Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy

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Background: Both gastrointestinal microbiota composition and cesarean section have been linked to atopic manifestations. However, results are inconsistent, and the hypothesized intermediate role of the microbiota in the association between birth mode and atopic manifestations has not been studied yet.
Objectives: We sought to investigate the relationship between microbiota composition, mode and place of delivery, and atopic manifestations.
Methods: The Child, Parent and Health: Lifestyle and Genetic Constitution Birth Cohort Study included data on birth characteristics, lifestyle factors, and atopic manifestations collected through repeated questionnaires from birth until age 7 years. Fecal samples were collected at age 1 month (n = 1176) to determine microbiota composition, and blood samples were collected at ages 1 (n = 921), 2 (n = 822), and 6 to 7 (n = 384) years to determine specific IgE levels.
Results: Colonization by *Clostridium difficile* at age 1 month was associated with wheeze and eczema throughout the first 6 to 7 years of life and with asthma at age 6 to 7 years. Vaginal home delivery compared with vaginal hospital delivery was associated with a decreased risk of eczema, sensitization to food allergens, and asthma. After stratification for parental history of atopy, the decreased risk of sensitization to food allergens (adjusted odds ratio, 0.52; 95% CI, 0.35-0.77) and asthma (adjusted odds ratio, 0.47; 95% CI, 0.29-0.77) among vaginally home-born infants was only found for children with atopic parents. Mediation analysis showed that the effects of mode and place of delivery on atopic outcomes were mediated by *C difficile* colonization.
Conclusion: Mode and place of delivery affect the gastrointestinal microbiota composition, which subsequently influences the risk of atopic manifestations. (J Allergy Clin Immunol 2011;128:948-55.)

Key words: Mode of delivery, cesarean section, hospital delivery, gastrointestinal microbiota composition, wheeze, eczema, asthma, atopy, mediation analysis, cohort study, KOALA study

Perturbations in the microbial colonization of an infant's gastrointestinal tract have been associated with an increased risk of atopic diseases in early life.[1-4] These alterations, such as a delayed colonization with beneficial bacteria, might interfere with the development of immunologic tolerance. The absence of a proper immunosuppressive mechanism by regulatory T cells can result in an imbalance between T_H1 and T_H2 cells and, in turn, T_H1- or T_H2-mediated inflammatory diseases.[5,6] Both cross-sectional and longitudinal studies have reported differences in gastrointestinal microbiota (GM) composition between allergic and nonallergic children.[1-3,7] Within the Child, Parent and Health: Lifestyle and Genetic Constitution (KOALA) Birth Cohort Study, we have previously found an association between GM composition and eczema, recurrent wheeze, and allergic sensitization in the first 2 years of life,[4,8] but the influence on the development of atopic diseases later in life, such as asthma, has not been studied yet.

Another perinatal risk factor for an increased risk of atopic disease is cesarean section, as shown in 2 meta-analyses conducted in 2008 by Bager et al[9] and Thavagnanam et al[10] (pooled odds ratios [ORs] of 1.18 [95% CI, 1.05-1.32] and 1.20 [95% CI, 1.14-1.26], respectively). There is evidence suggesting that cesarean section not only increases the risk of asthma but also of other allergic diseases, such as food allergy.[9]

It has been suggested that the neonatal GM composition has an intermediate role in the relationship between delivery characteristics and the development of atopic disease. This is based on the fact that children born by means of cesarean section are not exposed to their mother's vaginal and fecal bacteria, unlike children born vaginally. In a recent study on the bacterial composition of the skin, oral mucosa, and gut, vaginally delivered infants

Abbreviations used
  aOR: Adjusted odds ratio
   GM: Gastrointestinal microbiota
KOALA: Child, Parent and Health: Lifestyle and Genetic
        Constitution
    OR: Odds ratio

harbored bacterial communities that were most similar in composition to the vaginal communities of the mothers, whereas infants delivered by means of cesarean section lacked bacteria from their mother's vaginas.[11]

