics during Pregnancy	2.07 (0.98, 4.36)	0.056
Maternal Asthma	3.36 (1.59, 7.08)	0.002
Gestational Age	0.80 (0.66, 0.96)	0.016

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficient of elevated CRP by more than 10%.

TABLE XVI

MULTIVARIABLE[a] MODEL FOR WHEEZING IN YEAR THREE USING ELEVATED CRP LEVELS (≥10 mg/L) (N=244)

	OR (95% CI)	**P-Value**
CRP ≥10 mg/L	2.03 (0.88, 4.69)	0.097
Mexican Ethnicity	0.50 (0.26, 0.98)	0.044
Smoking during Pregnancy	3.87 (1.55, 9.68)	0.004
Gestational Age	0.79 (0.66, 0.94)	0.008
Baby Exposed to Smoke in Year 1	2.13 (1.09, 4.18)	0.028

[a]Variables were included in the multivariable logistic regression model if significant at the 0.05 level in a univariate model or if they altered the coefficient of elevated CRP by more than 10%.

3. **Discussion**

Our study is the first to look at the relationship between systemic maternal inflammation, as measured by prenatal CRP levels, and subsequent asthma and wheezing in the offspring within asthmatic or at-risk women. After risk adjustment, we found a significant relationship between prenatal continuous CRP levels and both asthma by year three and wheezing in year three. We also found a significant relationship between prenatal continuous CRP levels and asthma by year three within Mexicans. Mexicans have been found to have significantly lower prevalence of asthma than other populations within the United States (Rodriguez et al., 2002; Lara et al., 2006; Ledogar et al., 2000; Freeman, Schneider, and McGarvey, 2003), including other Hispanic subgroups. Asthma prevalence has been found to be highest in Puerto Ricans, followed by Cubans and Dominicans, and lowest among Central Americans and Mexicans (Carter-Pokras and Gergen, 1993; Koinis-Mitchell et al., 2011). The reason for this disparity has remained unclear, and it is debatable whether the protection is due to differences in early life exposures or due to lifestyle and environmental factors that are likely to change with acculturation. Our study is the first to find a relationship between prenatal CRP levels and subsequent asthma within Mexicans.

We also found an interaction between baby's exposure to smoke and wheezing, with CRP levels being significantly higher for babies with wheezing as compared to those without wheezing in babies not exposed to smoke. There was no difference between CRP levels and wheezing in babies exposed to smoke. Overall, we found CRP levels were not affected by smoking or Mexican ethnicity. However, after adjustment for other risk factors, we did find CRP levels were higher in children of Mexican ethnicity with asthma compared to those with no asthma, and were higher for babies with wheezing as compared to those without wheezing in babies not exposed to smoke. We speculate this effect modification may be related to the low risk of atopic disease in both Mexicans and babies not exposed to smoke. Inflammation in lower-risk individuals may indicate an environment that could affect the developing immune system more than it would for offspring already at risk for atopic disease. In high-risk children, the immune response may already be permissive toward the development of atopy and thus unaffected by maternal inflammation (Kozyrskyj, Ernst, and Becker, 2007). Due to low sample size, we could not look at the effect of a three-way interaction between Mexican ancestry, exposure to smoke, and CRP levels on our outcomes.

Our study found a median prenatal CRP level of 4.9 mg/L (IQR: 3.2–7.7 mg/L) at 29.5 ± 3.7 weeks gestation. Normal pregnancy has been shown to be an inflammatory stressor as measured by elevations in serum

CRP, with one study showing healthy pregnant women had a median CRP level of 4.8 mg/L (IQR: 0.63–15.7 mg/L) (Picklesimer et al., 2008). These elevations in CRP have been found to remain consistently high from the earliest stages of pregnancy through to childbirth (Sacks et al., 2004; Belo et al., 2005; Picklesimer et al., 2008). However, higher than usual inflammatory response in pregnant women, as measured by CRP, has also been related to adverse pregnancy outcomes including preeclampsia, neonatal sepsis (Jeon et al., 2014), preterm delivery (Ernst et al., 2011, Lohsoonthorn, Qiu, and Williams, 2007), and intrauterine growth restriction (Ernst et al., 2011; Morales et al., 2011; Tjoa et al., 2003). Our data did not reflect any differences in birth outcomes, as measured by preterm delivery and low birth weight, with increased CRP levels in unadjusted analysis.

Elevations in serum CRP levels are caused by stress and infection, and elevated with older age, obesity, smoking, lack of physical activity, and low SES (Picklesimer et al., 2008). A birth cohort study found that higher maternal CRP levels were associated with increasing maternal age, parity, pre-pregnancy obesity, and lack of physical activity. They found no association between CRP levels and maternal smoking, first-trimester infections, antibiotic use, or acetaminophen. Reproductive outcomes such as birth weight, prematurity, and small for gestational age were not related to maternal CRP levels. Our study found similar results with CRP levels increasing with increasing maternal age, increasing body mass index, and parity. We did not find an association between infections, antibiotic use, or acetaminophen by CRP levels. An analysis of NHANES data has shown that adults with asthma and asthma symptoms have higher levels of CRP (Arif, Delclos, and Colmer-Hamood, 2007). Elevated CRP levels have been demonstrated to be significantly higher in people with reduced lung function, independent of asthma (Rasmussen et al., 2009). There was no difference in prenatal CRP levels among mothers with asthma compared with mothers without asthma in our unadjusted analysis. Since CRP levels were already elevated due to pregnancy, it is possible that differences in pre-pregnancy CRP levels by asthma status were masked.

