

Maternal vaginal microflora during pregnancy and the risk of asthma hospitalization and use of antiasthma medication in early childhood

Christine Stabell Benn, MD,^a Poul Thorsen, MD, PhD,^b Jørgen Skov Jensen, MD, PhD,^c Birgitte Boysen Kjær, MD,^c Hans Bisgaard, MD, DMSc,^d Morten Andersen, MD, PhD,^e Klaus Rostgaard, MSc,^a Bengt Björkstén, MD, DMSc,^f and Mads Melbye, MD, DMSc^a
Copenhagen, Aarhus, and Odense, Denmark, and Stockholm, Sweden

Background: Infants with wheezing and allergic diseases have a microflora that differs from that of healthy infants. The fetus acquires microorganisms during birth when exposed to the maternal vaginal microflora. It is therefore conceivable that the maternal vaginal microflora might influence the establishment of the infant flora and, as a consequence, the development of wheezing and allergic diseases.

Objective: We sought to study the associations between the composition of the maternal vaginal microflora and the development of wheezing and asthma in childhood.

Methods: We performed a population-based cohort study in Denmark. Vaginal samples for bacterial analysis were obtained during pregnancy. A total of 2927 women (80% of the invited women) completed the study and had 3003 live infants. Infant wheezing was assessed as one or more hospitalizations for asthma between 0 and 3 years of age. Asthma was assessed as use of 3 or more packages of antiasthma medication between 4 and 5 years of age.

Results: Maternal vaginal colonization with *Ureaplasma urealyticum* during pregnancy was associated with infant wheezing (odds ratio [OR], 2.0; 95% CI, 1.2-3.6), but not with asthma, during the fifth year of life. Maternal colonization with staphylococci (OR, 2.2; 95% CI, 1.4-3.4) and use of antibiotics

in pregnancy (OR, 1.7; 95% CI, 1.1-2.6) were associated with asthma during the fifth year of life.

Conclusion: The composition of the maternal vaginal microflora might be associated with wheezing and asthma in the offspring up to 5 years of age. (J Allergy Clin Immunol 2002;110:72-7.)

Key words: Asthma, wheezing, childhood, maternal vaginal microflora, *Ureaplasma urealyticum*, staphylococci, antibiotics

The rising frequency of allergic diseases has been hypothesized to be due to changes in the indigenous intestinal flora, resulting in reduced microbial stimulation and maturation of the immune system.^{1,2} This hypothesis is supported by several recent observations of differences in the intestinal microflora of allergic and nonallergic infants and children.^{1,3-5} For example, allergic infants have been reported to be colonized less often with bacteroides,^{1,3} to have lower ratios of bifidobacteria to clostridia,⁵ and to have higher counts of aerobic bacteria, such as *Staphylococcus aureus*, than nonallergic children.^{1,3} Furthermore, several authors have reported an association between use of antibiotics in infancy and development of allergic diseases.⁶⁻⁸ Very recently, oral supplementation with lactobacilli of pregnant women and their offspring during the first 6 months of life was reported to prevent early atopic disease in infants with a family history of allergy.⁹

Wheezing in infants and children up to 3 years of age has been associated with colonization of the throat by *Ureaplasma urealyticum*,^{10,11} which is a common inhabitant of the vaginal microflora. Eradication of the bacteria before 1 year of age reduced the incidence of wheezing.^{10,11}

During delivery, the fetus acquires microorganisms from the mother's vaginal flora, and within a few days, the composition of the fecal flora seems to be well established.¹²⁻¹⁶ A disturbed maternal microflora has been offered as a possible explanation for a disturbed infant intestinal flora.¹⁴ In the present study we used data from a previously conducted study of maternal vaginal microflora and preterm delivery to explore this hypothesis. By linking data from that study with data from registries, we were able to study the associations between different microbes in the maternal vaginal microflora and the use of antiasthma medication between 4 and 5 years of age.

We used the same data set to study whether maternal vaginal colonization with *U. urealyticum* was associated

From ^athe Department of Epidemiology Research, Danish Epidemiology Science Centre, Statens Serum Institut, Copenhagen; ^bthe Department of Epidemiology and Social Medicine, Aarhus University, Aarhus; ^cMycoplasma Laboratory, Statens Serum Institut, Copenhagen; ^dthe Department of Pediatrics, Rigshospitalet, Copenhagen; ^ethe Department of Clinical Pharmacology, Institute of Public Health, University of Southern Denmark, Odense; and ^fthe Centre for Allergy Research and Institute of Environmental Medicines, Karolinska Institutet, Stockholm.

