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Original Contribution

Antibiotic Exposure by 6 Months and Asthma and Allergy at 6 Years: Findings in a Cohort of 1,401 US Children

Kari R. Risnes, Kathleen Belanger, William Murk, and Michael B. Bracken*

* Correspondence to Dr. Michael B. Bracken, Center for Perinatal, Pediatric, and Environmental Epidemiology, Yale University School of Public Health, 1 Church Street, Sixth Floor, New Haven, CT 06510 (e-mail: michael.bracken@yale.edu).

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Many studies have reported that antibiotic use may be associated with increased risk of childhood asthma. Respiratory tract infections in small children may be difficult to distinguish from early symptoms of asthma, and studies may have been confounded by "protopathic" bias, where antibiotics are used to treat early symptoms of asthma. These analyses of a cohort including 1,401 US children assess the association between antibiotic use within the first 6 months of life and asthma and allergy at 6 years of age between 2003 and 2007. Antibiotic exposure was associated with increased risk of asthma (adjusted odds ratio = 1.52, 95% confidence interval (CI): 1.07, 2.16). The odds ratio if asthma was first diagnosed after 3 years of age was 1.66 (95% CI: 0.99, 2.79) and, in children with no history of lower respiratory infection in the first year of life, the odds ratio was 1.66 (95% CI: 1.12, 3.46). The adverse effect of antibiotics was particularly strong in children with no family history of asthma (odds ratio = 1.89, 95% CI: 1.00, 3.58) ($P_{interaction} = 0.03$). The odds ratio for a positive allergy blood or skin test was 1.59 (95% CI: 1.10, 2.28). The results show that early antibiotic use was associated with asthma and allergy at 6 years of age, and that protopathic bias was unlikely to account for the main findings.

anti-bacterial agents; asthma; child; cohort studies; hypersensitivity

Abbreviations: CI, confidence interval; LRTI, lower respiratory tract infection; NICU, neonatal intensive care unit; OR, odds ratio.

Asthma is one of the most common chronic diseases of childhood, affecting an estimated 1 in 4 urban children in the developed world (1). The "hygiene hypothesis" is a frequently cited explanation for the increasing prevalence of allergic diseases. Initial interpretation suggested that reduced exposure to bacteria and viruses may delay development of the immune system and promote atopic immune responses (2, 3). A broader understanding is that microflora, especially gastrointestinal flora, are important for developing a healthy immune system with resistance to allergic sensitization (4). Thus, antibiotic exposure in early life could increase the risk of atopic diseases through altered microbial exposure (5). If antibiotic exposure in early life is associated with allergic diseases, it is an additional reason to reduce unnecessary antibiotic use in children (6).

Many studies report a positive association between antibiotic use and childhood asthma (7–12), including a large prospective cohort study with more than 5,000 cases of childhood asthma that determined early antibiotic exposure to be one of the most important predictors of childhood asthma (11). However, interpretation of individual studies is controversial (13, 14). Respiratory tract infections in small children may be difficult to distinguish from early symptoms of asthma, and many studies may be confounded by "protopathic" bias, where antibiotics are used to treat respiratory tract infections that could be early symptoms of asthma.

Some studies report lack of an association in cohorts of children with a genetic predisposition to asthma (10, 15, 16), which has suggested that children with no predisposition are more susceptible to early effects of antibiotics than high-risk children genetically predisposed to asthma (10).

We hypothesize that early antibiotic use is associated with increased risk of childhood asthma. To reduce risk of protopathic bias, we assessed the association of antibiotic use within the first 6 months of life with asthma and allergy at 6

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years of age. We considered whether the association differed according to parental history of asthma.

MATERIALS AND METHODS

Study cohort

Between April 1997 and June 2000, pregnant women were recruited from 56 private obstetric practices and 15 public clinics across southern New England. Details of the enrolment procedure have been previously published (17, 18). A flow chart for inclusion is presented in Figure 1. For the present analysis, the Perinatal Risk of Asthma in Infants of Asthmatic Mothers (PRAM) Study, 1,871 children, including those with mothers with physician-diagnosed asthma ($n = 872$), all children with mothers with asthma symptoms ($n = 449$), and a random sample of offspring of mothers without asthma or asthma symptoms ($n = 550$), were selected for follow-up (17). After non-English speakers ($n = 61$) and neonatal deaths ($n = 3$) were excluded, 1,807 women were eligible at the 6-year follow-up. Of the eligible women, 302 (16.7%) women were excluded because of refusal, inability to locate, and missed interviews. From September 2003 until January 2007, 1,505 women were interviewed at their child's sixth birthday (±3 months) to determine the child's asthma status. For the present analyses, we excluded participants who did not have complete information about antibiotic exposure before 6 months of age ($n = 31$) and 19 participants for whom perinatal information could not be retrieved. Children diagnosed with asthma before 6 months of age ($n = 54$) were excluded from all analysis with asthma as the outcome, leaving a total of 1,401 (93%) children for these analyses.