We additionally investigated quartiles of CRP and high-risk CRP as alternative ways of analyzing CRP levels. The highest quartile of CRP was weakly associated with asthma by year three (OR: 2.6, 95% CI: 0.9, 7.4) when compared to the lowest quartile, and the highest quartile of CRP was significantly predictive of wheezing in year three when compared to the lowest quartile (OR: 2.9, 95% CI: 1.1–7.7). When analyzing elevated CRP levels (≥10 mg/L), multivariable models indicated elevated CRP was a significant predictor for asthma (OR: 3.4, 95% CI: 1.4, 8.3) and weakly associated with wheezing. A birth cohort study of 504 babies found a significant increase in

wheezing for children in the highest tertile of maternal CRP serum levels, after adjusting for important confounders (OR: 2.9, 95% CI: 1.2–6.7) (Morales et al., 2011). Sonnenschein et al. (2013) analyzed CRP levels in cord-blood and found increased risk of wheezing and lower respiratory infections in the first four years of life (Sonnenschein-van der Voort et al., 2013). Their prospective study of 4,984 mother-child pairs also found that higher maternal CRP levels in early pregnancy were associated with a lower risk of wheezing by year two, and a higher risk of eczema (Sonnenschein-van der Voort et al., 2013). They attributed the difference in effects to the larger sample size and ability to adjust for more effect modifiers.

The prenatal environment is considered an important factor in the development of the fetal immune system and in the subsequent occurrence of allergic and respiratory diseases (Devereux, Barker, and Seaton, 2002). Elevated levels of CRP may indicate a process leading to a direct effect on the developing fetus (Sonnenschein-van der Voort et al., 2013). The pathways may include fetal growth restriction with smaller lungs and airways, an inflammatory fetal status, or other alterations in the infant's immune system that may affect the development of wheezing or asthma. Prenatal cytokines and immune modulators, Th1 and Th2, are thought to play important roles in controlling the maturation of the developing immune system and conditioning it for postnatal responses against allergens (Macaubas et al., 2003). Elevated prenatal CRP levels may be a marker of adverse prenatal conditions, influencing the fetal respiratory system development through alteration of the Th2 immune response.

Our study provides three adjusted analyses of prenatal CRP as a risk factor for subsequent asthma and wheezing in at-risk children. While our sample size is limited, the study design offered many pertinent risk factors for childhood asthma and wheezing, and the prospectively collected measures of maternal and child confounders strengthened the study results. This unique population of disadvantaged urban, mostly Mexican, mothers were closely followed from the first trimester of pregnancy through the child's third year of life. There are some limitations with this study, however. Asthma is a complex clinical disease which is difficult to critically assess before age six (Eder, Ege, and von Mutius, 2006). Given that our study included diagnoses prior to age three, the nature of the disease progression may still be unpredictable. C-reactive protein has a short half-life and we were only able to assess inflammation once throughout the pregnancy. Several studies, however, have shown that while CRP levels are elevated during pregnancy, and there is some fluctuation throughout the pregnancy, early-pregnancy CRP levels do correlate with those later in pregnancy (Picklesimer et al., 2008; Belo et al., 2005; Sacks et al., 2004). Additionally, the serum samples were collected 12 to 15 years prior to analysis of CRP. There have been several

studies that have measured the stability of CRP in serum over time and have shown levels remain stable as long as the sera are frozen and remain at -70°C (Aziz et al., 2003; Ishikawa et al., 2007). One study analyzed CRP levels at baseline and again after a median time of 13.5 years and found levels were significantly elevated over that time but remained highly correlated with their baseline measurement (Ishikawa et al., 2007). Any differences in CRP would be non-differential with respect to our outcomes so any potential bias would be towards the null. Finally, CRP measures the effects of the microbiome indirectly and we do not have biological data on direct microbiome measures. Nonetheless, our results indicate that elevated prenatal CRP levels may be associated with the subsequent development of asthma and wheezing in the offspring.

We conclude that systemic inflammation during pregnancy in at-risk mothers may reflect a prenatal environment that could increase offspring susceptibility to develop wheezing and asthma in early life. Further research is needed to explore the underlying mechanisms of the in utero environment on the subsequent development of atopy.

D. **Aim 3b Results. The Relationships among Psychosocial Factors, Maternal CRP Levels, and the Subsequent Development of Asthma by Year Three and Wheezing in Year Three**

Psychosocial measures were collected during pregnancy (FFFS) and during the child's third year visit (FHI and F-COPES). Table XVII shows the sample size, mean with standard deviation, range, and internal reliability as measured by Cronbach's alpha for each survey. FFFS was measured in 217 mothers during pregnancy and has a high internal reliability (Cronbach's alpha = 0.81). FHI and F-COPES were measured in mothers at the child's third year visit. There were 228 mothers who completed FHI and 183 who completed F-COPES.

TABLE XVII

SUMMARY OF PSYCHOSOCIAL MEASURES DURING PEPS

Psychosocial Measures	**N**	**Mean ± SD**	**Range**	**Cronbach's α**
Prenatal Measures				
FFFS	217	22.9 ± 14.7	0–69	0.81
Three Year Measures				
Family Hardiness Index	228	44.2 ± 8.4	4–59	0.84
Commitment subscale	227	18.9 ± 3.7	8–24	0.76
Challenge subscale	227	13.0 ± 3.3	1–18	0.74
Control subscale	228	12.4 ± 3.2	4–18	0.64
F-COPES	183	97.1 ± 19.3	16–131	0.84
Acquiring Social Support	183	27.4 ± 7.3	7–45	0.83
Reframing	183	30.4 ± 6.4	1–40	0.78
Seeking Spiritual Support	182	13.8 ± 4.0	2–20	0.75
Mobilizing Family Support	183	13.1 ± 3.7	3–20	0.75
Passive Appraisal	182	12.4 ± 3.8	1–20	0.42

Women in our PEPS study reported very high levels of family hardiness. For overall FHI, the mean was 44.2 (range 4–59). Internal reliability was high (Cronbach's alpha=.84), but was lower for the subscales. According to the developers, the overall internal reliability for FHI was 0.82 with the subscales of commitment, challenge, and control having an internal reliability of 0.81, 0.80, and 0.65, respectively (McCubbin, McCubbin, and Thompson, 1986). Our study showed similar internal reliability.

