

Prematurity, chorioamnionitis, and the development of recurrent wheezing: A prospective birth cohort study

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Background: Prematurity (<37 weeks) has been inconsistently associated with asthma and wheezing. Chorioamnionitis may promote both prematurity and inflammatory pathways in infants' airways.

Objective: To investigate the relationship of prematurity and chorioamnionitis with the development of early childhood recurrent wheezing.

Methods: The Boston Birth Cohort (n = 1096) were followed prospectively from birth to a mean age of 2.2 ± 2 years. Perinatal and postnatal clinical data and placental pathology were collected. The primary outcome was recurrent wheezing (≥ 2 physician documented episodes). Secondary outcomes included physician-diagnosed asthma, food allergy, and eczema. Preterm children were grouped by gestational age into moderately (33-36.9 weeks) and very preterm (<33 weeks) with and without chorioamnionitis, and compared with term children without chorioamnionitis (reference group). Chorioamnionitis was diagnosed either by intrapartum fever or by placental histology findings. Logistic regression models were performed to investigate the independent and joint associations of degree of prematurity and chorioamnionitis.

Results: Prematurity was associated with recurrent wheezing (odds ratio [OR], 1.7; 95% CI, 1.2-2.6). However, when subjects were grouped by degree of prematurity with or without chorioamnionitis, the highest risk of wheezing (OR, 4.0; 95% CI, 2.0-8.0) and physician-diagnosed asthma (OR, 4.4; 95% CI, 2.2-8.7) was present in the very preterm children with chorioamnionitis. The effect on both wheezing (OR, 5.4; 95% CI, 2.4-12.0) and asthma (OR, 5.2; 95% CI, 2.3-11.9) was greater in African Americans. Neither prematurity nor chorioamnionitis was associated with food allergy or eczema.

Conclusion: We found a strong joint effect of prematurity and chorioamnionitis on early childhood wheezing. This effect was stronger in African American subjects. (J Allergy Clin Immunol 2008;122:1001-1008.)

Key words: Chorioamnionitis, prematurity, recurrent wheezing

A limited number of studies have explored the role of prenatal factors on the development of respiratory morbidity or later asthma.¹⁻⁵ At least 1 study suggests that either programming *in utero* or immunologic changes determined *in utero* affect the risk for respiratory diseases.⁶ Given that a significant proportion of patients who develop asthma in childhood wheezed in early life,⁷ investigation of prenatal and early life factors is important to understand better the etiology of recurrent wheezing and possibly later development of asthma.

In this context, we chose to evaluate the role of prematurity and associated chorioamnionitis on recurrent wheezing in early life. Preterm births (<37 weeks gestation) account for 12.5% of all births in the United States, and prematurity has been identified as a risk factor for asthma in many⁸⁻¹¹ but not all studies.^{12,13} A major limitation of previous studies, which may have led to inconsistent findings, is that they did not consider the underlying pathogenesis of preterm birth. A great proportion of high-risk preterm births is associated with chorioamnionitis.¹⁴⁻¹⁶ In this regard, febrile episodes in pregnancy (especially the third trimester) have also been associated with subsequent development of asthma in offspring.^{17,18} Chorioamnionitis as a potential risk factor for wheezing illness becomes even more important because, like asthma,^{19,20} prematurity and chorioamnionitis disproportionately affect the African American community (Institute of Medicine [IOM] report 2006, www.iom.edu). This comorbid epidemic has not been evaluated as a contributor to asthma health disparities.

The purpose of this study was to evaluate the association between prematurity and wheezing accounting for the presence of perinatal chorioamnionitis using subjects enrolled in the Boston Birth Cohort, a large-scale, multiethnic, inner-city population-based study,²¹ which includes both term and preterm children.

METHODS

Patient population

The Boston Birth Cohort was initiated in 1998, and recruitment is ongoing at the Boston Medical Center (BMC). It consists of mother-infant pairs enrolled from a range of socioeconomic strata that includes inner-city poor up to middle-class subjects. It is a multiethnic population, and the 3 major racial groups are black (56%), white (11%), and Hispanic (20%).

The Boston Birth Cohort was originally designed to study adverse birth outcomes, particularly preterm birth and intrauterine fetal growth restriction. The inclusion and exclusion criteria of the parent study are as follows: any

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Abbreviations used

BMC: Boston Medical Center
IOM: Institute of Medicine
IRB: Institutional Review Board
OR: Odds ratio

woman admitted to the Labor and Delivery floor at BMC who delivered a singleton live infant and met our case (gestational age <37 weeks or birth weight <2500 g) or control (gestational age ≥37 weeks with birth weight ≥2500 g) criteria was eligible.²¹ For the purposes of this follow-up study, preterm status was solely determined by gestational age, not by case status as defined in the parent study. A small percentage of women (<1%) with pregnancies that involved *in vitro* fertilization, multiple gestation, chromosomal abnormalities, or major birth defects, or women with congenital or acquired uterine lesions or incompetent cervix were excluded. The Children's Memorial Hospital Institutional Review Board (IRB), the BMC IRB, and the Massachusetts Department of Public Health approved the parent study protocol. Under a separate Children's Memorial Hospital and BMC IRB-approved study protocol and consent process, all infants enrolled in the Boston Birth Cohort are eligible for the postnatal follow-up study.