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Reprint requests: Christine Stabell Benn, MD, Department of Epidemiology Research, Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen S, Denmark.

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Abbreviations used

ICD: International Classification of Diseases
OPED: Odense Pharmacoepidemiologic Database
OR: Odds ratio

with increased risk of infant wheezing measured by means of asthma hospitalizations up to 3 years of age.

The possible effect-measure modification by other factors of importance for the colonization of the offspring, such as maternal use of antibiotics in pregnancy¹⁷ and birth through cesarean section,¹⁸⁻²¹ was investigated. Because several studies have found different risk factors for children with and without a familiar disposition to allergy,^{6,22} we also looked for possible effect-measure modification by reported maternal allergy.

METHODS

Subjects

The present study is based on a previously conducted study of risk factors for preterm delivery. Pregnant women attending prenatal care at the Department of Obstetrics and Gynecology, Odense University Hospital, Denmark, were invited to participate in that study at their first antenatal care visit between 8 and 24 full gestational weeks (mean of 17 full gestational weeks plus 1 day, SD 2 weeks plus 4 days) during the study period from November 1992 to February 1994. The inclusion criteria were as follows: age 18 years and older, ability to understand Danish, and no history of severe fetal congenital malformations in previous pregnancies. Of the 3596 women meeting the inclusion criteria, 3174 (88%) participated. The present study is based on the 3003 infants of the 2927 women (80% of the 3596 invited women) for whom complete information was available on exposures during pregnancy and delivery.

Assessment of exposures

At enrollment, all participants had a pelvic examination, and vaginal-cervical samples were obtained. The sampling technique and the microbiologic methods have been described thoroughly elsewhere.²³ Examinations and collection of samples were done by one of the investigators (P.T.) in 98% of the patients and by specialists in gynecology and obstetrics in the remaining 2%.

The women were asked to complete a questionnaire at enrollment, in gestational week 30, and again at birth. Questions related to allergy included "Are you suffering from allergy (eg, toward penicillin, pollen, or nickel)?" and, if the answer was affirmative, the type of allergy was questioned. Some women answered by mentioning an allergic disease, and others indicated the provoking allergen or allergens. Women who reported asthma, hay fever, or atopic dermatitis or who mentioned relevant allergens, such as pollen, animal dander, house dust, or nuts, were considered to have an allergic disease. Furthermore, the women were asked about the size of the household, education, and smoking habits and, at each time point, about any use of antibiotics in the preceding 2 weeks and during pregnancy in general. The children were born between March 1993 and August 1994. Midwives collected data on the birth, including gestational age, birth weight, and mode of delivery. Data on feeding type during the first days of life were collected if the mother and child stayed at the hospital more than 6 hours after the delivery (n = 2098).

Assessment of outcomes

For the present study, data on hospitalizations for asthma were retrieved from The Danish Hospital Discharge Register. The 8th revision of the International Classification of Diseases (ICD) was

used until 1994, after which the 10th revision was used. The ICD-8 diagnoses 493.* (asthma bronchial) and 466.02 (bronchitis acuta asthmatica) and the ICD-10 diagnoses J45.* (asthma) and J46.* (status asthmaticus) were considered indicative of wheezing between 0 and 3 years of age.

Data on the use of antiasthma medication were retrieved from the Odense Pharmacoepidemiologic Database (OPED), a prescription database that has been described in detail elsewhere.²⁴ In brief, information on all prescription refunds in the county of Funen (covering the referring area of the Department of Obstetrics and Gynecology, Odense University Hospital, Denmark) since 1992 is included in the OPED. It covers prescriptions issued by general practitioners, specialists in private practice, and physicians in outpatient units. Medication during hospital admissions is not registered. Since April 1996, children have been registered under a personal registration number, making studies on their drug use possible. The analyses of the use of asthma medication were limited to the 2742 children who were still living in the county of Funen in the year 2000 and thus presumably covered by OPED in their fifth year of life, which fell between March 1998 and August 1999. The Anatomical Therapeutic Chemical classification index was used. The codes R03AC (inhaled selective β -adrenoceptor agonists), R03BA (inhaled glucocorticoids), and R03BB (inhaled anticholinergics) were considered to indicate antiasthma medications. Antiasthma medications dispensed as mixtures are often used to treat acute bronchitis and were therefore excluded from the analyses. Antiasthma medication in the fifth year of life was defined as the prescription of 3 or more packages of antiasthma medication. We did not use data on hospital admission for asthma because very few of the children were hospitalized for asthma in the fifth year of life, and they almost exclusively fell into the category of antiasthma medication users.