Initially, children born by means of cesarean section are colonized by bacteria originating from the hospital environment and nonmaternal skin bacteria,[11-13] resulting in a delayed colonization of the gut by beneficial bacteria and a different bacterial composition compared with that seen in vaginally delivered infants. Infants born by means of cesarean section have an especially lower abundance of *Bifidobacterium* and *Bacteroides* species and are more frequently colonized by *Clostridium difficile*.[12,14,15] The place of delivery has an additionally important effect on the establishment of the GM composition because infants born vaginally in a hospital have a different GM composition, including more *C difficile*, compared with home-born infants.[11,14]

Although the GM is hypothesized to constitute an intermediate factor in the causal pathway between delivery characteristics and atopic diseases, this intermediate role has not been studied. We therefore investigated whether GM composition and mode and place of delivery are associated with the development of atopic manifestations, including asthma in childhood. Additionally, we investigated whether the GM composition is an intermediate factor in the relationship between mode and place of delivery and the development of atopic manifestations.

METHODS
Study population and design

The KOALA study is a prospective birth cohort study enrolling pregnant women at 34 weeks of gestation. From an existing cohort, 2343 healthy pregnant women with a conventional lifestyle were recruited.[16] Furthermore, 491 women with alternative lifestyles regarding child-rearing practices, dietary habits, restricted use of medication, and vaccination schemes were included in the cohort. Newborns with congenital abnormalities (eg, Down syndrome) and premature newborns (gestational age, <37 weeks) were excluded. Children who received antibiotics in the first month of life were excluded from the analyses that included GM composition. More details of the study population and methods have been described previously.[17]

Questionnaires on demographic and perinatal and lifestyle factors were sent to all parents at 34 weeks of gestation and at 3, 7, 12, and 24 months and at 4 to 5 and 6 to 7 years postpartum. The questionnaires from 7 months postpartum and onward included questions adapted from the International Study of Asthma and Allergies in Childhood to collect data on atopic manifestations and wheeze.[18] Parents recruited from January 2002 onward were also asked for informed consent for sampling the feces of the child at the age of 1 month postpartum (n = 1176) to determine gastrointestinal microbial composition. Children in this subgroup, if still participating, were also eligible for blood sampling at the ages of 1, 2, and 6 to 7 years to determine specific IgE levels.

Informed consent forms were signed by all parents. The KOALA study was approved by the Medical Ethical Committee of the Maastricht University/ University Hospital of Maastricht and the National Ethical Committee for Medical Research.

Microbial analysis

Infants' fecal samples were collected at age 1 month by their parents and immediately sent to the laboratory by post. The methods of fecal sampling and subsequent DNA extraction have been described in detail elsewhere.[14]

Fecal samples were subjected to real-time PCR for detection of *Bifidobacterium* species, *Escherichia coli*, *C difficile*, *Bacteroides fragilis* group, and *Lactobacillus* species.[14]

Definition of mode and place of delivery

Mode and place of delivery were measured in the 3-month postpartum questionnaire as "vaginal delivery at home," "vaginal delivery in the hospital," or "cesarean section delivery in the hospital." Artificial deliveries (eg, use of forceps or vacuum pump) were considered vaginal delivery in the hospital because those deliveries took place in a hospital.

Definition of parentally reported atopic outcomes

The presence of asthma, wheeze, and eczema was based on parentally completed questionnaires on atopic symptoms adapted from the International Study of Asthma and Allergies in Childhood questionnaire.[18]

Asthma at the age of 6 to 7 years was defined as a parentally reported doctor's diagnosis of asthma in combination with clinical symptoms in the last 12 months or having used asthma medications in the last 12 months. Clinical symptoms were defined as at least 1 attack of wheeze or dyspnea. Asthma medication was defined as regular use of short-acting bronchodilators or use of inhaled corticosteroids according to the Dutch guidelines of treatment of bronchial asthma in children. Regular use was defined as everyday use for a period of at least 2 months during the last 12 months or use associated with the physical activity of the child.[19]

The presence of wheeze was defined as parentally reported wheeze since the last follow-up moment for questionnaires in the first year of life (questionnaires at 7 and 12 months) and in the last 12 months for questionnaires after the first year of life (24 months, 4-5 years, and 6-7 years).