Data collection and measurements

Recruitment of the birth cohort. For the parent study, mother-infant pairs were recruited 24 to 48 hours postdelivery. After obtaining signed written informed consent, we interviewed subjects by using a standardized questionnaire to gather epidemiologic data. We also reviewed maternal and infant medical records by using a standardized abstraction form to obtain clinical data including ultrasound findings, placental pathology reports, laboratory reports, pregnancy complications, labor and delivery course, and birth outcomes.

Postnatal follow-up study. In the postnatal follow-up study, after written informed consent was obtained from the biological mother, visits were conducted at 6 to 12 months, 2 years, 4 years, and 6 years in alignment with the child's pediatric primary care visit schedule. Mothers were interviewed by using standardized postnatal health questionnaires to gather important postnatal epidemiologic data. We also reviewed the study child's medical record by using a standard medical record abstraction form to obtain clinical data including type and date of visits, clinical diagnosis including International Classification of Diseases, 9th Revision codes, and growth parameters.

Assessment of gestational age. Precise estimation of gestational age is essential for defining degree of prematurity. In this cohort, gestational age was assessed on the basis of both the first day of the last menstrual period as recorded in the maternal medical record and early (<20 weeks) prenatal ultrasound. This approach has been used in large hospital-based preterm studies and in our ongoing funded preterm studies.²¹

Determination of presence of chorioamnionitis. For this study, chorioamnionitis was considered present if the pregnancy was associated with either the presence of intrapartum fever (>38°C)²² based on review of medical record or by the presence of placental histological changes associated with chorioamnionitis. A detailed description of placental pathology criteria has been published by our group elsewhere.²³ Briefly, all placentas sent for pathology were reviewed by the hospital's perinatal pathologist, and a subset was independently reviewed by a second pathologist to confirm reliability. The presence and location of inflammation were reported by using well established algorithms. For example, types of inflammatory cells were identified and reported to designate acute versus chronic inflammation, and the location of inflammatory infiltrate was denoted in these reports to specify decidual, chorionic, amnionic, chorionic plate, and umbilical cord involvement. These guidelines for histologic reporting were consistent with recommendations developed by the College of American Pathologists and have been used consistently in the literature to classify types of placental infection.^{24,25}

Outcome measures

The primary outcome was recurrent wheezing (≥2 episodes of physician-documented wheezing). Secondary outcomes included physician diagnosis of

asthma, eczema, and/or food allergy. These outcomes were determined by standardized abstraction of the medical record. The follow-up of these children and determination of the outcomes were greatly facilitated by the fact that approximately 60% of subjects who are recruited in the Boston Birth Cohort continue pediatric primary care at BMC, and 38% continue at a BMC-affiliated neighborhood health center. The availability of a unified electronic medical record allows for abstraction of data with efficiency and accuracy.

Statistical analyses

We used a χ^2 test to compare the baseline characteristics of term and preterm children in terms of demographic measures, socioeconomic status measures, and maternal and *in utero* risk factors and complications of pregnancy. We also performed this analysis comparing children whose mothers did and did not meet our criteria for chorioamnionitis.

Potential confounding variables included infant and maternal factors. Infant factors included child's current age and sex, breast-feeding, infection during pregnancy, preterm delivery, postnatal passive smoking, and type of delivery. Maternal factors included maternal ethnicity, maternal smoking during pregnancy, maternal asthma and maternal atopy, stress during pregnancy, parity, and SES as measured by highest level of maternal education achieved. To determine the best fitted and most parsimonious model, we performed a 2-step process. First, certain variables that have been strongly associated with early childhood wheezing in the literature were retained in the model regardless of significance. These included tobacco smoke exposure *in utero*, postnatal passive smoke exposure, breast-feeding, maternal asthma, and the child's age. This resulted in 3 variables that were not statistically significant being kept in the model (intrapartum antibiotics, breast-feeding, and postnatal passive smoking). Second, the other variables listed were retained in the model depending on the level of significance in the bivariate analyses. A backward stepwise regression model, forcing inclusion of the covariates thought to be important from the literature, was used as a tool to assist in this selection process.

This process resulted in the inclusion of the following covariates: child's age and sex, postnatal passive smoke exposure, maternal ethnicity, presence of maternal atopic diseases, maternal education, maternal smoking postnatally, intrapartum antibiotics, and breast-feeding status. Of note, all variables that differed between those with and without chorioamnionitis (delivery type, antibiotic use, and gestational age) were accounted for in our models. (Gestational age group was used to stratify the comparison groups, and the other 2 variables were included in the model.)