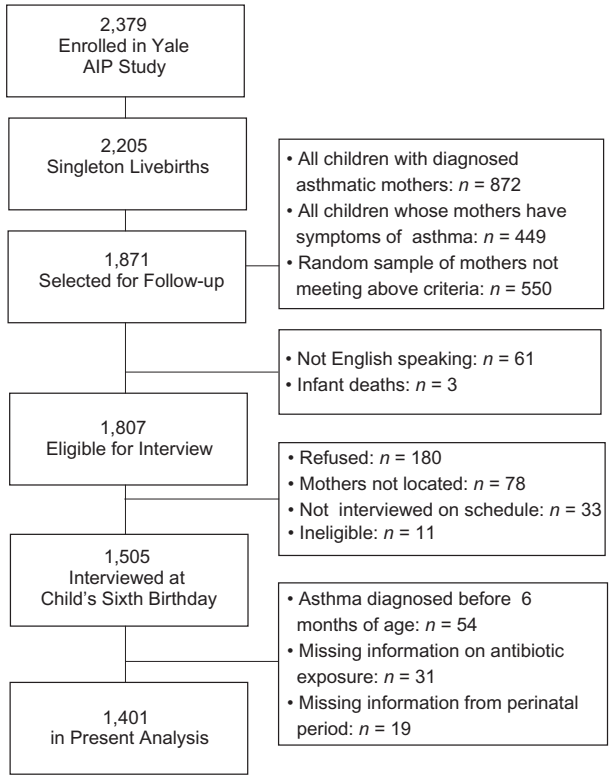

Figure 1. Number of subjects enrolled in the Yale Asthma in Pregnancy (AIP) Study, with flow diagram for inclusion in the present analysis, the PRAM Study, 1997–2007. PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers.

Data collection

Four discrete sources of data were used in this analysis. Questionnaires are available upon request.

Mothers were interviewed before 24 weeks' gestation, usually at home. A standardized pregnancy interview included marital status, age, education, household characteristics, yearly income, lifestyle risk factors, medical conditions, medication during pregnancy, maternal anthropometry, and pregnancy complications. Detailed information about respiratory symptoms, hospitalization, and medication use the year before pregnancy was retrieved (19).

A postpartum interview was conducted within 1 month of delivery. Mothers were asked about pregnancy and delivery complications, asthma symptoms, medication use, and environmental exposures during late pregnancy.

Pregnancy, labor, delivery, and neonatal information was abstracted from hospital charts. Information included birth weight, gestational age at delivery, and complications at delivery. Gestational age in completed weeks was based on the earliest ultrasound or on the last menstrual period when information from ultrasound was not available. Preterm birth was defined as less than 37 weeks' completed gestation, and low birth weight was defined as birth weight less than 2,500 g. Detailed neonatal information was abstracted from the hospital records: transfer to a neonatal intensive care unit (NICU), length of stay in NICU, Apgar score, resuscitation, and the use of mechanical ventilation. We abstracted the neonatal diagnosis from hospital charts. Respiratory problems were defined according to *International Classification of Diseases*, Ninth Revision, codes 770.0–770.9, and neonatal bacterial infections included diagnosis of sepsis or meningitis defined by positive culture of blood or cerebrospinal fluid.

At the child's sixth birthday (±3 months), standardized maternal interviews were conducted at home (87.4%) or by telephone. Trained interviewers unfamiliar with the research hypotheses and not performing the initial interview completed the interview using a standardized 70-item questionnaire. Questions included the child's medical history, asthma diagnosis, asthma symptoms, and medication use during the child's lifetime and the health of the child's biologic father, including asthma status. The mother was asked about the child's number of biologic siblings and whether each sibling had a physician's diagnosis of asthma. The mother was asked about nutrition, including months of breastfeeding, medication use, and antibiotic use while breastfeeding. She was asked whether the child had been diagnosed by a physician as having lower respiratory infections (bronchiolitis, bronchitis, pneumonia, or respiratory syncytial virus), ear infection, strep throat,

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Table 1. Characteristics of the Study Population in 1,401 Children Followed From the First Trimester in Utero for Antibiotic Use by 6 Months and Asthma at 6 Years of Life, the PRAM Study, 1997–2007[a]