Women in our sample also reported very high coping with total scores averaging 97.1 (range 16–131). The developers of F-COPES list the overall internal reliability as 0.86. For the subscales of acquiring social support, reframing, seeking spiritual support, mobilizing family support, and passive appraisal, the internal reliability was 0.83, 0.82, 0.80, 0.71, and 0.63, respectively (McCubbin, McCubbin, and Thompson, 1986). Other than for the subscale of passive appraisal, which has low internal reliability in our cohort, the other subscales showed similar internal reliability.

Table XVIII shows Pearson correlation coefficients between psychosocial factors during pregnancy and at year three. Lower scores on FFFS indicate higher family functioning. FFFS is negatively correlated with all FHI scores, and with all F-COPES scores and subscales except for family support and passive appraisal. The FHI and F-COPES measures are also significantly correlated in this cohort.

TABLE XVIII

PEARSON CORRELATION COEFFICIENTS BETWEEN FFFS, FHI, AND F-COPES

	During Pregnancy	**At Year Three**									
	FFFS	Overall FHI	Commitment	Challenge	Control	Overall F-COPES	Social	Reframing	Spiritual	Family	Passive
FFFS		-0.31**	-0.30**	-0.19*	-0.23*	-0.22*	-0.19*	-0.17	-0.19*	-0.16	-0.03
Overall FHI			0.84**	0.80**	0.71**	0.43**	0.40**	0.40**	0.22*	0.24*	0.24*
Commitment				0.55**	0.38**	0.38**	0.26**	0.45**	0.19*	0.25**	0.18*
Challenge					0.32**	0.36**	0.39**	0.36**	0.19*	0.21*	0.08
Control						0.29**	0.23*	0.24*	0.17*	0.11	0.32**
Overall F-COPES							0.82**	0.84**	0.76**	0.74**	0.47**
Social								0.54**	0.48**	0.65**	0.11
Reframing									0.55**	0.48**	0.42**
Spiritual										0.53**	0.30**
Family											0.10
Passive											

*p<.05; **p<.001

TABLE XIX

CORRELATIONS BETWEEN CRP LEVELS AND PSYCHOSOCIAL MEASURES

	During Pregnancy	**At Year Three**									
	FFFS	Overall FHI	Commitment	Challenge	Control	Overall F-COPES	Social	Reframing	Spiritual	Family	Passive
CRP levels	-0.14	-0.01	0.09	-0.12	0.02	-0.04	-0.13	0.04	0.08	-0.06	-0.01
Log(CRP) levels	-0.19*	0.07	0.17	-0.03	0.02	-0.02	-0.12	0.03	0.11	-0.04	0.01
CRP ≥10 mg/L	-0.07	-0.06	-0.05	-0.05	-0.04	-0.10	-0.09	0.01	-0.05	0.04	0.01

*p<.05

TABLE XX

PSYCHOSOCIAL MEASURES BY OUTCOMES

Psychosocial Outcomes	**Asthma by Year 3**			**Wheezing in Year 3**		
	Asthma Mean ± SD	**No Asthma** Mean ± SD	**P-Value**	**Wheezing** Mean ± SD	**No Wheezing** Mean ± SD	**P-Value**
During Pregnancy						
FFFS	27.1 ± 15.5	22.1 ± 14.4	0.068	25.8 ± 14.8	22.1 ± 14.6	0.130
At Year Three						
Family Hardiness Index	43.1 ± 8.2	44.4 ± 8.4	0.400	42.9 ± 7.9	44.6 ± 8.5	0.205
Commitment subscale	17.6 ± 4.3	19.2 ± 3.5	0.015	17.9 ± 4.1	19.2 ± 3.5	0.028
Challenge subscale	12.9 ± 3.5	13.0 ± 3.3	0.806	12.8 ± 3.4	13.1 ± 3.3	0.562
Control subscale	12.7 ± 3.1	12.4 ± 3.2	0.573	12.2 ± 2.8	12.5 ± 3.3	0.577
F-COPES	100.7 ± 14.3	96.4 ± 20.1	0.167	100.8 ± 13.0	95.9 ± 20.8	0.065
Acquiring Social Support	29.3 ± 5.9	27.0 ± 7.5	0.120	28.7 ± 6.0	27.0 ± 7.6	0.177
Reframing	30.5 ± 4.5	30.4 ± 6.7	0.913	31.0 ± 4.7	30.2 ± 6.8	0.446
Seeking Spiritual Support	14.1 ± 3.5	13.8 ± 4.1	0.732	14.3 ± 3.7	13.7 ± 4.1	0.435
Mobilizing Family Support	14.2 ± 3.3	12.9 ± 3.7	0.093	14.2 ± 3.2	12.8 ± 3.8	0.022
Passive Appraisal	12.6 ± 3.4	12.4 ± 3.9	0.741	12.7 ± 2.3	12.4 ± 4.2	0.550

Table XIX shows Pearson and Spearman correlation coefficients between psychosocial factors during pregnancy and at year three with maternal CRP levels. Logarithmic-transformed CRP levels are negatively correlated with FFFS scores, meaning higher CRP levels are associated with better family functioning. There are no other statistically significant correlations with maternal CRP levels and psychosocial survey measures.

Table XX shows psychosocial outcomes (mean ± SD) by the primary endpoints of asthma by year three and wheezing in year three. Higher FFFS scores indicate worse family functioning and there is a trend towards worse FFFS scores within families where the child has asthma compared to families where the child does not have asthma (27.1 ± 15.5 versus 22.1 ± 14.4, p=.068). At year three, families where the child has asthma have significantly worse FHI commitment subscale scores compared with families where the child does not have asthma (17.6 ± 4.3 versus 19.2 ± 3.5, p=.015). This relationship is similar in families where the child has wheezing (17.9 ± 4.1 versus 19.2 ± 3.5, p=.028). In families where the child has wheezing, the F-COPES mobilizing family support subscale is higher than in families where the child does not have wheezing (14.2 ± 3.2 versus 12.8 ± 3.8, p=.022). There are no other differences between psychosocial outcomes and endpoints. After risk adjustment, the significant relationships between psychosocial outcomes and endpoints are eliminated (data not shown).