We performed an initial logistic regression analysis to evaluate the effects of prematurity overall (<37 weeks gestation) on the outcomes, with adjustment for chorioamnionitis and the other covariates listed. We further evaluated how the variables of degree of prematurity and chorioamnionitis jointly affect the risk of early childhood wheezing. By using term pregnancies (≥37 weeks) without chorioamnionitis as the reference group, the moderate preterm (33-36.9 weeks) and very preterm (<33 weeks) were further stratified by the presence of chorioamnionitis. Finally, we performed race-specific analysis to determine whether these associations were consistent in African American children. For this subgroup analysis, we removed the variable of maternal ethnicity.

Two other secondary analyses were performed by using the same covariates. The first divided the deliveries into spontaneous and medically indicated deliveries to evaluate the role of other perinatal morbidities on wheezing. The other secondary analysis evaluated the joint effect of fever and placental inflammation on the odds of recurrent wheezing or asthma. This was performed to evaluate the relative effect of these 2 variables, which were used to construct the chorioamnionitis variable.

All analyses were performed by using statistical software SAS for Windows 8.20 (SAS Institute, NC).

RESULTS

This analysis included 771 term and 325 preterm infants enrolled from the Boston Birth Cohort who completed of at least 1 postnatal follow-up study visit. Recruitment from the Boston

Birth Cohort to the follow-up study has been approximately 90% of those approached. No differences in key variables including birth weight, gestation, and prevalence of chorioamnionitis have been found from those infants yet to be enrolled in the follow-up study (data not shown).

The mean age of children at the point of last follow-up was 2.2 ± 2.0 years. Baseline characteristics of the study children, including demographic measures, SES measures, and maternal/*in utero* factors, are displayed in Table I, stratified by term and preterm birth. Compared with the term children, preterm children had higher frequencies of maternal cigarette smoking during pregnancy, cesarean sections, prenatal antibiotic and steroid use, and chorioamnionitis as well as other pregnancy complications. Preterm children also had a higher frequency of wheezing (19.7 % vs 10.2% in term infants) and a slightly higher prevalence of maternal asthma (15.7 vs 11.2 in term infants). We also compared subjects with and without chorioamnionitis in this article's Table E1 in the Online Repository at www.jacionline.org. Subjects with chorioamnionitis were similar to those without chorioamnionitis in demographic variables, SES variables, and maternal *in utero* factors, but had lower mean gestational age, more frequent delivery by cesarean section, and a higher frequency of antibiotic use.

This article's Table E2 in the Online Repository at www.jacionline.org displays the results of a stepwise logistic regression to evaluate major covariates, in addition to prematurity and chorioamnionitis, for the outcomes of interest. We evaluated the association of prematurity overall (<37 weeks gestational age) with recurrent wheezing. We found that prematurity was associated with increased risk of recurrent wheezing (odds ratio [OR], 1.7; 95% CI, 1.2-2.6), even after adjusting for child's age, sex, breast-feeding, postnatal exposure to maternal cigarette smoking, and chorioamnionitis as well as maternal ethnicity, education, prenatal smoke exposure, and history of atopy. In contrast, prematurity did not seem to increase the risk of eczema (OR, 0.6; 95% CI, 0.5-0.9) or food allergy (OR, 0.8; 95% CI, 0.4-1.5).

We further evaluated the joint association of degree of prematurity and presence of chorioamnionitis with the outcomes of interest as shown in Table II. Our data suggest a joint effect when gestational age and chorioamnionitis are combined. With the exception of the moderately preterm group, children with chorioamnionitis had an increased risk of recurrent wheezing compared with term children without chorioamnionitis. Of note, the moderately preterm group with chorioamnionitis had smaller numbers of subjects. Children who were very preterm and had chorioamnionitis had the highest risk of recurrent wheezing (OR, 4.0; 95% CI, 2.0-8.0). Similarly, in this group, the likelihood of physician-diagnosed asthma was increased (OR, 4.4; 95% CI, 2.2-8.7). Neither prematurity nor chorioamnionitis were associated with an increased risk of food allergy or eczema. There was no evidence of a statistically significant effect modification of chorioamnionitis on the associations of prematurity with the outcomes studied. Specifically, this is evidence of a joint effect of these variables, but not an interaction.

Finally, we evaluated if these associations were consistently observed if restricted to African American children. As shown in Table III, we found a similar association greater in magnitude than that seen in the analysis of all the study subjects. For example, African American children who were very preterm with chorioamnionitis had a 5.4-fold increased risk of recurrent wheezing (OR, 5.4; 95% CI, 2.4-12.0) and a 5.2-fold increased risk of physician-diagnosed asthma (OR, 5.2; 95% CI, 2.3-11.9). Again,