	Total (*N* = 1,401)		Antibiotic Exposure by 6 Months (*n* = 464; 33.1%)			Asthma at 6 Years (*n* = 164; 11.7%)		
	No.	%	Yes, %	OR	95% CI	Yes, %	OR	95% CI
Maternal age, years								
<25	300	21.4	36.7	1.15	0.89, 1.52	16.0	1.51	1.04, 2.20
25–35	838	59.8	33.4	1.00	Referent	11.2	1.00	Referent
>35	263	18.8	28.1	0.78	0.57, 1.05	8.4	0.72	0.44, 1.18
Maternal marital status								
Married	1,044	74.5	31.4	1.00	Referent	9.9	1.00	Referent
Single/divorced	357	25.5	38.1	1.34	1.05, 1.73	17.1	1.88	1.33, 2.65
Maternal ethnicity								
White	1,032	73.7	33.5	1.00	Referent	10.0	1.00	Referent
African American	134	9.6	26.1	0.70	0.47, 1.05	17.9	1.97	1.21, 3.20
Hispanic	178	12.7	38.8	1.26	0.90, 1.74	16.9	1.83	1.18, 2.84
Asian/other	57	4.1	24.6	0.65	0.35, 1.20	12.3	1.26	0.56, 2.86
Maternal education								
≤12 years	372	26.6	32.5	0.90	0.69, 1.17	14.8	1.33	0.92, 1.92
At least some college	692	49.4	35.0	1.00	Referent	11.6	1.00	Referent
At least some graduate school	337	24.0	30.0	0.80	0.60, 1.05	8.6	0.72	0.46, 1.12
Household income, US dollars								
≥40,000	1,008	72.0	31.8	1.00	Referent	9.52	1.00	Referent
<40,000	348	24.8	37.4	1.28	1.00, 1.65	17.5	2.02	1.43, 2.86
Not answered	45	3.2	31.1	0.97	0.51, 1.85	15.6	1.75	0.76, 4.03
Smoking								
Never	868	62.0	32.6	1.00	Referent	11.1	1.00	Referent
Quit before pregnant	298	21.3	35.6	1.14	0.87, 1.50	10.1	0.90	0.58, 1.40
First trimester	154	11.0	30.5	0.91	0.63, 1.32	17.5	1.71	1.07, 2.73
First and third trimesters	81	5.7	34.6	1.09	0.68, 1.76	13.6	1.26	0.65, 2.47
Maternal asthma symptoms[b]								
No symptoms	734	51.6	30.7	1.00	Referent	7.6	1.00	Referent
Intermittent	410	29.7	33.9	1.16	0.90, 1.50	13.4	1.88	1.27, 2.78
Persistent	233	17.5	39.5	1.47	1.09, 2.00	20.6	3.14	2.07, 4.77
No information for classification	24	1.7	33.3	1.13	0.48, 2.68	20.8	1.01	0.48, 2.68
Parent with asthma								
None	681	48.6	30.0	1.00	Referent	6.9	1.00	Referent
1	641	45.8	36.2	1.33	1.05, 1.67	15.1	2.40	1.67, 3.47
2	79	5.6	35.4	1.28	0.78, 2.09	25.3	4.57	2.54, 8.22

Table continues

sinus infection, croup, or tonsillitis. For each condition, she was asked how many times, in the first year of life and in the most recent 12 months, the child had been diagnosed with each condition.

Exposure variables

When the child was 6 years (±3 months) of age, the mother was asked about the medication the child had taken and, specifically, ''Has your child ever taken antibiotics?'' If yes, she responded to whether antibiotics were taken within the specified age intervals (0–6 months, 7–12 months, >1–2 years, and >2–6 years) and how many times at each age interval antibiotics were taken (once, twice, or 3 or more times). This information was used to create the primary exposure variable of whether or not the child had taken antibiotics and the number of antibiotic courses (0, 1, ≥2 courses) before 6 months of age.

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Table 1. Continued

	Total (N = 1,401)		Antibiotic Exposure by 6 Months (n = 464; 33.1%)			Asthma at 6 Years (n = 164; 11.7%)		
	No.	%	Yes, %	OR	95% CI	Yes, %	OR	95% CI
Sibling with asthma								
No	1,150	82.1	32.3	1.00	Referent	9.4	1.00	Referent
Yes	251	17.9	37.1	1.24	0.93, 1.64	22.3	2.77	1.94, 3.96
Maternal diabetes								
No	1,379	98.4	32.7	1.00	Referent	11.4	1.00	Referent
Yes	22	1.6	59.1	2.97	1.26, 7.00	31.8	3.63	1.46, 9.04
Delivery mode[c]								
Vaginal	1,088	80.6	32.1	1.00	Referent	12.0	1.00	Referent
Cesarean	262	19.4	40.5	1.44	1.09, 1.90	10.3	0.85	0.54, 1.30
Gender								
Boys	720	51.4	37.2	1.00	Referent	12.2	1.00	Referent
Girls	681	48.6	31.8	0.83	0.66, 1.04	11.2	0.90	0.65, 1.25
Gestational age, weeks								
≥37	1,324	94.5	34.1	1.00	Referent	11.7	1.00	Referent
<37	77	5.5	41.6	1.16	0.72, 1.88	11.2	1.83	1.15, 3.14
Birth weight, g								
≥2,500	1,349	96.3	32.2	1.00	Referent	11.4	1.00	Referent
<2,500	52	3.7	40.4	1.38	0.79, 2.44	19.2	1.85	0.91, 3.76
Diagnosis of respiratory problem in NICU								
No	1,169	86.6	32.3	1.00	Referent	11.3	1.00	Referent
Yes	181	13.4	43.1	1.59	1.16, 2.19	14.4	1.61	1.10, 2.36
Antibiotics to mother while breastfeeding								
No	1,240	88.5	32.4	1.00	Referent	11.8	1.00	Referent
Yes	161	11.5	38.5	1.30	0.93, 1.83	11.2	0.94	0.56, 1.59
LRTI in first year of life								
No	1,197	85.4	28.5	1.00	Referent	10.4	1.00	Referent
Yes	204	14.6	67.7	4.90	3.58, 6.71	19.6	2.11	1.42, 3.12
Otitis in first year of life								
No	641	45.8	9.4	1.00	Referent	11.4	1.00	Referent
Yes	760	54.3	53.2	11.00	8.13, 14.86	12.0	1.06	0.76, 1.47

Abbreviations: CI, confidence interval; LRTI, lower respiratory tract infection (mother responded that child had a physician's diagnosis of ≥1 of the following before 1 year of age: bronchitis, bronchiolitis, pneumonia, respiratory syncytial virus); NICU, neonatal intensive care unit; OR, odds ratio; PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers.