Children were regarded as having eczema when their parents provided an affirmative answer to the following question: "Did your child suffer from an itchy rash that was coming and going over the last 12 months (or since the last follow-up in the first year of life)?" Children for whom only diaper rash, rash around the eyes, and/or scalp scaling was reported were not regarded as having had eczema. Eczema was measured at 7, 12, and 24 months and 6 to 7 years of age.

Determination of atopic sensitization

At 1 year postpartum, infants' capillary blood spots were collected during home visits. Levels of specific IgE against hen's eggs, cow's milk, and peanut were measured as described earlier,[20,21] with modifications to accommodate the use of capillary blood samples.[22] The detection limit for specific IgE was less than 0.36 IU/mL. Children were considered sensitized if specific serum IgE levels were greater than the detection limit for at least 1 allergen.

At 2 and 6 to 7 years postpartum, children's venous blood samples were collected during home visits and stored at −80°C until analysis. Samples were analyzed for specific IgE against hen's egg, cow's milk, peanut, birch, grass pollen, cat, dog, and house dust mite by using RASTs, as described earlier.[23] The detection limit for specific IgE was 0.10 IU/mL. Children were considered sensitized if specific serum IgE levels were greater than 0.35 IU/mL against at least 1 allergen.

All IgE measurements were done by Sanquin Research (Amsterdam, The Netherlands).

Statistical analyses

Logistic regression models were used to estimate unadjusted and adjusted odds ratios (ORs) with 95% CIs for the outcome of asthma at 6 to 7 years.

Generalized estimating equation models with exchangeable correlation structures were used for the repeated outcome measurements of eczema, wheeze, and sensitization to food and inhalant allergens.

Because participants were recruited from 2 different sources (conventional and alternative recruitment groups), interaction between main determinants
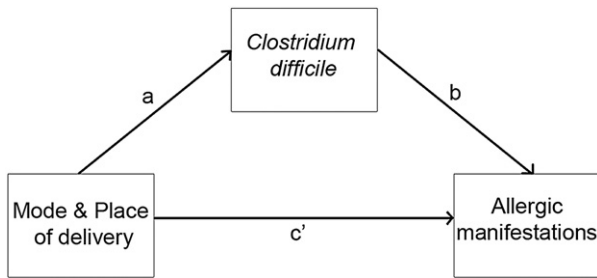
FIG 1. Mediation model. The relation between the antecedent variable mode of delivery on the dependent variable allergic manifestations is mediated through *C difficile*. *a* Represents the strength of the relation between mode of delivery and *C difficile*, *b* represents the strength of the association between *C difficile* and the allergic outcomes, and *c'* represents the strength of the relation between mode of delivery and the allergic outcomes.

and the recruitment group was tested in all models. Because none of the interaction terms reached statistical significance, the groups were combined and treated as 1 cohort in the analyses.

The following variables were included in the analyses to control for potential confounding: recruitment group (conventional, alternative), sex (male, female), birth weight (in grams), breast-feeding (never, 0-3 months, 3-6 months, >6 months of breast-feeding), maternal smoking during pregnancy (yes, no), maternal age at estimated date of delivery (in years), highest achieved maternal education level (low: primary school, preparatory vocational or lower general secondary education; middle: vocational education, higher general secondary or preuniversity education; high: higher vocational or academic education), and age at collection fecal sample (in days). Furthermore, we controlled for siblings' atopic history, which was defined as parent-reported doctor-diagnosed food allergy, eczema, hay fever, asthma, and pet and/or house dust mite allergy (no siblings; ≥1 sibling, none atopic; or ≥1 sibling, at least 1 atopic).

Because previous studies have shown an effect modification by parental history of atopy,[24-26] all analyses were conducted both unstratified and stratified for parental history of atopy. In the unstratified analyses parental atopic history was included as an additional confounder. Parental history of atopy was defined as follows: self-reported doctor-diagnosed eczema, hay fever, asthma, and pet and/or house dust mite allergy (no atopic parent; at least 1 parent atopic) were taken into account.