TABLE I. Baseline characteristics of Boston birth cohort by gestational age

Characteristic	Term	Preterm	P value
N	771	325	
Demographic measures			
Sex male (%)	384 (49.8)	158 (48.6)	.72
Age (y), mean $\pm$ SD	2.2 $\pm$ 2.0	2.2 $\pm$ 2.0	.74
Overall age (y), mean $\pm$ SD	2.2 $\pm$ 2.0		
Age <3 y (%)	467 (60.6)	195 (60.0)	.86
Age $\geq$ 3 y	304 (39.4)	130 (40.0)	
Maternal race/ethnicity (%)			
African American	477 (61.9)	206 (63.4)	.392
White	47 (6.1)	26 (8.0)	
Hispanic	154 (20.0)	65 (20.0)	
Asian/Pacific Islander	18 (2.3)	4 (1.2)	
Other	75 (9.7)	24 (7.4)	
SES measures			
Annual household income (%)			
<\$15,000	226 (47.2)	107 (52.7)	.40
\$15,000 to \$30,000	164 (34.2)	64 (31.5)	
$\geq$ \$30,000	89 (18.6)	32 (15.8)	
Maternal education level (%)			
<High school	230 (29.8)	103 (31.7)	.53
High school	267 (34.6)	118 (36.3)	
>High school	274 (35.5)	104 (32.0)	
Maternal/ <i>in utero</i> factors			
Maternal ever smoking (%)	101 (13.1)	76 (23.4)	<.001
Postnatal passive smoking (%)	141 (18.3)	90 (27.7)	<.001
Maternal asthma (%)	86 (11.2)	51 (15.7)	.04
Any maternal atopic disease (%)	204 (26.5)	102 (31.4)	.10
High stress during pregnancy (%)	152 (19.7)	64 (19.7)	.99
Breast-feed (%)			
Breast only	46 (6.0)	26 (8.0)	.08
Bottle only	122 (15.8)	66 (20.3)	
Both	603 (78.2)	233 (71.7)	
Delivery by cesarean section (%)	226 (29.3)	132 (40.6)	<.001
First child (nulliparity) (%)	319 (41.4)	144 (44.3)	.43
Placental evidence of chorioamnionitis (%)	88 (11.4)	84 (25.8)	<.001
Intrapartum antibiotic use (%)	107 (13.9)	104 (32.2)	<.001
Steroid use (%)	3 (0.4)	109 (33.5)	<.001
Complications during pregnancy* (%)	82 (10.6)	100 (30.8)	<.001
$\geq$ 2 Episodes of wheezing (%)	79 (10.2)	64 (19.7)	<.001

*Complications during pregnancy include chronic hypertension; pre-eclampsia; eclampsia; hemolysis, elevated liver enzymes, and low platelets syndrome; diabetes (gestational diabetes); placental abruption; or incompetent cervix.

neither prematurity nor chorioamnionitis was associated with food allergy or eczema.

As a secondary analysis, deliveries were divided into spontaneous births and medically indicated deliveries to evaluate whether other perinatal morbidities confounded the effect of chorioamnionitis on wheezing. As expected, both hypertensive disorders (including pre-eclampsia; eclampsia; hemolysis, elevated liver enzymes, and low platelets syndrome) and other morbidities (including gestational diabetes, abruption placenta, placenta previa, or incompetent cervix) were more common in medically indicated deliveries (see this article's Table E3 in the Online Repository at www.jacionline.org). However, the relationships of wheezing and asthma with chorioamnionitis and prematurity persisted despite stratification by type of delivery (see this article's Table E4 in the Online Repository at www.jacionline.org).

TABLE II. The joint association of degree of prematurity with chorioamnionitis and recurrent wheezing, asthma, eczema, and food allergy

Chorioamnionitis	Degree of prematurity (weeks gestation)	Total, N	Case, %	OR (95% CI)	P value
Wheezing*					
No	≥37	683	9.2	1.0	—
	33-36.9	178	15.7	1.7 (1.0-2.9)	.03
	<33	63	22.2	2.7 (1.3-5.5)	.005
Yes	≥37	88	18.2	2.0 (1.1-3.8)	.03
	33-36.9	32	15.6	1.3 (0.4-3.6)	.67
	<33	52	32.7	4.0 (2.0-8.0)	.0001
Asthma					
No	≥37	683	12.9	1.0	—
	33-36.9	179	13.5	1.0 (0.6-1.7)	.95
	<33	63	19	1.6 (0.8-3.5)	.18
Yes	≥37	88	17.0	1.2 (0.6-2.2)	.67
	33-36.9	32	21.9	1.4 (0.6-3.6)	.47
	<33	52	42.3	4.4 (2.2-8.7)	3.2×10^{-5}
Eczema					
No	≥37	683	32.1	1.0	—
	33-36.9	178	23.6	0.6 (0.4-0.9)	.02
	<33	63	23.8	0.7 (0.4-1.2)	.20
Yes	≥37	88	43.2	1.5 (0.9-2.4)	.07
	33-36.9	32	25.0	0.6 (0.3-1.4)	.24
	<33	52	37.2	0.9 (0.5-1.7)	.74
Food allergy					
No	≥37	683	6.3	1.0	—
	33-36.9	178	5.6	0.8 (0.4-1.7)	.55
	<33	63	3.2	0.4 (0.1-1.9)	.25
Yes	≥37	88	5.7	0.7 (0.3-2.0)	.53
	33-36.9	32	3.1	0.4 (0.1-2.8)	.34
	<33	52	5.8	0.8 (0.2-2.7)	.67

Covariates adjusted in the model: infant factors: child's age and sex, breast-feeding, postnatal passive smoking; maternal factors: maternal ethnicity, maternal atopic diseases, maternal smoking during pregnancy, antibiotics, and maternal educational status.