[a] Additional variables were evaluated for confounding: infant's/child's race, maternal parity, number in household, passive smoke in pregnancy, alcohol exposure in pregnancy, maternal hypertension, preterm labor, resuscitation at birth, admitted to NICU, length of NICU stay, mechanical ventilation in NICU, neonatal sepsis or bacteremia, neonatal meningitis, and breastfeeding.

[b] Symptoms and medication use in year before pregnancy. Severity score adapted from Global Initiative for Asthma Severity Classification Guidelines (18).

[c] n = 1,350.

Outcome variables

In the 6-year interview, mothers were asked, ''Has the child ever been diagnosed by a doctor or health professional as having asthma?'' and ''How old was your child when s/he was first diagnosed as having asthma'' (completed years and months). Mothers were asked, ''Has your child had wheezing or whistling in the chest in the last 12 months?'' The primary outcome in this analysis was physician-diagnosed asthma after 6 months of age with history of wheezing in the sixth year of life.

The mother was asked if the child had ever had an allergic reaction, whether blood immunoglobulin E or skin prick

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tests were performed, and the results of the tests. An allergic reaction and positive blood or skin prick test were considered a positive allergy outcome. Children with asthma at 6 years and a positive allergy test were classified as having allergic asthma.

Statistical analysis

We used logistic regression to calculate odds ratios with 95% confidence intervals for each potential confounding factor and for antibiotic exposure before 6 months of age. The same procedure was repeated for outcomes: asthma at 6 years of age and a positive allergy test. Covariates were initially selected for model inclusion by identifying those associated with both the exposure and the outcome at $P \leq 0.20$. Adjusted models used a backward elimination procedure that preserved in the final models variables producing a 10% or greater change in the estimated association. Antibiotic exposure was analyzed as a dichotomous variable and as a categorical variable for the number of courses of antibiotics (0, 1, $\geq$2 courses). Two-sided P values from linear trend tests were calculated by treating dose categories as ordinal variables in the regression model.

To reduce the likelihood of protopathic bias, we repeated the analyses to assess the association between antibiotic exposure before 6 months of age and asthma using the first diagnosis after the age of 3 years.

Possible effect modification by history of parental asthma or by lower respiratory tract infection (LRTI) during the first year of life was evaluated by using a likelihood ratio test. We included a product term between antibiotic exposure and parental asthma in the analysis and, respectively, between antibiotic exposure and LRTI.

To evaluate potential bias from missing values, we conducted sensitivity analyses, assessing the association between antibiotic exposure and asthma in excluded individuals. To evaluate the accuracy of maternal recall, we compared selected answers given in the 6-year interview with information abstracted from hospital records after birth. Agreement is reported by the percentage of participants responding similarly. All statistical analyses were conducted by using STATA, release 10, statistical software (StataCorp LP, College Station, Texas).

The Human Investigations Committee at Yale University approved the study.

RESULTS

The majority of the population was white and had at least some college education (Table 1). One fourth of participants had a household income below 40,000 US dollars per year, the median US income at the time of the study. By design, a high proportion of the participants were children with a genetic predisposition for asthma; more than 50% of the participants had 1 or 2 parents with asthma. Half the children had 1 or more episodes of physician-diagnosed otitis, and 15% had 1 or more episodes of physician-diagnosed LRTI during their first year of life.

One third of the children had been exposed to antibiotics by 6 months of age (Table 1). Antibiotic use was more common for children of single mothers, children of lower income families, and children with parental asthma, maternal diabetes, low birth weight, and a history of respiratory diagnosis in the neonatal period. Nearly 70% of children with a history of lower respiratory infections and more than 50% with otitis in the first year of life had received antibiotics.

Of the 1,401 children studied, 164 met the criteria for asthma. In unadjusted analyses, children of African-American or Hispanic mothers had twice the risk of asthma compared with children of white mothers (Table 1). Children of younger or single mothers and in lower income families were at increased risk of asthma. Maternal diabetes was associated with increased asthma risk in the child (odds ratio (OR) = 3.63, 95% confidence interval (CI): 1.46, 9.04). If one parent had a history of asthma, the odds ratio for asthma at 6 years of age was 2.40 (95% CI: 1.67, 3.47) compared with children with no history of parental asthma. The corresponding odds ratio if both parents had a history of asthma was 4.57 (95% CI: 2.54, 8.22). The odds ratios for childhood asthma associated with parental smoking in the first trimester were 1.71 (95% CI: 1.07, 2.73); with preterm birth, 1.83 (95% CI: 1.15, 3.14); and with respiratory diagnosis in the neonatal period, 1.61 (95% CI: 1.10, 2.36).