SPSS 15.0 for Windows (SPSS, Inc, Chicago, Ill) was used to perform the statistical analyses. *P* values of less than .05 were considered statistically significant.

To investigate whether *C difficile* mediated the associations between mode of delivery and the atopic manifestations, we used the *ab* product coefficient method. This entails estimating the product of 2 coefficients: that of the association between mode of delivery and *C difficile* (the *a* path) and that of the association between *C difficile* and study outcomes (the *b* path, Fig 1).[27] We obtained standardized coefficients and SEs in these analyses.[27] These data were then entered into the RMediation package written for use in the R statistical software program to calculate 95% CIs around the *ab* estimate.[28] The *ab* estimate indicates the magnitude of the mediated or indirect "effect," which is significant if 0 is not included in the CI. Note that we used the method of distribution of the product to estimate the 95% CI; this yields asymmetric confidence limits to properly reflect the distribution of the *ab* estimate, which is often skewed.[27]

All mediation analyses were confined to the subsets of study participants with complete data on the 3 primary variables considered in each model (ie, mode of delivery, *C difficile*, and study outcome).

RESULTS

Of the 2834 children enrolled in the cohort, 8 were excluded because of congenital abnormalities, 91 because of prematurity (gestational age, <37 weeks), and 2 because of both, resulting in 2733 children included in the study (Fig E1 in this article's Online

Repository at www.jacionline.org). Table I shows the baseline characteristics for the total study cohort, the children for whom the 6- to 7-year questionnaire was available (n = 1926), and the children from whom an appropriate fecal sample was received at age 1 month (n = 952). Because recruitment of the alternative group coincided with the start of biosampling, the subgroup from whom fecal samples had been collected consisted of a higher proportion of participants from the alternative recruitment group than the group recruited earlier. This difference is also reflected in the duration of breast-feeding, smoking during pregnancy, and maternal educational level.

At age 6 to 7 years, 6.9% (133/1915) of the children had parent-reported asthma. At the age of 12 months, the prevalence of wheeze was 16.9% and decreased with age to 8.0% at age 6 to 7 years (see Fig E2 in this article's Online Repository at www.jacionline.org). For eczema, the prevalence peaked at 24 months at 23.3% and decreased to 12.4% at age 6 to 7 years (see Fig E3 in this article's Online Repository at www.jacionline.org). At the age of 1 year, 13.9% of the children were sensitized to food allergens compared with 21.5% and 21.6% at the ages of 2 and 6 to 7 years, respectively. Sensitization to inhalant allergens increased from 10.7% at age 2 years to 28.9% at age 6 to 7 years.

GM composition and atopic outcomes

Birthplace and mode were strongly associated with colonization by *C difficile*: colonization rates for vaginally home-born, vaginally hospital-born, and cesarean section–delivered children were 19.1%, 27.2%, and 43.4%, respectively (P_{trend} < .001).

C difficile was also the single gut microbe investigated that was associated with an increased risk for asthma (adjusted odds ratio [aOR], 2.06; 95% CI, 1.16-3.64; Table II). Stratified results showed that this association was restricted to those children with atopic parents (aOR, 2.27; 95% CI, 1.22-4.21).

The previously reported associations between the presence of *C difficile* in the feces at 1 month postpartum and the development of wheeze, eczema, and sensitization to food allergens in the first 2 years of life[4] were also found in this study and persisted throughout the first 7 years of life (Tables II and III). For eczema and sensitization to food allergens, we also found that the association with *C difficile* was limited to children with a family history of atopy, whereas the association between this bacterium and wheeze was more pronounced in the children without atopic parents.

None of the other bacterial species and genera under study were associated with the atopic outcomes in either the unstratified (Tables II and III) or stratified (data not shown) analysis.

Mode and place of delivery and atopic outcomes

Children with at least 1 atopic parent who were born vaginally at home had a lower odds of having asthma than children born vaginally at the hospital (reference category; aOR, 0.47; 95% CI, 0.29-0.77; Table IV). The risk of asthma for children born by means of cesarean section did not differ significantly compared with that of children born vaginally in the hospital. These associations were absent in children without atopic parents.