*Wheezing: episodes of wheezing ≥2.

TABLE III. The joint association of degree of prematurity and chorioamnionitis with recurrent wheezing and asthma among African American children

Chorioamnionitis	Degree of prematurity (weeks gestation)	Total, N	Case, %	OR (95% CI)	P value
Wheezing*					
No	≥37	423	10.4	1.0	—
	33-36.9	103	16.5	1.7 (0.9-3.3)	.10
	<33	43	30.2	3.8 (1.7-8.5)	.0009
Yes	≥37	54	20.4	2.0 (0.9-4.3)	.09
	33-36.9	23	21.7	1.8 (0.6-5.3)	.31
	<33	37	42.3	5.4 (2.4-12.0)	4.6×10^{-5}
Asthma					
No	≥37	423	14.7	1.0	—
	33-36.9	103	13.6	0.9 (0.5-1.8)	.74
	<33	43	23.3	1.9 (0.8-4.4)	.45
Yes	≥37	54	22.2	1.3 (0.6-2.9)	.45
	33-36.9	23	30.4	2.0 (0.7-5.7)	.18
	<33	37	51.4	5.2 (2.3-11.9)	.0001

Covariates adjusted in the model: infant factors: child's age and sex, breast-feeding, postnatal passive smoking; maternal factors: maternal atopic diseases, maternal smoking during pregnancy, antibiotics, and maternal educational status.

*Wheezing: episodes of wheezing ≥2.

Finally, for this analysis, a total of 36 children had chorioamnionitis determined by maternal fever alone, 106 children had chorioamnionitis defined by placental histopathology alone (sub-clinical chorioamnionitis), and 30 children had chorioamnionitis determined by both. We further examined this classification in

2 ways. First, we evaluated the proportion of elevated white counts at delivery by these variables. All the groups that would have been identified as chorioamnionitis in our main analysis had a higher proportion of elevated white counts at delivery (>15,000 by Gibbs criteria)²² than individuals who did not meet our criteria

of chorioamnionitis (see this article's Table E5 in the Online Repository at www.jacionline.org). Second, we performed a subgroup analysis by using groupings defined by maternal fever and placental pathology in preterm infants with available data on both placental pathology and peripartum fever. As noted in this article's Table E6 in the Online Repository at www.jacionline.org, those with placental inflammation and no fever showed higher risk of asthma compared with those without inflammation and fever. A similar trend was observed for the outcome of recurrent wheezing but was not significant because of the smaller sample size in the subgroup analysis. The group at highest risk was those with both inflammation and fever. Our data indicates that placental inflammation appears to be a more sensitive and robust risk factor for wheezing or asthma than fever alone.

DISCUSSION

In utero events can affect early life immune responses^{26,27} and the development of atopic diseases in the offspring²⁻⁵ and can even result in epigenetic effects on subsequent generations.⁶ To date, no prospective studies have examined the joint effect of prematurity and chorioamnionitis in relation to the development of respiratory morbidity and atopic diseases in early childhood. In this study, we found that although prematurity was associated with recurrent wheezing in this cohort of children in the first few years of life, this association was greatly augmented by the severity of prematurity and presence of chorioamnionitis. The children who were very preterm and had chorioamnionitis had the greatest risk of recurrent wheezing. Furthermore, this association was much stronger in African American children. Our findings provide new information to a long-standing clinical and public health challenge—that is, increased risk of respiratory morbidities in African American children.

There were a number of features of our cohort that allowed us to carry this study out. We performed a thorough evaluation of prenatal factors, including placental pathology, which provided a very sensitive indicator of intrauterine infection or inflammation. Ours is one of the few prospective studies with large enough numbers to allow stratification by severity of prematurity and presence of chorioamnionitis. Our study also has significant minority representation, with a high frequency of African American subjects. Like asthma,^{28,29} prematurity and chorioamnionitis disproportionately affect the African American community (IOM report 2006, www.iom.edu). Our findings underscore the importance of examining the role of *in utero* factors on the later development of respiratory morbidities. They also raise questions about whether the persistent and high prevalence of prematurity and chorioamnionitis, especially in African Americans, is linked in a causal fashion to the asthma epidemic and ethnic disparity in respiratory outcomes.