The unadjusted odds ratio for asthma associated with antibiotics exposure was 1.81 (95% CI: 1.31, 2.52), and the adjusted odds ratio was 1.52 (95% CI: 1.07, 2.16) (Table 2). Restricting the analyses to asthma cases diagnosed after 3 years of age did not attenuate the associations, but fewer cases yielded less precise estimates, and the result is no longer statistically significant (adjusted OR = 1.66, 95% CI: 0.99, 2.79). The adjusted odds ratio for a positive blood immunoglobulin E or skin prick allergy test was 1.59 (95% CI: 1.10, 2.28), and the adjusted odds ratio for allergic asthma (current asthma at 6 years and a positive allergy test by 6 years of age) was 1.76 (95% CI: 1.01, 3.09).

The number of antibiotic courses before 6 months of age and asthma show a dose-response relation ($P_{\text{trend}} = 0.01$) (Table 3). Compared with that for no exposure to antibiotics, the adjusted odds ratio associated with 1 antibiotic course was 1.40 (95% CI: 0.90, 2.15) and, for 2 or more courses, the odds ratio was 1.72 (95% CI: 1.11, 2.65).

Stratified analyses (Table 4) show no strong evidence of an association between antibiotic exposure and the risk of asthma if at least 1 parent had asthma (adjusted OR = 1.35, 95% CI: 0.88, 2.06). The adjusted odds ratio for asthma in children who had no parental history of asthma was 1.89 (95% CI: 1.00, 3.58) ($P_{\text{interaction}} = 0.03$). Analyses showed evidence of increased risk of asthma associated with antibiotic exposure in children who had no report of LRTI (adjusted OR = 1.66, 95% CI: 1.12, 3.46). There was no evidence of interaction by LRTI ($P_{\text{interaction}} = 0.28$).

Incomplete antibiotic information was not associated with asthma in the child ($P = 0.54$). Analyses were rerun while assuming that all missing antibiotic exposures before 6 months were negative, then positive. These analyses did not meaningfully alter the association between antibiotic exposure and asthma. The association between antibiotic exposure and asthma did not change substantially when

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Table 2. Results Assessing Associations of Antibiotic Exposure Before 6 Months of Age With Current Asthma and Allergy by 6 Years in 1,401 Children, the PRAM Study, 1997–2007

	No. of Cases	Unadjusted OR	95% CI	Adjusted OR	95% CI
Asthma, with first diagnosis at age					
>6 months	164	1.81	1.31, 2.52	1.52[a]	1.07, 2.16
>3 years	66	1.78	1.09, 2.90	1.66[a]	0.99, 2.79
Allergy					
Positive allergy test[b]	152	1.93	1.38, 2.71	1.59[c]	1.10, 2.28
Allergic asthma[d]	58	2.44	1.32, 3.80	1.76[c]	1.01, 3.09

Abbreviations: CI, confidence interval; OR, odds ratio; PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers.

[a] Adjusted for household income (</≥40,000 US dollars), parental asthma (none, 1, 2), and physician's diagnosis of lower respiratory tract infections in first year of life (yes/no).

[b] Report of a positive blood or skin prick test for 1 or more of the following allergens by 6 years of age: eggs, food, medication, cats, dogs, dust, mites, cockroaches, pollens, mold, trees, grass, ragweed, or others versus negative test/not tested.

[c] Adjusted for parental asthma (none, 1, 2), maternal age (<25, 25–35, >35 years), and physician's diagnosis of lower respiratory tract infections in first year of life (yes/no).

[d] Report of a physician's diagnosis of asthma after 6 months of age and current wheeze at 6 years of age combined with a positive allergy test as defined in footnote b.

the individuals excluded because of missing values were included in the analysis (unadjusted OR = 1.81, 95% CI: 1.31, 2.50). Proportions of agreement between questionnaire data and hospital record data were 95% for NICU admission, 60% for length of stay in NICU, and 90% for the use of mechanical ventilation. Proportions of agreement did not differ by the child's asthma status. Information about pet ownership in the child's first year of life, first recorded in the postpartum interview and repeated at the 6-year interview, yielded agreement in 88% of participants.

DISCUSSION

There were several strengths to our study. We recruited pregnant women from many different clinics and pregnancy care providers. The original cohort was enriched so that 40% of mothers had asthma, providing increased power and enabling us to study the effects related to family history of asthma. In other respects, the study cohort is broadly representative of the southern New England population. The study was designed to determine pre- and perinatal risk factors for childhood asthma, and the detailed, prospectively collected measures of potential pre- and perinatal confounders are strengths of these analyses.

Asthma is a complex clinical manifestation that is difficult to diagnose reliably before the age of 6 years (1, 5). Our analyses included as cases children with a physician-verified diagnosis of asthma and current symptoms during the sixth year of life.