Vaginal hospital delivery was also associated with a reduced risk of eczema in the period between 0 and 7 years, although results were only statistically significant in the unstratified analyses. No significant associations were found between the mode and place of delivery and wheeze (Table IV).

TABLE I. Baseline characteristics

	Total study cohort (n = 2733)		Children with question-naire at 6-7 y available (n = 1926)		Children with GM composition determined (n = 952)	
	No.*	Percent	No.*	Percent	No.*	Percent
Mode and place of delivery						
Vaginal at home	1179	46.1	862	46.0	450	48.3
Vaginal in hospital	1092	42.7	795	42.4	382	41.0
Cesarean section	288	11.2	216	11.5	99	10.6
Conventional recruitment group	2257	82.6	1570	81.5	649	68.2
Male sex	1385	50.7	961	49.9	479	50.3
Maternal atopy (yes)	986	37.4	712	37.3	368	38.7
Paternal atopy (yes)	959	36.7	697	36.5	361	38.2
Siblings with atopy						
No siblings	2170	83.0	1599	83.7	789	82.9
No atopic siblings	319	12.2	225	11.8	101	10.6
≥1 Atopic sibling	126	4.8	87	4.5	61	6.4
Breast-feeding						
None	399	15.3	252	13.3	115	12.3
0-3 mo	941	36.0	637	33.7	264	28.2
4-6 mo	417	15.9	326	17.2	170	18.1
>6 mo	858	32.8	677	35.8	388	41.4
Maternal education level						
Low	274	10.2	154	8.1	68	7.2
Middle	1125	41.7	756	39.8	345	36.6
High	1299	48.1	991	52.1	529	56.2
Smoking during pregnancy (yes)	198	7.2	98	5.1	35	3.7
	Mean	SD	Mean	SD	Mean	SD
Maternal age at delivery (y)	32.0	3.79	32.3	3.71	32.7	3.85
Birth weight (g)	3537.1	476.3	3549.3	467.9	3562.5	459.9

*Numbers might not add up to the total number because of missing values.

In children with a positive family history of atopy, vaginal home delivery decreased the odds for sensitization to food allergens (aOR, 0.52; 95% CI, 0.35-0.77) compared with vaginal birth in the hospital. The odds of sensitization to food allergens did not differ significantly between children born by means of cesarean section or born vaginally in the hospital (Table V).

In contrast to sensitization to food allergens, mode and place of delivery were not associated with sensitization to inhalant allergens (Table V), although it has to be noted that these latter analyses showed a statistically significant interaction with time, meaning that results differed for sensitization at the different time points (2 and 6-7 years, respectively). Further analyses showed that, although not statistically significant, home delivery tended toward decreased risk of sensitization to inhalant allergens in children with atopic parents only at age 6 to 7 years (aOR, 0.65; 95% CI, 0.35-1.21).

Mediation analyses

Because mode and place of delivery strongly influence *C difficile* colonization and this bacterium is also associated with the atopic manifestations under study, colonization by *C difficile* might act as an intermediate factor in the association between birth mode and atopic diseases.

Mediation analyses (Table VI) indeed showed that *C difficile* mediated the relation between mode and place of delivery and both asthma and eczema. Statistically significant *ab* cross-products were found not only for birthplace in relation to these

atopic outcomes but also for birth mode (cesarean section vs vaginal hospital delivery).

Although stratified analyses did not always show statistically significant *ab* cross-products in the group with parental atopy, the direction and order of magnitude were comparable with the over-all analyses. Additional adjustment for the confounders included in previous analyses (Tables II-V) generated similar results (data not shown).

DISCUSSION

In this prospective birth cohort study, colonization with *C difficile* was associated with an increased risk of asthma at 6 to 7 years of age and wheeze, eczema, and sensitization to food allergens until the age of 6 to 7 years. Furthermore, in children with a positive family history of atopy, vaginal home delivery was associated with a decreased risk of asthma and sensitization to food allergens when compared with vaginal hospital delivery. Results for sensitization to inhalant allergens show the same trend as the results for asthma; however, they lacked statistical significance. It has to be noted that the group for whom venous blood was available was much smaller than the group for whom questionnaire data were available, thereby resulting in a lower power.