Statistical methods

All associations were measured in terms of odds ratios (ORs) and assessed by means of logistic regression with asthma hospitalizations and use of antiasthma medication, respectively, as outcomes. First, the associations between the outcomes and potential confounders were assessed in univariate analyses. We tested whether potential confounders with more than 2 levels could be reduced to binary form without loss of information. If so, subsequent analyses were made by using the binary form. A logistic regression with stepwise elimination to exclude potential confounders that did not contribute significantly ($P > .10$) was performed for each of the outcomes to obtain a parsimonious adjusted model. The associations between each outcome and the exposures of interest were subsequently assessed in the obtained adjusted model. Effect-measure modification was investigated by examining the multiplicative interaction term. P values were based on likelihood ratio tests, and 95% CIs were based on Wald tests. All calculations were carried out with the SAS software package (version 6.12).

Ethical aspects

The Ethical Committee for Vejle and Funen Counties and the Danish Data Protection Board approved the original study. The present study, which did not include additional contacts with the original participants, was approved by the Danish Data Protection Board according to Danish law.

RESULTS

Clinical outcomes

Fifty-one (1.7%) of the 3003 children were hospitalized for asthma during the first 3 years of life, and 93 (3.4%) of the 2742 children who still lived in the county in 2000 used 3 or more packages of antiasthma medica-

Asthma, rhinitis,
other respiratory
diseases

TABLE I. Potential confounding factors and risk of asthma hospitalization and use of antiasthma medication

	Distribution (%)	Hospital admission for asthma, age 0-3 y, OR (95% CI)	Use of antiasthma medication, age 4 to 5 y, OR (95% CI)
Boys	1501 (50)	1.4 (0.8-2.5)	1.2 (0.8-1.9)
Girls	1501 (50)	1.0 (ref)	1.0 (ref)
Gestational age (wk)			
<37	202 (7)	2.3 (1.0-5.2)	2.2 (1.2-4.2)
37-41	2518 (84)	1.0 (ref)	1.0 (ref)
>41	283 (9)	1.1 (0.5-2.9)	1.2 (0.6-2.4)
Birth weight (g)			
≤2500	211 (7)	2.4 (1.1-5.2)	1.7 (0.9-3.3)
2501-4000	2280 (76)	1.0 (ref)	1.0 (ref)
>4000	512 (17)	0.7 (0.3-1.7)	0.8 (0.4-1.4)
Maternal age (y)			
≤20	32 (1)	2.2 (0.3-16.5)	0.8 (0.1-6.3)
21-25	475 (16)	1.0 (0.4-2.4)	1.0 (0.6-1.7)
26-30	1352 (45)	1.0 (ref)	1.0 (ref)
31-35	884 (29)	1.4 (0.7-2.6)	0.5 (0.3-0.9)
>35	260 (9)	1.3 (0.5-3.5)	0.7 (0.3-1.6)
Mother <12 y school			
Yes	1370 (47)	1.7 (1.0-3.0)	1.7 (1.1-2.5)
No	1552 (53)	1.0 (ref)	1.0 (ref)
Smoking in pregnancy			
Yes	887 (30)	2.1 (1.2-3.7)	1.0 (0.6-1.5)
No	2080 (70)	1.0 (ref)	1.0 (ref)
Maternal allergic disease			
Yes	430 (15)	1.5 (0.8-3.1)	2.6 (1.7-4.2)
No	2533 (85)	1.0 (ref)	1.0 (ref)
First days' feeding			
Formula	174 (8)	0.8 (0.3-2.7)	2.6 (1.3-5.1)
Mixed	275 (13)	1.0 (0.4-2.5)	1.9 (1.0-3.6)
Breast	1649 (79)	1.0 (ref)	1.0 (ref)

TABLE II. Maternal colonization with *U urealyticum* and risk of asthma hospitalization

	No. (% of all)	Asthma hospitalization, age 0-3 y, adjusted OR (95% CI)*
<i>U urealyticum</i>	1225 (41)	2.0 (1.2-3.6)
Maternal allergic disease		
Yes	424 (14)	13.2 (1.6-105)†
No	2503 (86)	1.4 (0.7-2.6)†
Cesarean delivery		
Yes	498 (17)	1.2 (0.3-4.3)
No	2466 (83)	2.3 (1.2-4.4)

*Adjusted for low birth weight (≤2500 g) and maternal smoking in pregnancy.