Many studies that have attempted to assess the association between antibiotic use and the development of asthma have been confounded by possible protopathic bias that applies if early asthma symptoms are the reason for antibiotic treatment (20). Protopathic bias may be minimized by considering only antibiotic exposure that occurs several years before the onset of asthma symptoms (21). Protopathic bias may also be avoided by studying the associations when antibiotics are used for indications that do not include symptoms similar to asthma, typically lower respiratory tract infections. The present study developed several strategies to reduce the influence of protopathic bias: We excluded from all analyses cases of asthma diagnosed within the first 6

Table 3. Associations Between the Number of Courses of Antibiotics Before 6 Months of Age and Current Asthma at 6 Years of Age in 1,401 Children, the PRAM Study, 1997–2007

No. of Antibiotic Courses Within First 6 Months of Life	No. of Asthma Cases	No. of Individuals	OR[a]	95% CI	P_{trend}
0	99	937	1.00	Referent	
1	37	242	1.40	0.90, 2.15	
≥2	38	216	1.72	1.11, 2.65	0.01

Abbreviations: CI, confidence interval; OR, odds ratio; PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers.

[a] Adjusted for household income (</≥40,000 US dollars, parental asthma (none, 1, 2), and physician's diagnosis of lower respiratory tract infections in first year of life (yes/no).

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Table 4. Stratified Analyses for the Associations of Antibiotic Exposure Within 6 Months of Age and Current Asthma at 6 Years in 1,401 Children, the PRAM Study, 1997–2007

	No. of Asthma Cases	No. of Individuals	OR	95% CI	$P_{interaction}$
Parental asthma[a]					
Yes	117	720	1.35[b]	0.88, 2.06	
No	47	681	1.89[b]	1.00, 3.58	0.03
LRTI[c] in first year of life					
Yes	40	204	1.03[d]	0.49, 2.17	
No	124	1,197	1.66[d]	1.12, 3.46	0.28

Abbreviations: CI, confidence interval; LRTI, lower respiratory tract infection; OR, odds ratio; PRAM, Perinatal Risk of Asthma in Infants of Asthmatic Mothers.

[a] One or both parents have physician-diagnosed asthma.

[b] Adjusted for household income (</≥40,000 US dollars) and lower respiratory tract infections in first year of life (yes/no).

[c] Report of physician's diagnosis of bronchiolitis, respiratory syncytial virus infection, pneumonia, or bronchitis in first year of life.

[d] Adjusted for household income (</≥40,000 US dollars) and parental asthma (none, 1, 2).

months of age, we conducted separate analyses for asthma first diagnosed after 3 years of age, and we assessed the association in children who had not reported a LRTI in the first year of life. The association between antibiotic exposure before 6 months age and asthma diagnosed after 3 years of age was quite strong. The strong association in children who did not report any diagnosis of lower respiratory infections during the first year of life further supports the interpretation that protopathic bias was limited. Other studies have found weaker results in asthma diagnosed after 3 years age compared with an earlier diagnosis (11, 22). In a large Canadian database study, however, age at diagnosis did not affect the estimated associations (12).

Ascertainment of antibiotic use in this study is dependent upon maternal recall after 5.5 years, warranting cautious interpretation of results. Inaccuracy in these retrospectively collected exposure data could bias estimated associations if mothers of asthmatic children overreported—or if mothers of nonasthmatic children underreported—antibiotic use. Inaccurate maternal recall that is unrelated to the child's asthma status will usually bias estimates toward the null. We did not have access to medical records to evaluate information on antibiotic exposure. The number of factors that could be validated for accuracy was limited, but the high level of observed agreement between interview data and hospital data and the agreement between cases and controls are reassuring regarding the quality of the data in the 6-year questionnaire. Several studies evaluating patient recall (23–25) report that agreement with medical records varies by medical condition. In one study, parental reports of asthma and bronchitis reached agreement with medical records by 85%–90% (24), while another study found that one third of parents had forgotten about first-year-of-life wheezing by the time the child was 11 years of age (26). Another report specifically validated parental antibiotic recall in a study about childhood atopic diseases and found agreement between questionnaire and medical records of 91% for antibiotic use (27). In the present study, it is reassuring that the level of exposure, about 30% of children receiving antibiotics by 6 months of age, corresponds well with findings from the largest and most recent database study (11) that also assessed antibiotic exposure before 6 months in their analyses. We conclude that the likelihood that associations are overestimated because of recall bias is limited.

The association of antibiotics and asthma in the present study was particularly strong in children with no family history of asthma. A similar finding was reported in a Canadian cohort (10) and corresponds to negative findings in cohorts that included only children with a family history of asthma (15, 16). One study reported a strong association in children of asthmatic parents (28), but no test for interaction was performed, and the study included only 53 cases of asthma.