Since FFFS showed mild associations with the outcomes, as well as a negative correlation with CRP, we added the psychosocial survey measure to the elevated CRP multivariable models for asthma and wheezing from Aim 3a (Table XXI). After accounting for the smaller sample size within these models due to the limited psychosocial data, Model 1 shows the adjusted OR for elevated CRP in our model from Aim 3a. Model 2 shows the adjusted OR for elevated CRP when FFFS has been added to the model, and Model 3 shows the OR for the model including an interaction term between elevated CRP and FFFS. FFFS did not confound or modify the relationship between CRP and the third-year outcomes.

TABLE XXI

MULTIVARIABLE MODELS FOR ASTHMA BY YEAR THREE AND WHEEZING IN YEAR THREE USING ELEVATED CRP LEVELS AND FFFS (n=180)

	Asthma by Year 3		**Wheezing in Year 3**	
	aOR[a] (95% CI)	**P-Value**	**aOR[b] (95% CI)**	**P-Value**
Model 1				
CRP ≥10 mg/L	4.96 (1.75, 14.05)	0.003	3.17 (1.23, 8.16)	0.017
Model 2				
CRP ≥10 mg/L	5.02 (1.75, 14.39)	0.003	3.29 (1.27, 8.53)	0.015
FFFS	1.02 (0.99, 1.05)	0.241	1.02 (0.99, 1.04)	0.241
Model 3				
Interaction: CRP*FFFS	1.03 (0.96, 1.11)	0.346	1.01 (0.95, 1.08)	0.720

[a]Models adjusted for Mexican ethnicity, prenatal antibiotics, prenatal smoking, maternal asthma, and gestational age
[b]Models adjusted for Mexican ethnicity, prenatal smoking, gestational age, and baby exposed to smoke

There was also no confounding or moderation by FFFS within the models looking at continuous log-transformed CRP or by CRP quartile (data not shown). We did not find that FHI or F-COPES confounded or modified the relationship between CRP and asthma or wheezing (data not shown). The relationship between FFFS and CRP was in the unexpected direction in unadjusted analyses. There is also little-to-no association between these psychosocial measures and our primary outcomes of asthma by year three and wheezing in year three. Given the lack of statistical significance between these variables, further mediation analysis was unwarranted.

We found no significant relationship between maternal CRP, psychosocial factors, and subsequent asthma and wheezing within this exploratory study. Our psychosocial measurements did not affect the outcomes, nor did they moderate the relationship between CRP and asthma. Other psychosocial measures may yield a stronger association with subsequent asthma and wheezing. Our limited sample size with the psychosocial measures may also have contributed to our null findings.

E. **Strengths and Limitations**

Our study design offered pertinent temporal risk factors for childhood asthma and wheezing, and the prospectively collected measures of maternal and child confounders strengthened the study results. This unique population of disadvantaged urban, mostly Hispanic, mothers was closely followed from the first trimester of pregnancy through the child's third year of life.

There are some limitations with our study, however. Given the highly selected population, the generalizability of the study results is limited to urban children at risk for allergy and asthma. Asthma is a complex clinical disease that is difficult to assess before age six (Eder, Ege, and von Mutius, 2006). Our study included diagnoses prior to age three when the nature of the disease progression may still be unpredictable. Since there was a high rate of infections in this cohort, there may be a covariation effect between infections and antibiotic use that we were unable to separate. Data on specific infections for which antibiotics were used in the PEPS were not precise enough to rule out the possibility of confounding by infection.

The use of antibiotics and type of infections noted were based on parental recall and so are prone to recall bias. However, nurses administered the surveys multiple times throughout the pregnancy and five times within the child's first year to ensure recall was limited to the prior few months. Another limitation is that we do not have access to the number of days antibiotics were taken or the reason antibiotics were taken by the mothers. We also did not capture reason for antibiotic use in the mothers or information on labor and delivery. Nor do we know the types of antibiotics used in either the children or the pregnant mothers. Broad-spectrum antibiotics became more prevalent in the 1980s and may alter microbiomes more than the narrow-spectrum antibiotics used in the past (Kozyrskyj, Ernst, and Becker, 2007). The study findings may be limited to certain types of antibiotics, which should be investigated further. We do not have information on confounders such as chorioamnionitis during pregnancy or information on childbirth such as C-section, which may be associated with prophylactic use of antibiotics and with altered microbiomes in infants. Finally, we do not have information on chronic disease in the mothers, aside from asthma. This study cohort focuses on high-risk urban children and results apply only to high-risk urban children. Prior literature mostly focuses on low-risk children, and we cannot make direct comparisons between these two groups.

Effects of the microbiome are measured indirectly by CRP and we do not have biological data on direct microbiome measures. C-reactive protein has a short half-life and we were only able to assess inflammation once throughout the pregnancy. Several studies, however, have shown that while CRP levels are elevated during pregnancy, and there is some fluctuation throughout the pregnancy, early-pregnancy CRP levels do correlate with those later in pregnancy (Picklesimer et al., 2008; Belo et al., 2005; Sacks et al., 2004). Additionally, the serum samples were collected 12 to 15 years prior to analysis of CRP. There have been several studies that have measured

the stability of CRP in serum over time and have shown levels remain stable as long as the sera are frozen and remain at -70°C (Aziz et al., 2003; Ishikawa et al., 2007). One study analyzed CRP levels at baseline and again after a median time of 13.5 years and found levels were significantly elevated over that time but remained highly correlated with their baseline measurement (Ishikawa et al., 2007).