The associations found between colonization with *C difficile* and atopic manifestations and the associations between mode and place of delivery and atopic manifestations are partly in line with the results of previous studies.[9,10,24] Although meta-analyses showed a significant effect of delivery by means of

TABLE II. Associations between GM composition and asthma, wheeze, and eczema

		Asthma 6-7 y* (n = 728)		Wheeze‡ (n = 942)§		Eczema‡ (n = 942)§	
	n/N	cOR (95% CI)	aOR† (95% CI)	cOR (95% CI)	aOR† (95% CI)	cOR (95% CI)	aOR† (95% CI)
C difficile (unstratified)							
− (reference)	35/547	1	1	1	1	1	1
+	23/181	**2.13 (1.22-3.70)‖**	**2.06 (1.16-3.64)‖**	**1.37 (1.05-1.79)‖**	**1.38 (1.05-1.81)‖**	**1.45 (1.10-1.90)‖**	**1.43 (1.09-1.88)‖**
Parental atopy							
C difficile							
− (reference)	27/325	1	1	1	1	1	1
+	21/127	**2.19 (1.19-4.03)‖**	**2.27 (1.22-4.21)‖**	1.20 (0.88-1.64)	1.26 (0.92-1.73)	**1.48 (1.07-2.03)‖**	**1.52 (1.10-2.09)‖**
No parental atopy							
C difficile							
− (reference)	8/222	1	1	1	1	1	1
+	2/54	1.02 (0.21-4.97)	1.11 (0.22-5.66)	**1.73 (1.03-2.90)‖**	**1.82 (1.09-3.05)‖**	1.20 (0.71-2.02)	1.23 (0.73-2.08)
Bifidobacterium species (unstratified)							
− (reference)	2/9	1	1	1	1	1	1
+	56/719	0.30 (0.06-1.46)	0.45 (0.09-2.29)	1.74 (0.67-4.53)	1.96 (0.71-5.39)	0.98 (0.30-3.19)	1.17 (0.36-3.79)
Escherichia coli (unstratified)							
− (reference)	5/85	1	1	1	1	1	1
+	53/640	1.45 (0.56-3.73)	1.16 (0.44-3.05)	1.33 (0.88-2.02)	1.22 (0.81-1.84)	1.15 (0.75-1.74)	1.10 (0.72-1.67)
Bacteroides fragilis (unstratified)							
− (reference)	8/135	1	1	1	1	1	1
+	50/593	1.46 (0.68-3.17)	1.49 (0.68-3.26)	0.88 (0.65-1.18)	0.89 (0.66-1.21)	1.02 (0.75-1.39)	1.03 (0.76-1.40)
Lactobacillus species (unstratified)							
− (reference)	38/495	1	1	1	1	1	1
+	20/233	1.13 (0.64-1.98)	1.20 (0.67-2.14)	0.94 (0.72-1.24)	0.96 (0.73-1.26)	1.14 (0.88-1.48)	1.15 (0.89-1.50)

Boldfaced text indicates statistically significant associations at $P < .05$.

− *(reference)*, Colonization absent (reference category); +, colonization present; *cOR*, crude odds ratio.

*Logistic regression analyses.

†Adjusted for recruitment group, parental history of atopy (included only in unstratified analyses), sibling history of atopy, and age at collection of fecal sample and sex.

‡Generalized estimating equations (measurements of wheeze at 7, 12, and 24 months and 4 to 5 and 6 to 7 years and eczema at 7, 12, and 24 months and 6 to 7 years).

§Outcome data are available for at least 1 time point in generalized estimating equation analyses.

‖$P < .05$.

cesarean section on asthma risk, we only found a statistically significant association for the place of delivery (home vs hospital). Thus our results demonstrate, for the first time, that not only the mode but also especially the place of delivery play an important role in the development of atopic manifestations in childhood.