The hygiene hypothesis, although controversial (29, 30), suggests that microbial exposure in early life enhances postnatal maturation of the immune system that may protect against development of allergic diseases (2, 4, 31). Normal postnatal development incurs a change from fetal predominantly T helper 2 (known as "T_H2") to more mature T helper 1 (known as "T_H1") immunity (4, 32). Overexpression of T_H2 responses to allergens is the hallmark of allergic diseases (33). One important mechanism to support the hygiene hypothesis is that microbial exposure, particularly in the intestinal tract, is necessary for postnatal transition to a balanced immune response in healthy children (3, 34). It has been suggested that the early postnatal period is particularly vulnerable to imbalances in immune response, and that delayed postnatal maturation of T_H1 cell function is a key component of genetic risk for atopy (35, 36). The present findings are compatible with an interpretation that children with no family history of asthma are more susceptible to the proatopic effects of antibiotics than children with a genetic predisposition to asthma.

Information on antibiotic use did not include details about the type of antibiotics, and this may be a limitation for the biologic interpretation of our findings. Broad-spectrum antibiotics potentially alter microflora more than

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narrow-spectrum antibiotics. Some studies that could separately assess the effects of broad-spectrum antibiotics found stronger associations with atopic disease than did those with narrow spectrum (9, 10), possibly supporting the interpretation that early antibiotic exposure alters atopic disease risk through alterations in microflora. In contrast, a large database study (12) reported that penicillin exposure was associated with a particularly high risk of asthma. It may be of relevance to the strong association found in the present study that US outpatient data from the period of our study show increased use of broad-spectrum antibiotics in small children (37).

In our data, we found a strong association between early antibiotic exposure with reported positive immunoglobulin E blood or skin test reactivity. Studies that have assessed the association of antibiotic exposure with immunoglobulin E levels in children (13, 15, 16, 38, 39) did not report evidence of an association. Two studies assessed a possible association with a positive skin prick test (28, 39) and found none. Two studies included only high-risk children with a family history of asthma (15, 16). If antibiotics do not affect the immune response in children with a genetic predisposition to asthma, this could explain the negative findings in these studies. However, the same explanation cannot apply to the negative findings of studies in children more representative of the general population (28, 38–40). These studies allergy tested all participants at 7–8 years of age, and the study outcomes were positive allergy tests, regardless of allergy symptoms. The validity of blood or skin prick tests to diagnose allergy in a general population is limited. The positive predictive value for allergic reactions can be as low as 50% (41). The diagnosis of allergy, therefore, has to be based on clinical history, with supplementary testing (42). Our study was observational, and tests were performed in children who reported an allergic reaction, suggesting that a positive test result was a reasonably valid indicator of allergy in this cohort. Further studies are warranted to establish whether early antibiotic exposure is associated with childhood allergy.

We conclude that antibiotic exposure before 6 months of age is associated with asthma and allergy at 6 years of age and that protopathic bias is unlikely to account for the main findings. The adverse effect of antibiotics on asthma risk was particularly strong in children with no parental history of asthma, which should encourage physicians to avoid unnecessary antibiotic use in low-risk children with no genetic predisposition to asthma.

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Author affiliations: Center for Perinatal, Pediatric, and Environmental Epidemiology, Yale University Schools of Public Health and Medicine, New Haven, Connecticut (Kari R. Risnes, Kathleen Belanger, William Murk, Michael B. Bracken); Department of Pediatrics, St. Olav University Hospital, Trondheim, Norway (Kari R. Risnes); and Department of Public Health, Norwegian University of Science and Technology, Trondheim, Norway (Kari R. Risnes).

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REFERENCES

1. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med*. 2006;355(21):2226–2235.
2. Strachan DP. Hay fever, hygiene, and household size. *BMJ*. 1989;299(6710):1259–1260.
3. Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J Allergy Clin Immunol*. 2006;117(5):969–977, quiz 978.
4. Holt PG, van den Biggelaar AH. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: the role of infections in allergy: atopic asthma as a paradigm. *Clin Exp Immunol*. 2010;160(1):22–26.
5. Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet*. 1999;354(suppl 2):SII12–SII15.
6. Nyquist AC, Gonzales R, Steiner JF, et al. Antibiotic prescribing for children with colds, upper respiratory tract infections, and bronchitis. *JAMA*. 1998;279(11):875–877.
7. Cohet C, Cheng S, MacDonald C, et al. Infections, medication use, and the prevalence of symptoms of asthma, rhinitis, and eczema in childhood. *J Epidemiol Community Health*. 2004;58(10):852–857.
8. Ahn KM, Lee MS, Hong SJ, et al. Fever, use of antibiotics, and acute gastroenteritis during infancy as risk factors for the development of asthma in Korean school-age children. *J Asthma*. 2005;42(9):745–750.
9. McKeever TM, Lewis SA, Smith C, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol*. 2002;109(1):43–50.
10. Kozyrskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest*. 2007;131(6):1753–1759.
11. Martel MJ, Rey E, Malo JL, et al. Determinants of the incidence of childhood asthma: a two-stage case-control study. *Am J Epidemiol*. 2009;169(2):195–205.
12. Marra F, Marra CA, Richardson K, et al. Antibiotic use in children is associated with increased risk of asthma. *Pediatrics*. 2009;123(3):1003–1010.
13. Mai XM, Kull I, Wickman M, et al. Antibiotic use in early life and development of allergic diseases: respiratory infection as the explanation. *Clin Exp Allergy*. 2010;40(8):1230–1237.
14. Marra F, Lynd L, Coombes M, et al. Does antibiotic exposure during infancy lead to development of asthma?: a systematic review and metaanalysis. *Chest*. 2006;129(3):610–618.
15. Celedón JC, Litonjua AA, Ryan L, et al. Lack of association between antibiotic use in the first year of life and asthma, allergic rhinitis, or eczema at age 5 years. *Am J Respir Crit Care Med*. 2002;166(1):72–75.
16. Kusel MM, de Klerk N, Holt PG, et al. Antibiotic use in the first year of life and risk of atopic disease in early childhood. *Clin Exp Allergy*. 2008;38(12):1921–1928.
17. Kang EM, Lundsberg LS, Illuzzi JL, et al. Prenatal exposure to acetaminophen and asthma in children. *Obstet Gynecol*. 2009;114(6):1295–1306.