Caution is needed in interpreting our findings because of a number of potential limitations. Although we have shown associations with physician diagnosed asthma, we focused on recurrent wheezing given the average age of the children (around 2 years). It would be important to evaluate whether the associations persist as we continue to follow these children and whether increased rates of recurrent wheezing correlate with increased rates of asthma diagnoses at later ages. We were not able to follow all of the patients in the initial cohort because recruitment into the follow-up cohort is ongoing. Subjects already enrolled in the follow-up study showed no differences from those not yet

recruited in key variables including birth weight, gestation, and percentage of the cohort with chorioamnionitis.

This study focused on 2 prominent prenatal factors (prematurity and chorioamnionitis). Although our analyses attempted to adjust for many potential covariates, we need to recognize that there are many other important prenatal factors that could affect the associations, such as intrapartum antibiotics and mode of delivery. Intrapartum antibiotics and mode of delivery can affect types of commensal organisms present in the mother³⁰ and infant³¹ and atopic disease prevalence.³²⁻³⁴ However, we do not think mode of delivery or intrapartum use of antibiotics influenced our findings; intrapartum antibiotics was included as a covariate in our analyses. Also, modification of the commensal flora by antibiotics or mode of delivery should affect allergic diseases in general, not just wheezing, because a significant proportion of the diagnoses of chorioamnionitis was made by histological diagnosis alone and represents subclinical inflammation, these mothers would not have had a clinical indication to receive intrapartum antibiotics. Notably, subclinical chorioamnionitis, determined by placental pathology, makes up the majority of our cases, and our analyses suggest that this is a stronger determinant than maternal fever alone. Finally, our findings compare subjects of similar gestational age, thereby decreasing any differential distribution of delivery mode. Although we have controlled for modifiable risk factors such as smoking and breast-feeding in the analysis, we have insufficient sample size to stratify by all of these variables or evaluate interactions between all of these variables in addition to our main variables of interest, prematurity and chorioamnionitis. Further studies with increased sample size may be able to address these potential interactions.

Other potential mechanisms that need to be considered include possibilities such as increased susceptibility to early life respiratory infections and pneumonias such as respiratory syncytial virus and the effect of these events on our findings. Also, further studies are needed to determine whether the effects associated with chorioamnionitis are a result of the administration of antipyretics in the perinatal period.

Several important points merit further discussion with respect to the previous literature. Prematurity has been associated with later childhood asthma in a number of studies.^{3,9-11} Although the effect of gestational age has not been consistent in all studies,^{12,13} a recent meta-analysis of 19 studies reported that preterm infants are more likely than term infants to develop asthma, with preterm infants having a 7% increased risk.⁸ One potential reason for discrepant results in the literature is that these studies did not evaluate the causes of prematurity. As emphasized in the 2006 IOM report, chorioamnionitis is a major pathway leading to the development of preterm birth.¹⁴⁻¹⁶ The potential role of chorioamnionitis as a cause of respiratory morbidity has been evaluated in only a few studies to date. In these studies, febrile episodes in pregnancy (especially the third trimester) were associated with subsequent development of asthma in offspring.^{17,18} In keeping with the fact that chorioamnionitis is much more common in very preterm infants,³⁵ we found our greatest effect in this group of infants.

Second, we did not find an association of prematurity and chorioamnionitis with atopic dermatitis or food allergy. This is also consistent with other published studies. A number of studies of prematurity suggest either no association with atopic diseases³⁶ or decreased risk of atopic outcomes including atopic dermatitis³⁷ and allergic rhinitis.³⁸ The differential associations for respiratory

morbidities versus nonrespiratory atopic diseases suggest differential pathways of disease promotion. In our study, chorioamnionitis increased the risk of wheezing and physician-diagnosed asthma, but not atopy. Although this outcome is important in itself, it may or may not equate to asthma later in life. If it does predispose to persistent asthma, it would suggest that some prenatal factors may lead to asthma via nonatopic pathways such as fetal programming or epigenetic effects *in utero*. However, the biological links between prematurity or chorioamnionitis and wheezing are not well established.

Finally, there is some biological rationale for our findings. Chorioamnionitis is associated with a strong proinflammatory response with increased levels of TNF- α , IL-6, and IL-8.^{39,40} These inflammatory cytokines may be a response to infection and also a trigger for premature delivery. Specifically, human decidual cells increase the production of delivery promoting factors such as prostaglandin E₂ and prostaglandin F₂ in response to inflammatory cytokines including IL-1 α , IL-1 β , and TNF- α .⁴¹ In animal models, administration of a phosphodiesterase (PDE) 4 inhibitor prevents preterm delivery.⁴² Some of the same cytokines that are a response to infection may be involved in chronic respiratory disease development. Of these inflammatory cytokines, TNF- α variants have been found to be associated with recurrent wheezing after respiratory syncytial virus bronchiolitis.⁴³ IL-8 levels were increased in children who developed wheezing illnesses in response to rhinovirus,⁴⁴ and in another cohort, individuals who wheezed with rhinovirus were at additional risk of persistent wheezing until follow-up at age 3 years.⁴⁵ Thus, the same cytokines generated as part of the inflammatory response to chorioamnionitis may play a role in asthma and responses to respiratory viral illness.