†Significant interaction, $P = .01$.

tion between 4 and 5 years of age. Nine (20%) of the 46 children with a history of hospitalization still living in the county in 2000 used antiasthma medication.

The potential confounders—single motherhood, number of other children in the home, and season of birth—were not associated with any of the outcomes. The associations between the remaining potential confounding factors and the outcomes are shown in Table I. By using logistic regression with stepwise elimination of noncontributing variables, low birth weight (≤2500 g) and maternal smoking during pregnancy contributed independently to the risk of early asthma hospitalizations, and low ges-

tational age at less than 37 weeks, maternal age, maternal education, and maternal allergic disease contributed independently to the risk of antiasthma medication use between 4 and 5 years of age. Accordingly, an adjusted model was developed for each of the outcomes. Feeding type during the first days of life was associated with use of antiasthma medication between 4 and 5 years of age, but the information was only available for a selected subgroup. Therefore we made 2 adjusted models, one without and one with first days' feeding included.

Hospital admission for asthma, age 0-3 years

Data on hospital admission for asthma from age 0 to 3 years are given in Table II. Maternal colonization with *U urealyticum* was associated with an increased likelihood of hospitalization with asthma in the offspring in univariate analysis (OR, 2.3; 95% CI, 1.3-4.0). The association between *U urealyticum* and asthma hospitalization remained significant when low birth weight (≤2500 g) and maternal smoking were included in the same model (OR, 2.0; 95% CI, 1.2-3.6). In such a model, smoking in pregnancy (OR, 1.8; 95% CI, 1.0-3.2) and birth weight (OR, 2.1; 95% CI, 1.0-4.7) also remained independently associated with hospitalization.

The causative fraction contributed by *U urealyticum* was 0.3 (0.1-0.5), which is equivalent to a reduction by 15 (5-26) of the 51 asthma hospitalizations in the cohort had *U urealyticum* been eradicated. [The risk among

TABLE III. Colonization-associated factors and risk of use of antiasthma medication

	Frequency (%)	Antiasthma medication use, age 4-5 y, adjusted OR (95% CI)*	Antiasthma medication use, age 4-5 y, adjusted OR (95% CI)†
<i>Lactobacillus</i> species	2080/3003 (69)	0.8 (0.5-1.3)	0.8 (0.5-1.4)
<i>Bacteroides</i> species	158/3003 (5)	0.4 (0.1-1.7)	0.0 (0.0-1.0)
<i>Staphylococcus</i> species	1354/3003 (45)	2.2 (1.4-3.4)	1.9 (1.1-3.1)
<i>Streptococcus</i> species	424/3003 (14)	1.5 (0.9-2.6)	1.5 (0.8-2.9)
<i>Candida albicans</i>	409/3003 (14)	1.1 (0.6-2.1)	1.4 (0.7-2.8)
<i>Escherichia coli</i>	276/3003 (9)	1.1 (0.6-2.2)	1.1 (0.5-2.4)
<i>Mycoplasma hominis</i>	203/3003 (7)	1.3 (0.6-2.8)	1.0 (0.4-2.5)
<i>Gardnerella vaginalis</i>	1014/3003 (34)	0.9 (0.6-1.4)	0.7 (0.4-1.3)
Antibiotics in pregnancy	757/2838 (27)	1.7 (1.1-2.6)	1.8 (1.0-3.1)
Cesarean section	504/3000 (17)	1.1 (0.6-1.9)	1.3 (0.7-2.4)

*Adjusted for low gestational age (<37 weeks), maternal age, maternal education, and maternal allergic disease.

†Further adjusted for breast-feeding and thus including only the 2098 cases in which the mother and child stayed at the hospital for more than 6 hours.

children of *U urealyticum*-positive mothers was not affected by treatment in pregnancy with antibiotics considered efficient against *U urealyticum* in vitro (in the present study, almost exclusively erythromycin) after the smear had been taken (data not shown).] Colonization with *U urealyticum* was associated with an increased risk of asthma hospitalization in children of allergic mothers compared with that in children of nonallergic mothers ($P = .01$).

There were no significant associations between asthma hospitalization between 0 and 3 years of age in the offspring and the presence of any of the other microbes in the vaginal microflora, use of antibiotics in pregnancy, or mode of delivery (data not shown).