Finally, our psychosocial measures (FFFS, FHI, and F-COPES) were not validated within Hispanic populations.

F. **Conclusion**

Asthma and wheezing in children are still not well understood but are the result of interactions between genetic, environmental, and microbiome factors prenatally, perinatally, and postnatally. We focused on three main risk factors and our research has helped elucidate some aspects of the development of asthma and wheezing within an at-risk urban, mostly Hispanic, cohort.

We conclude the associations found between antibiotic use in young children and subsequent development of asthma and wheezing may be due to confounding by respiratory infections. However, our study suggests an association between prenatal antibiotic use and the development of asthma in at-risk children. Modification of microbial load may be occurring prenatally, affecting the maturation of the infant immune system and increasing a child's risk for developing asthma. While the relationship with prenatal antibiotics does not hold for wheezing in our study, there may be a trend that could be further delineated within a larger cohort study.

We conclude that systemic inflammation during pregnancy in at-risk mothers may reflect a prenatal environment that could increase offspring susceptibility to develop wheezing and asthma in early life. Further research is needed to explore the underlying mechanisms of the in utero environment on the subsequent development of atopy and asthma.

In an exploratory study, we did not find an association between maternal psychosocial factors, specifically family functioning, hardiness, and coping, and subsequent asthma or wheezing in the offspring. We also did not find

that psychosocial factors affected systemic maternal inflammation, as measured by CRP, or moderated or mediated the relationship between CRP and the primary outcomes.

By investigating three primary exposures, we sought to identify relationships between prenatal risk factors, early life events, and subsequent asthma and wheezing. We hope this research will aid in the progression of understanding the complex interactions in the development of asthma and wheezing.

G. **Financial Support**

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APPENDICES

APPENDIX A

Surveys

Marin Bidimensional Acculturation Scale

Response categories: *Items 1–6*: 1 = Almost never; 2 = Sometimes; 3 = Often; 4 = Almost always

Language Use Subscale

1. How often do you speak English?
2. How often do you speak in English with your friends?
3. How often do you think in English?
4. How often do you speak Spanish?
5. How often do you speak in Spanish with your friends?
6. How often do you think in Spanish?

Response categories: *Items 7–18*: 1 = Very poorly; 2 = Poorly; 3 = Well; 4 = Very well

Linguistic Proficiency Subscale

7. How well do you speak English?
8. How well do you read in English?
9. How well do you understand TV programs in English?
10. How well do you understand radio programs in English?
11. How well do you write in English?
12. How well do you understand music in English?
13. How well do you speak Spanish?
14. How well do you read in Spanish?
15. How well do you understand TV programs in Spanish?
16. How well do you understand radio programs in Spanish?
17. How well do you write in Spanish?
18. How well do you understand music in Spanish?

Response categories: *Items 19–24*: 1 = Almost never; 2 = Sometimes; 3 = Often; 4 = Almost always

Electronic Media Subscale

19. How often do you watch TV programs in English?
20. How often do you listen to radio programs in English?
21. How often do you listen to music in English?
22. How often do you watch TV programs in Spanish?
23. How often do you listen to radio programs in Spanish?
24. How often do you listen to music in Spanish?

APPENDIX A (continued)

Feetham Family Functioning Survey

PEPS FORM: Feetham Family Functioning

Participant's ID#: _____ Participant's Name:_____

Date: _____/_____/_____ Nurse Name:_____

Note: This should be a SELF-ADMINISTERED survey if possible. Please check–off method of administration:

☐ Self-Administered ☐ Nurse-Administered

DIRECTIONS: In this survey you are asked to rate activities (functions) that occur in your family and with family members. For ***each*** family function you are asked to answer three questions.

How much is there now?
How much should there be?
How important is this to you?

Please answer ***all three*** questions for ***each*** family function by **circling the number** which represents how you feel ***now*** about the family function.
The term spouse refers to your husband or the person who assumes the functions of a spouse. If you do not have a person in the spouse role answer the questions based on how much you want the functions met.

Please try to answer all items.

1. The amount of discussion with your ***friends*** regarding your concerns and problems.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

2. The amount of discussion with your ***relatives*** regarding your concerns and problems *(do not include your spouse).*

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

APPENDIX A (continued)

3. The amount of time your spend with your ***spouse.***

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

4. The amount of discussion of your concerns and problems with your ***spouse***.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

5. The amount of time you spend with ***neighbors.***

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

6. The amount of time ***you*** spend in leisure/recreational activities.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

APPENDIX A (continued)

7. The amount of help from your ***spouse*** with family tasks such as care of children, house repairs, household chores, etc.

a. How much is there now?

Little 1 2 3 4 5 6 7 Much

b. How much should there be?

Little 1 2 3 4 5 6 7 Much

c. How important is this to you?

Little 1 2 3 4 5 6 7 Much

8. The amount of help from ***relatives*** with family tasks such as care of children, house repairs, household chores, etc. *(do not include spouse).*

a. How much is there now?

Little 1 2 3 4 5 6 7 Much

b. How much should there be?

Little 1 2 3 4 5 6 7 Much

c. How important is this to you?

Little 1 2 3 4 5 6 7 Much

9. The amount of time with ***health professionals*** (*doctors, nurses, social workers, etc.).*

a. How much is there now?

Little 1 2 3 4 5 6 7 Much

b. How much should there be?

Little 1 2 3 4 5 6 7 Much

c. How important is this to you?

Little 1 2 3 4 5 6 7 Much

10. The amount of help from your ***friends*** with family tasks such as care of children, house repairs, household chores, etc. **a.** How much is there now?

Little 1 2 3 4 5 6 7 Much

b. How much should there be?

Little 1 2 3 4 5 6 7 Much

c. How important is this to you?