In summary, we found a strong joint effect of prematurity and chorioamnionitis on early childhood recurrent wheezing. This effect was even stronger in African American subjects. Further investigation is warranted to evaluate whether the associations persist as we continue to follow these children to older age and whether increased rates of recurrent wheezing correlate with increased rates of later asthma. Our findings remain to be confirmed by future studies in other populations.

Clinical implications: Chorioamnionitis may increase the risk of recurrent wheezing in very-low-birth-weight infants.

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TABLE E1. Baseline characteristics of Boston birth cohort by chorioamnionitis

Characteristic	No chorioamnionitis	Chorioamnionitis	P value
N	764	160	
Demographic factors			
Maternal age (y), mean $\pm$ SD	29.0 $\pm$ 6.6	28.0 $\pm$ 6.2	.09
Gestational age (wk), mean $\pm$ SD	38.0 $\pm$ 3.0	35.1 $\pm$ 5.5	<.001
Child's sex: male (%)	464 (50.2)	78 (45.3)	.24
Child's age grouped by year, n (%)			
<1	276 (29.9)	33 (19.2)	.002
1	185 (20.0)	26 (15.1)	
2	117 (12.7)	25 (14.5)	
3	100 (10.8)	19 (11.0)	
4	102 (11.0)	21 (12.2)	
5	66 (7.1)	20 (11.6)	
≥ 6	78 (8.4)	28 (16.3)	
Maternal race/ethnicity (%)			
African American	569 (61.6)	114 (66.3)	.81
White	62 (6.7)	11 (6.4)	
Hispanic	190 (20.6)	29 (16.9)	
Asian/Pacific Islander	19 (2.1)	3 (1.7)	
Other	84 (9.1)	15 (8.7)	
SES factors			
Annual household income (%)			
<\$15,000	278 (48.2)	55 (52.4)	.52
\$15,000 to \$30,000	198 (34.3)	30 (28.6)	
$\geq$ \$30,000	101 (17.5)	20 (19.0)	
Maternal education level (%)			
<High school	286 (31.0)	47 (27.3)	.59
High school	320 (34.6)	65 (37.8)	
>High school	318 (34.4)	60 (34.9)	
Maternal/in utero factors			
Maternal ever smoking (%)	145 (15.7)	32 (18.6)	.34
Postnatal passive smoking (%)	192 (20.8)	39 (22.7)	.58
Maternal asthma (%)	118 (12.8)	19 (11.0)	.53
Any maternal atopic disease (%)	257 (27.8)	49 (28.5)	.86
High stress during pregnancy (%)	177 (19.2)	39 (22.7)	.29
Breast-feeding patterns in infancy (%)			
Breast only	57 (6.2)	15 (8.7)	.46
Bottle only	159 (17.2)	29 (16.9)	
Both	708 (76.6)	128 (74.4)	
Delivery by cesarean section (%)	279 (30.2)	79 (45.9)	<.001
First child (nulliparity) (%)	374 (40.5)	89 (51.7)	.02
Antibiotic use (%)	147 (15.9)	64 (37.2)	<.001
Steroid use (%)	66 (7.1)	46 (26.7)	<.001

*Complications during pregnancy include chronic hypertension; pre-eclampsia; eclampsia; hemolysis, elevated liver enzymes, and low platelets syndrome; diabetes (gestational diabetes); abruptio placenta; placenta previa; or incompetent cervix.

TABLE E2. The covariates associated with recurrent wheezing, asthma, eczema, and food allergy, using 1 stepwise logistic regression model†

Variable	Wheezing*		Asthma		Eczema		Food allergy	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Child's age, y	1.2 (1.1-1.4)	<.0001	1.4 (1.3-1.5)	<.0001	1.1 (1.1-1.2)	<.0001	1.2 (1.1-1.4)	.001
Child's sex	1.3 (0.9-2.0)	.12	1.6 (1.1-2.2)	.01	1.2 (0.9-1.6)	.12		
Maternal asthma	2.1 (1.3-3.4)	.003	2.0 (1.2-3.2)	.005				
Infection	1.6 (1.0-2.5)	.05	1.7 (1.1-2.5)	.02	1.4 (0.9-1.9)	.10		
Race	0.8 (0.7-1.0)	.05	0.9 (0.7-1.0)	.06				
Education	1.4 (1.1-1.8)	.006	1.3 (1.0-1.6)	.04			1.4 (1.0-1.9)	.06
Preterm	1.7 (1.2-2.6)	.005	1.4 (1.0-2.1)	.06	0.6 (0.5-0.9)	.003		
Breast-feeding	0.9 (0.4-2.0)	.79	0.8 (0.4-1.9)	.66	1.3 (0.8-2.1)	.35	2.1 (0.9-4.9)	.08
Maternal smoking	2.0 (1.3-3.1)	.002	1.5 (0.9-2.3)	.09	1.0 (0.7-1.5)	.93	1.1 (0.6-2.2)	.75
Intrapartum antibiotics	1.4 (0.9-2.2)	.12						

*Wheezing: episodes of wheezing ≥ 2 .