Use of antiasthma medication, age 4-5 years

Data on the use of antiasthma medication from age 4 to 5 years are given in Table III. Colonization with staphylococci was associated with use of antiasthma medication in the offspring in univariate analysis (*S aureus*: OR, 1.6; 95% CI, 0.8-3.3; other staphylococci species: OR, 2.2; 95% CI, 1.4-3.4, as compared with no colonization with staphylococci). The effects of *S aureus* and other staphylococci were not statistically significantly different and were therefore combined into one group (OR, 2.1; 95% CI, 1.4-3.2). In the adjusted model the association remained significant (OR, 2.2; 95% CI, 1.4-3.4).

Maternal use of antibiotics in pregnancy was associated with the use of antiasthma medication during the fifth year of life in univariate analysis (OR, 1.7; 95% CI, 1.1-2.6). The association remained significant (OR, 1.7; 95% CI, 1.1-2.6) in the adjusted model, including low gestational age (<37 weeks), maternal age, maternal education, and maternal allergic disease.

There were no significant associations between any of the other microbes in the vaginal microflora or mode of delivery and use of antiasthma medication in the adjusted model. In particular, there was no association between maternal colonization with *U urealyticum* and use of antiasthma medication during the fifth year of life (OR, 1.0; 95% CI, 0.7-1.6).

Effect-measure modification by use of antibiotics in pregnancy, cesarean section, and maternal allergic disease

Apart from the above-mentioned effects, there were no differential effects of the different microbes in the maternal vaginal microflora on the outcomes according to use of antibiotics in pregnancy, cesarean section, or maternal allergic disease.

DISCUSSION

As hypothesized a priori, we found that maternal colonization with *U urealyticum* in pregnancy was a risk factor for infant wheezing. Maternal colonization with staphylococci and use of antibiotics in pregnancy were risk factors for asthma during the fifth year of life.

U urealyticum is a sexually transmitted human pathogen that can be found in the cervix or vagina of 40% to 80% of asymptomatic women of reproductive ages. Colonization is linked to young age, low socioeconomic status, and multiple partners.²⁵ It can be transmitted vertically, and it has been isolated from the respiratory tract of 40% to 51% of newborn babies.²⁶⁻²⁸ The transmission rate does not seem to be affected by mode of delivery.²⁸ This is probably because of the fact that *U urealyticum* might be present in amniotic fluid as early as week 15²⁹ and weeks 24 to 43 of gestation,^{29,30} indicating in utero infection.

Recently, *U urealyticum* was associated with wheezing at up to 3 years of age.^{10,11} The association between maternal vaginal colonization with *U urealyticum* and asthma hospitalizations at 0 to 3 years of age in our study suggests that *U urealyticum* acquired before or at the time of birth is associated with reactive airway disease in infants. The absence of any significant modifying effect of cesarean section suggests in utero infection of the fetus.³¹

If *U urealyticum* was eradicated, our data suggest a possible reduction by nearly one third of hospitalizations for wheezing. [However, erythromycin, which is generally considered to be the macrolide of choice during preg-

Asthma, rhinitis,
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nancy, does not eradicate *U urealyticum* in vivo.³² This might explain why antibiotic treatment did not modify the effect of *U urealyticum* in the present study.] Perhaps treatment of the colonized child might be a more efficient prophylactic measure.¹¹

It is noteworthy that although infant wheezing before 3 years of age is not generally considered to be of allergic origin, we found an indication that the effect of *U urealyticum* might be stronger in children of allergic mothers. If true, this could suggest an increased susceptibility of children with a genetic disposition to allergic disease to either infection with or reaction toward *U urealyticum*. The observation is similar to the recently observed association between respiratory syncytial virus and atopy.³³ There was no association between maternal colonization with *U urealyticum* and use of antiasthma medication at 4 to 5 years of age. On the basis of this observation, we do not believe that *U urealyticum* is associated with long-lasting reactive airways or with asthma.