Little 1 2 3 4 5 6 7 Much

APPENDIX A (continued)

If you don't have a ***child(ren)***, check here _____ and skip questions 11, 12, 13.

11. The number of problems with your ***child(ren).***

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

12. The amount of time you spend with your ***child(ren)***?

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

If you do not have a **child** in school, check here _____ and omit question 13.

13. The amount of time your ***child(ren)*** miss school.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

APPENDIX A (continued)

14. The number of disagreements with your ***spouse.***

a. How much is there now?

Little — Much

1 2 3 4 5 6 7

b. How much should there be?

Little — Much

1 2 3 4 5 6 7

c. How important is this to you?

Little — Much

1 2 3 4 5 6 7

15. The amount of time ***you*** are ill.

a. How much is there now?

Little — Much

1 2 3 4 5 6 7

b. How much should there be?

Little — Much

1 2 3 4 5 6 7

c. How important is this to you?

Little — Much

1 2 3 4 5 6 7

16. The amount of time ***you*** spend doing housework *(cooking, cleaning, washing, yard work, etc.).*

a. How much is there now?

Little — Much

1 2 3 4 5 6 7

b. How much should there be?

Little — Much

1 2 3 4 5 6 7

c. How important is this to you?

Little — Much

1 2 3 4 5 6 7

17. The amount of time ***you*** miss work *(including housework).*

a. How much is there now?

Little — Much

1 2 3 4 5 6 7

b. How much should there be?

Little — Much

1 2 3 4 5 6 7

c. How important is this to you?

Little — Much

1 2 3 4 5 6 7

APPENDIX A (continued)

18. The amount of time your ***spouse*** misses work *(including housework).*

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

19. The amount of emotional support from ***friends***.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

20. The amount of emotional support form ***relatives.***

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

21. The amount of emotional support from your ***spouse.***

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

APPENDIX A (continued)

22. The amount of time ***your*** work routine is disrupted *(including housework).*

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

23. The amount of time your ***spouse's*** work routine is disrupted *(including housework).*

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

24. The amount of satisfaction with ***your*** marriage.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

25. The amount of satisfaction with the sexual relations with your ***spouse***.

a. How much is there now?

Little Much
1 2 3 4 5 6 7

b. How much should there be?

Little Much
1 2 3 4 5 6 7

c. How important is this to you?

Little Much
1 2 3 4 5 6 7

APPENDIX A (continued)

26. What is most ***difficult*** for you now?

_____

_____

_____

_____

_____

27. What is most ***helpful*** for you now?

_____

_____

_____

_____

_____

APPENDIX A (continued)

Family Hardiness Index (FHI) Questionnaire

PEPS FORM: Family Hardiness Index Questionnaire

Participant's ID#: _____ Participant's Name:_____

Date: _____/_____/_____ Nurse Name:_____

Note: This should be a SELF-ADMINISTERED survey if possible. Please check–off method of administration:

☐ Self-Administered ☐ Nurse-Administered

DIRECTIONS: Please read each statement below and decide to what degree each describes your family. Is the statemen **False (0), Mostly False (1), Mostly True (2), or True (3)** about your family? Circle a number 0 to 3 to match your feeli about each statement. Please respond to each and every statement.

In our family…	False	Mostly False	Mostly True	True
1) Trouble results from mistakes we make.	0	1	2	3
2) It is not wise to plan ahead and hope because things do not turn out anyway.	0	1	2	3
3) Our work and efforts are not appreciated no matter how hard we try and work.	0	1	2	3
4) In the long run, the bad things that happen to us are balanced by the good things that happen.	0	1	2	3
5) We have a sense of being strong even when we face big problems.	0	1	2	3
6) Many times I feel I can trust that even in difficult times things will work out.	0	1	2	3
7) While we don't always agree, we can count on each other to stand by us in times of need.	0	1	2	3
8) We do not feel we can survive if another problem hits us.	0	1	2	3
9) We believe that things will work out for the better if we work together as a family.	0	1	2	3
10) Life seems dull and meaningless.	0	1	2	3
11) We strive together and help each other no matter what.	0	1	2	3
12) When our family plans activities we try new and exciting things.	0	1	2	3
13) We listen to each other's problems, hurts, and fears.	0	1	2	3
14) We tend to do the same things over and over…it's boring.	0	1	2	3
15) We seem to encourage each other to try new things and experiences.	0	1	2	3
16) It is better to stay at home than go out and do things with others.	0	1	2	3
17) Being active and learning new things are encouraged.	0	1	2	3
18) We work together to solve problems.	0	1	2	3
19) Most of the unhappy things that happen are due to bad luck.	0	1	2	3
20) We realize our lives are controlled by accidents and luck.	0	1	2	3

APPENDIX A (continued)

Family Crisis Oriented Personal Evaluation Scales (F-COPES) Survey

PEPS FORM: Family Crisis Oriented Personal Evaluation Scales

Participant's ID#: _____ Participant's Name:_____

Date: _____/_____/_____ Nurse Name:_____

Note: This should be a SELF-ADMINISTERED survey if possible. Please check–off method of administration:

☐ Self-Administered ☐ Nurse-Administered

DIRECTIONS: First, read the list of "Response Choices" one at a time. Second, decide how well each statement describes your attitudes and behavior in response to problems or difficulties. If the statement describes your response very well, then circle the number 5 indicating that you strongly agree; if the statement does not describe your response at all, then circle the number 1 indicating that you strongly disagree; if the statement describes your response to some degree, then select a number 2, 3, or 4 to indicate how much you agree or disagree with the statement about your response. Please circle a number (1, 2, 3, 4, or 5) to match your response to each statement. Thank you.