†The following variables were included in stepwise models: infant factors: breast-feeding, infection during pregnancy, preterm delivery, child's current age and sex, postnatal passive smoking, and type of delivery; maternal factors: maternal race and educational status, maternal smoking during pregnancy, maternal asthma, maternal atopy, stress during the pregnancy, intrapartum antibiotics, and parity.

TABLE E3. The distribution of hypertensive disorders, other pregnancy associated complications, and chorioamnionitis among the preterm birth mothers by delivery type

Variables	Group	Spontaneous delivery	Medically indicated	P value
Hypertensive disorders	No	538 (97.3)	200 (91.7)	.001
	Yes	15 (2.7)	18 (8.3)	
Other complications	No	523 (94.6)	199 (91.3)	.09
	Yes	30 (5.4)	19 (8.7)	
Chorioamnionitis	No	489 (88.4)	194 (89.0)	.82
	Yes	64 (11.6)	24 (11.0)	

Other complications during pregnancy include diabetes (gestational diabetes), abruptio placenta, placenta previa, or incompetent cervix.

TABLE E4. The joint association of degree of prematurity with chorioamnionitis on recurrent wheezing, asthma, stratified by delivery type

		Spontaneous delivery				Medically induced			
Chorioamnionitis	Degree of prematurity	Total, N	Case, %	OR (95% CI)	P value	Total, N	Case, %	OR (95% CI)	P value
Wheezing*									
No	≥37	489	9.0	1.0	—	194	9.8	1.0	—
	33-36.9	117	17.1	2.1 (1.1-3.9)	.02	61	13.1	1.2 (0.5-3.5)	.68
	<33	33	18.2	2.7 (1.0-7.4)	.0476	30	26.7	2.0 (0.6-6.4)	.25
Yes	≥37	64	20.3	2.7 (1.3-5.6)	.01	24	12.5	0.7 (0.1-3.2)	.62
	33-36.9	27	18.5	1.7 (0.6-4.9)	.36	5	0.0	—	.99
	<33	44	31.8	4.5 (2.0-10.1)	.0003	8	37.5	5.8 (1.0-33.2)	.05
Asthma									
No	≥37	489	13.9	1.0	—	194	10.3	1.0	—
	33-36.9	117	13.7	1.0 (0.5-1.9)	.99	61	13.1	1.2 (0.4-3.5)	.69
	<33	33	18.2	2.0 (0.7-5.5)	.20	30	20.0	1.6 (0.4-5.6)	.48
Yes	≥37	64	17.2	1.3 (0.6-2.9)	.45	24	16.7	1.2 (0.3-4.8)	.80
	33-36.9	27	25.9	2.0 (0.7-5.5)	.17	5	0.0	—	.99
	<33	44	40.9	4.3 (1.9-9.5)	.0003	8	50.0	9.1 (1.5-55.7)	.02

Covariates adjusted in the model: infant factors: child's age and sex, breast-feeding, postnatal passive smoking; maternal factors: maternal ethnicity, maternal atopic diseases, maternal smoking during pregnancy, antibiotics, and maternal educational status.

*Wheezing: episodes of wheezing ≥2.

TABLE E5. The distribution of high white blood cell counts (≥ 15 k/L) among infant groups based on the presence of placental inflammation and maternal fever

Placental inflammation	Fever	Total, N	High WBC*	Low WBC
CBC after delivery				
No	No	287	93 (32.4)	194 (67.6)
	Yes	13	7 (53.8)	6 (46.2)
Yes	No	47	21 (44.7)	26 (55.3)
	Yes	17	10 (58.8)	7 (41.2)

WBC, White blood cell count; CBC, complete blood count.

*This represents a WBC of $>15,000$ by Gibbs criteria.

TABLE E6. The joint association of placental inflammation and maternal fever on recurrent wheezing and asthma in preterm children

		Preterm			
Placental inflammation	Fever	Total, N	Case, %	OR (95% CI)	P value
Wheezing*					
No	No	241	17.4	1.0	—
	Yes	8	12.5	0.4 (0.1-4.3)	.48
Yes	No	60	26.7	1.2 (0.6-2.5)	.67
	Yes	16	31.3	2.8 (0.8-10.5)	.12
Asthma					
No	No	241	14.9	1.0	—
	Yes	8	25.0	1.0 (0.1-6.5)	.97
Yes	No	60	36.7	2.6 (1.2-5.6)	.01
	Yes	16	31.3	3.5 (0.9-13.8)	.07

Covariates adjusted in the model: infant factors: child's age and sex, breast-feeding, postnatal passive smoking; maternal factors: maternal ethnicity, maternal atopic diseases, maternal smoking during pregnancy, antibiotics, and maternal educational status.

*Wheezing: episodes of wheezing ≥ 2 .