During the last decades, changes in the intestinal flora have been observed among infants from high-income countries in parallel with the rise in prevalence of allergic diseases in these countries. The changes include later and less colonization with *Escherichia coli*, other enterobacteria, enterococci, bifidobacteria, and lactobacilli and an increased prevalence of colonization with *Clostridium difficile* and *S aureus*. The changes have been ascribed to cesarean section, use of antibiotics and neonatal intensive care, the overly hygienic lifestyle in the countries in general, and disturbed vaginal microflora of the mothers.^{1,2} Lactobacilli are markers of a normal vaginal microflora, but there was no significantly decreased risk of asthma associated with maternal colonization with lactobacilli in our study. However, maternal colonization with staphylococci was associated with an increased risk of asthma. This observation is in line with previous findings of a higher rate of intestinal colonization with *S aureus* in allergic children.^{1,3} The increasing prevalence of *S aureus* in the intestine has been suggested to be due to lack of competition from other bacterial species, which are better suited for colonization of the intestine.³⁴ Thus intestinal colonization with staphylococci can be regarded as a marker of lack of a normal intestinal microflora. Maternal vaginal staphylococci in midpregnancy might be a marker of lack of normal microflora and diversity. The transfer of staphylococci and the lack of transfer of other microorganisms might influence the child's gut flora and thereby the normal immunologic development, leading to asthma.

Also in line with previous observations,^{1,3} although not statistically significant, lack of colonization with bacteroides seemed to be associated with asthma.

Use of antibiotics, especially broad-spectrum antibiotics, affects the gram-negative flora of the intestine profoundly. Gram-negative bacteria have been reported to induce the IL-10³⁵ cytokine, which inhibits skin reactivity to allergens,³⁶ presumably by inhibiting IgE-induced activation of mast cells.³⁷ In the present study use of antibiotics in pregnancy was weakly associated with asthma in

the fifth year of life. Use of antibiotics in infancy is a possible confounder; mothers who used antibiotics in pregnancy might be more likely to ask for antibiotic treatment of their infant, and thus we cannot exclude that the association between maternal use of antibiotics in pregnancy and asthma in the fifth year of life might be explained by use of antibiotics in infancy. Thus we do think that the association should be interpreted with caution.

It is not known which species of intestinal microorganisms are particularly important for the maturation of the immune system in infants and how and when this is achieved. However, it seems unlikely that the maturation would depend on the presence or absence of single microbes. Rather, the diversity of the flora would be expected to be important. This could not be addressed in the present study.

Our outcome measures are register based. This raises the question of outcome misclassification. We selected specific outcomes by considering only hospital-verified diagnoses of asthma and by requiring use of at least 3 packages of antiasthma medication in 1 year. Thus we believe the specificity of the outcomes to be high, whereas the sensitivity was probably low. Women who used antibiotics in pregnancy might be more likely to ask for antiasthma medication for their children, but otherwise there seems to be no reason to suspect differential misclassification with respect to any of the other exposures. Thus for both outcomes, this type of nondifferential misclassification would result in underestimation of the true exposure effects. There was only a small overlap between children hospitalized for asthma during the first 3 years and children using antiasthma medication during the fifth year of life. This can be explained by the fact that infant wheezing is different from asthma in older children, with the latter often being of allergic origin.^{38,39} Supporting this observation, maternal smoking and low birth weight are strong risk factors for early wheezing, but not for asthma,^{40,41} whereas maternal allergy is a stronger risk factor and high maternal age a more protective factor for asthma than for wheezing.⁴¹

Maternal allergic disease was diagnosed by means of 2 simple questions, and there might be some misclassification. However, by including only women who gave answers indicating asthma, hay fever, atopic dermatitis, or relevant provoking allergens, we find that we have matched the criteria used in other epidemiologic studies. The strong association between maternal allergic disease and use of antiasthma medication between 4 and 5 years of age supports the validity of the diagnosis.

The composition of the maternal vaginal microflora was determined between the 8th and the 24th gestational week and might not be representative of the maternal vaginal microflora at the time of delivery. However, the presence of many of the microbes, including *U urealyticum*, is relatively stable over time.⁴² Even if representative of the maternal vaginal microflora at birth, this study cannot prove that the maternal vaginal microflora was actually conferred to the infant, although there is considerable evidence that maternal vaginal flora colonizes

the infant.¹²⁻¹⁶ Regardless, the observed associations need not be causal; it cannot be excluded that maternal vaginal microflora is merely a marker of something else.

In conclusion, maternal colonization with *U urealyticum* in pregnancy was associated with infant wheezing but did not seem to be associated with long-lasting reactive airways or with asthma. Furthermore, this study adds maternal colonization with staphylococci and use of antibiotics in pregnancy to the possible risk factors for asthma. The composition of the maternal vaginal microflora might be associated with infant wheezing and asthma during early childhood.

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