When we face problems or difficulties in our family, we respond by:	Strongly Disagree	Moderately Disagree	Neither Agree Nor Disagree	Moderately Agree	Strongly Agree
1) Sharing our difficulties with relatives.	1	2	3	4	5
2) Seeking encouragement and support from friends.	1	2	3	4	5
3) Knowing we have the power to solve major problems.	1	2	3	4	5
4) Seeking information and advice from persons in other families who have faced the same or similar problems.	1	2	3	4	5
5) Seeking advice from relatives (grandparents, etc.).	1	2	3	4	5
6) Seeking assistance from community agencies and programs designed to help families in our situation.	1	2	3	4	5
7) Knowing that we have the strength within our own family to solve our problems.	1	2	3	4	5
8) Receiving gifts and favors from neighbors (e.g. food, taking in mail, etc.).	1	2	3	4	5
9) Seeking information and advice from the family doctor.	1	2	3	4	5
10) Asking neighbors for favors and assistance.	1	2	3	4	5
11) Facing the problems "head-on" and trying to get solution right away.	1	2	3	4	5
12) Watching television.	1	2	3	4	5
13) Showing that we are strong.	1	2	3	4	5
14) Attending church services.	1	2	3	4	5
15) Accepting stressful events as a fact of life.	1	2	3	4	5
16) Sharing concerns with close friends.	1	2	3	4	5

Please continue on next page →

APPENDIX A (continued)

When we face problems or difficulties in our family, we respond by:	Strongly Disagree	Moderately Disagree	Neither Agree Nor Disagree	Moderately Agree	Strongly Agree
17) Knowing luck plays a big part in how well we are able to solve family problems.	1	2	3	4	5
18) Exercising with friends to stay fit and reduce tension.	1	2	3	4	5
19) Accepting that difficulties occur unexpectedly.	1	2	3	4	5
20) Doing things with relatives (get-togethers, dinners, etc.).	1	2	3	4	5
21) Seeking professional counseling and help for family difficulties.	1	2	3	4	5
22) Believing we can handle our own problems.	1	2	3	4	5
23) Participating in church activities.	1	2	3	4	5
24) Defining the family problem in a more positive way so that we do not become too discouraged.	1	2	3	4	5
25) Asking relatives how they feel about problems we face.	1	2	3	4	5
26) Feeling that no matter what we do to prepare, we still have difficulty handling problems.	1	2	3	4	5
27) Seeking advice from a minister.	1	2	3	4	5
28) Believing if we wait long enough, the problem will go away.	1	2	3	4	5
29) Sharing problems with neighbors.	1	2	3	4	5
30) Having faith in God.	1	2	3	4	5

APPENDIX B

IRB

Notice of Original Approval

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office of the Vice Chancellor for Research (MC 672)
210 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

NOTICE OF APPROVALFOR AN
EXPERIMENTALPROJECT
ON HUMAN SUBJECTS

INSTITUTIONALREVIEWBOARD

Board #1
IRBNO: H-98-655

Project Title: Peer Education in Pregnancy Study

Principal Investigator: Victoria Persky,
Noel Chavez, Sally Freels
Department: Epi Bio

Sponsoring Agency:
NIEHS
Grant or Contract No:

Campus: Chicago/W

Approved: 08/07/98
Renew: 08/99; 08/00
Resubmit: 08/01

New X

The above research project has been reviewed by the Institutional Review Board. The Board approved, as appropriate and ethical:

(1) the procedures to be used to protect the rights and welfare of the human subjects involved;
(2) the method(s) for obtaining informed consent of the participants.

The Board concurs that the risks to the human subjects involved are consonant with the potential benefit of the knowledge to be derived.

It is the investigator's responsibility to note and abide by the dates specified above. Approval of a protocol which is not renewed or which has been active three (3) years is automatically terminated. The investigator has the individual responsibility for securing the above rights and consent for using procedures that involve minimum risk. The investigator is to be guided in his or her conduct by The NIH OPRR Protecting Human Research Subjects IRB Guidebook available in the UIC Office for Protection from Research Risks. It is understood that review of this experimental project for conformity with policy regarding use of human subjects will be the responsibility of the following members of the Departmental Review and Surveillance Committee:

Fred Kviz Judith Munson Michele Issel

The Institutional Review Board trusts that all concerned parties fulfill the responsibilities which they have accepted, and that they will notify the chairman of the board of any unanticipated problems involving risks to the subjects or any changes in the project.

Comments:

Larry Danzig
Chairman, Institutional Review Board Date
08/07/98

UIC

aprr/jal
cc:

Phone (312) 996-4995

APPENDIX B (continued)

Most Recent Continuing Review (through June 4, 2015)

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

May 9, 2014

Victoria W. Persky, MD, MPH
Epidemiology and Biostatistics
1603 W. Taylor Street
878-A S.P.H.P.I., M/C 923
Chicago, IL 60612-4394
Phone: (312) 996-4783 / Fax: (312) 996-0064

RE: **Protocol # 1998-0655**
"Peer Education in Pregnancy Study"

Dear Dr. Persky:

Your Continuing Review was reviewed and approved by the Expedited review process on May 8, 2014. You may now continue your research.

Please note the following information about your approved research protocol:

Please note that Investigator's training for Shannon Brunner expired May 3, 2014 and 2 hours of continuing education are needed for them to continue in the research. Please see the link below for more information regarding UIC investigator training policies: http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/education/continuing.shtml

Please note that the following personnel should be removed from Appendix P -Lenore Coover, Linda McCauley, Peter Thorne, Dennis Ownby, Cynthia Wagner, Silva Gutierrez, and Juan Muniz. They were removed from the study 2/26/14 because their training had expired.