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18. Bracken MB, Triche EW, Belanger K, et al. Asthma symptoms, severity, and drug therapy: a prospective study of effects on 2205 pregnancies. *Obstet Gynecol*. 2003;102(4):739–752.
19. Belanger K, Hellenbrand ME, Holford TR, et al. Effect of pregnancy on maternal asthma symptoms and medication use. *Obstet Gynecol*. 2010;115(3):559–567.
20. Salas M, Hofman A, Stricker BH. Confounding by indication: an example of variation in the use of epidemiologic terminology. *Am J Epidemiol*. 1999;149(11):981–983.
21. Tamim H, Monfared AA, LeLorier J. Application of lag-time into exposure definitions to control for protopathic bias. *Pharmacoepidemiol Drug Saf*. 2007;16(3):250–258.
22. Celedón JC, Fuhlbrigge A, Rifas-Shiman S, et al. Antibiotic use in the first year of life and asthma in early childhood. *Clin Exp Allergy*. 2004;34(7):1011–1016.
23. Harlow SD, Linet MS. Agreement between questionnaire data and medical records. The evidence for accuracy of recall. *Am J Epidemiol*. 1989;129(2):233–248.
24. Pless CE, Pless IB. How well they remember. The accuracy of parent reports. *Arch Pediatr Adolesc Med*. 1995;149(5):553–538.
25. Nicholas C, Wegienka G, Havstad S, et al. How accurately do young adults recall childhood pets? A validation study. *Am J Epidemiol*. 2009;170(3):388–392.
26. Kjellman NI, Croner S, Gustafsson PM. Development of asthma in children. *Allerg Immunol (Paris)*. 1991;23(8):351–357.
27. Øien T, Storrø O, Johnsen R. Assessing atopic disease in children two to six years old: reliability of a revised questionnaire. *Prim Care Respir J*. 2008;17(3):164–168.
28. Droste JH, Wieringa MH, Weyler JJ, et al. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? *Clin Exp Allergy*. 2000;30(11):1547–1553.
29. Platts-Mills TA, Woodfolk JA, Sporik RB. Con: the increase in asthma cannot be ascribed to cleanliness. *Am J Respir Crit Care Med*. 2001;164(7):1107–1108, discussion 1108–1109.
30. Björkstén B. The hygiene hypothesis: do we still believe in it? *Nestle Nutr Workshop Ser Pediatr Program*. 2009;64:11–18, discussion 18–22.
31. von Mutius E. Pro: the increase in asthma can be ascribed to cleanliness. *Am J Respir Crit Care Med*. 2001;164(7):1106–1107, discussion 1108–1109.
32. Ribeiro-do-Couto LM, Boeije LC, Kroon JS, et al. High IL-13 production by human neonatal T cells: neonate immune system regulator? *Eur J Immunol*. 2001;31(11):3394–3402.
33. Prescott SL. Early origins of allergic disease: a review of processes and influences during early immune development. *Curr Opin Allergy Clin Immunol*. 2003;3(2):125–132.
34. von Mutius E. Allergies, infections and the hygiene hypothesis—the epidemiological evidence. *Immunobiology*. 2007;212(6):433–439.
35. Holt PG, Clough JB, Holt BJ, et al. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy*. 1992;22(12):1093–1099.
36. Prescott SL, Macaubas C, Smallacombe T, et al. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet*. 1999;353(9148):196–200.
37. Stille CJ, Andrade SE, Huang SS, et al. Increased use of second-generation macrolide antibiotics for children in nine health plans in the United States. *Pediatrics*. 2004;114(5):1206–1211.
38. Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ*. 2001;322(7283):390–395.
39. von Mutius E, Illi S, Hirsch T, et al. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. *Eur Respir J*. 1999;14(1):4–11.
40. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol*. 2009;123(4):735–746, quiz 747–748.
41. Stevenson MD, Sellins S, Grube E, et al. Aeroallergen sensitization in healthy children: racial and socioeconomic correlates. *J Pediatr*. 2007;151(2):187–191.
42. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol*. 2010;125(2 suppl. 2):S284–S296.

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