Protocol Approval Period: June 4, 2014 - June 4, 2015
Approved Subject Enrollment #: 720 (Limited to data analysis only from 483 subjects)
Additional Determinations for Research Involving Minors: The Board determined that this research satisfies 45CFR46.404, research not involving greater than minimal risk.
Performance Sites: UIC, Lawndale Christian Health Center, Erie Family Health Center, Chicago Commons (Formerly Emerson House), Family Focus - Lawndale, Teen Health Center Westside
Sponsor: NIEHS - National Institute of Environmental Health Sciences
PAF#: Not applicable

APPENDIX B (continued)

1998-0655 Page 2 of 3 May 9, 2014

Grant/Contract No: 1 R21 ES08716-03
Grant/Contract Title: Community Based Asthma Intervention in Pregnant Women

Research Protocol:

a) Research Protocol: Peer Education in Pregnancy Study; 07/02/2009

Recruitment Material:

a) N/A- Limited to data analysis only

Informed Consent:

a) N/A- Limited to data analysis only

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b) (1) under the following specific category:

(9) Continuing review of research, not conducted under an investigational new drug application or investigational device exemption where categories two (2) through eight (8) do not apply but IRB has determined and documented at a convened meeting that the research involves no greater than minimal risk and no additional risks have been identified.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
05/05/2014	Continuing Review	Expedited	05/08/2014	Approved

Please remember to:

→ Use your **research protocol number** (1998-0655) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the right to seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 355-2764. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

APPENDIX B (continued)

Sincerely,

Betty Mayberry, B.S.
IRB Coordinator, IRB # 2
Office for the Protection of Research Subjects

Enclosure: None

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923
OVCR Administration, M/C 672
Privacy Office, Health Information Management Department, M/C 772

APPENDIX B (continued)

Amendment for Analyzing Maternal Serum Samples

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Amendment to Research Protocol and/or Consent Document – Expedited Review
UIC Amendment # 38

March 17, 2014

Victoria W. Persky, MD, MPH
Epidemiology and Biostatistics
1603 W. Taylor Street
878-A S.P.H.P.I., M/C 923
Chicago, IL 60612-4394
Phone: (312) 996-4783 / Fax: (312) 996-0064

RE: Protocol # 1998-0655
"Peer Education in Pregnancy Study"

Dear Dr. Persky:

Please note that Cynthia Wagner-Casanova has been removed as key research personnel due to expired investigator training.

Please note that the research training for ***Shannon Brunner*** **will** expire on **05/03/2014** and she must complete a minimum of two hours of continuing education prior to the expiration date in order to continue to participate in the conduct of the research. You may refer her to the OPRS website, where continuing education offerings are available:
http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/education/2-2-2/ce_requirements.shtml

Members of Institutional Review Board (IRB) #2 have reviewed this amendment to your research and/or consent form under expedited procedures for minor changes to previously approved research allowed by Federal regulations [45 CFR 46.110(b)(2)]. The amendment to your research was determined to be acceptable and may now be implemented.

Please note the following information about your approved amendment:

Amendment Approval Date: March 10, 2014
Amendment:
Summary: UIC Amendment #38, dated 19 February 2014 and submitted to OPRS 20 February 2014, is an investigator-initiated amendment requesting approval to analyze stored serum samples drawn from pregnant participants early in the study; serum samples contain ID

APPENDIX B (continued)

numbers and date of collection; the analysis of the biologic samples for risk factors for asthma was part of the original plan of study as reflected in the protocol and attached consent document (PEPS Participation Consent, v4, 7/29/2003); departmental funding has been obtained to test the samples for c-reactive protein levels, which is a marker of inflammation; higher levels of c-reactive protein have been associated with asthma and investigators hypothesize that the levels in the samples may be an indicator for subsequent asthma development in the child.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
02/20/2014	Amendment	Expedited	02/27/2014	Modifications Required
03/04/2014	Response To Modifications	Expedited	03/10/2014	Approved

Please be sure to:
→ **Use only the IRB-approved and stamped consent document(s) and/or HIPAA Authorization form(s) enclosed with this letter when enrolling subjects.**

→ Use your research protocol number (1998-0655) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"UIC Investigator Responsibilities, Protection of Human Research Subjects"
(http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB #2 has the right to ask further questions, seek additional information, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the OPRS at (312) 996-1711 or me at (312) 355-2939. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jewell Hamilton, MSW
IRB Coordinator, IRB # 2
Office for the Protection of Research Subjects

Enclosure(s): None

cc: Ronald C. Hershow, Epidemiology and Biostatistics, M/C 923
Privacy Office, Health Information Management Department, M/C 772

CITED LITERATURE

Aagaard, K., J. Ma, K. M. Antony, R. Ganu, J. Petrosino, and J. Versalovic. 2014. "The placenta harbors a unique microbiome." *Sci Transl Med* 6 (237):237ra65. doi: 10.1126/scitranslmed.3008599.

Abdou, C. M., T. P. Dominguez, and H. F. Myers. 2013. "Maternal familism predicts birthweight and asthma symptoms three years later." *Soc Sci Med* 76 (1):28–38. doi: 10.1016/j.socscimed.2012.07.041.

Adlerberth, I., E. Lindberg, N. Aberg, B. Hesselmar, R. Saalman, I. L. Strannegard, and A. E. Wold. 2006. "Reduced enterobacterial and increased staphylococcal colonization of the infantile bowel: An effect of hygienic lifestyle?" *Pediatr Res* 59 (1):96–101. doi: 10.1203/01.pdr.0000191137.12774.b2.

Alegria, M., P. E. Shrout, M. Woo, P. Guarnaccia, W. Sribney, D. Vila, A. Polo, Z. Cao, N. Mulvaney-Day, M. Torres et al. 2007. "Understanding differences in past year psychiatric disorders for Latinos living in the US." *Soc Sci Med* 65 (2):214–30. doi: 10.1016/j.socscimed.2007.03.026.

Arif, A. A., G. L. Delclos, and J. Colmer-Hamood. 2007. "Association between asthma, asthma symptoms and C-reactive protein in US adults: Data from the National Health and Nutrition Examination Survey, 1999–2002." *Respirology* 12 (5):675–82. doi: 10.1111/j.1440-1843.2007.01122.x.

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