The associations between place of delivery on the one hand and asthma and sensitization to food allergens on the other hand were modified by parental atopic status. This effect-modifying role has also been shown previously regarding birth mode.[24-26]

We were not able to take head circumference into account. It has been shown that an increased head circumference is associated with increased IgE levels to inhalant allergens and asthma.[29-31] Therefore increased head circumference could possibly cause confounding by indication because large head circumference increases the risk of obstetric complications and thus the chance of delivery by means of cesarean section. However, our finding that increased risks of atopic manifestations were also found for vaginal delivery in the hospital makes it unlikely that the associations between delivery by means of cesarean section and atopic diseases can be explained only by head circumference.

We investigated not only the separate associations between delivery characteristics and atopy development but also a hypothetical underlying mechanism. Mediation analyses confirmed an intermediate role for *C difficile* in the association between mode

and place of delivery and atopic outcomes. Statistically significant products of *ab* coefficients were found for the outcomes asthma and eczema, whereas borderline significant results were found for the other outcomes. Notably, mediation was not only found for vaginal home delivery but also for cesarean section compared with vaginal hospital delivery in association with both asthma and eczema. This means that mediation exists for birth by cesarean section, whereas a significant relation between birth by cesarean section and atopic outcomes in the main analyses was not obtained. However, this is not uncommon; there are many cases in which significant mediation exists while a significant relation between the dependent and independent variables is not obtained.[32]

The findings of mediation by *C difficile* strengthen the evidence for a causal role of birth mode on atopic manifestations through influencing the GM composition. The fact that mediation only party explained the association between delivery characteristics and atopic manifestations (associations remained significant after adjusting for *C difficile*) might indicate that other intestinal microorganisms are also involved in this relationship.

We conclude that the mode and place of delivery affect the GM composition, which subsequently influences the risk of atopic manifestations, as demonstrated for *C difficile*. These findings strengthen the evidence for a causal relationship between birth

TABLE III. Associations between GM composition and sensitization to food and inhalant allergens

	Sensitization to food allergens* (n = 764)†		Sensitization to inhalant allergens* (n = 634)†	
	cOR (95% CI)	aOR (95% CI)‡	cOR (95% CI)	aOR (95% CI)‡
C difficile (unstratified)				
− (reference)	1	1	1	1
+	1.40 (0.97-2.02)	**1.43 (1.00-2.06)**	1.30 (0.83-2.05)	1.22 (0.77-1.92)
Parental atopy				
C difficile				
− (reference)	1	1	1	1
+	**1.63 (1.04-2.55)§**	**1.68 (1.07-2.65)§**	1.40 (0.83-2.38)	1.40 (0.82-2.39)
No parental atopy				
C difficile				
− (reference)	1	1	1	1
+	1.02 (0.55-1.89)	1.03 (0.55-1.91)	0.96 (0.39-2.37)	0.85 (0.35-2.04)
Bifidobacterium species (unstratified)				
− (reference)	1	1	1	1
+	−	−	0.60 (0.11-3.20)	0.56 (0.10-3.10)
Escherichia coli (unstratified)				
− (reference)	1	1	1	1
+	0.97 (0.58-1.61)	0.87 (0.52-1.47)	1.08 (0.56-2.09)	1.02 (0.53-1.97)
Bacteroides fragilis (unstratified)				
− (reference)	1	1	1	1
+	0.95 (0.62-1.44)	0.95 (0.63-1.45)	1.03 (0.60-1.76)	1.08 (0.63-1.87)
Lactobacillus species (unstratified)				
− (reference)	1	1	1	1
+	1.15 (0.82-1.63)	1.14 (0.81-1.62)	0.80 (0.51-1.27)	0.79 (0.50-1.24)

Boldfaced text indicates statistically significant associations at $P < .05$.

− *(reference)*, Colonization absent (reference category); +, colonization present; *cOR*, crude odds ratio.

*Generalized estimating equations (measurements at 12 months [only food allergens], 24 months, and 6 to 7 years).

†Outcome data are available for at least 1 time point in generalized estimating equation analyses.

‡Adjusted for recruitment group, parental history of atopy (included only in unstratified analyses), sibling history of atopy, age at collection of fecal sample, and sex.

§$P < .05$.

TABLE IV. Association of mode and place of delivery with asthma, wheeze, and eczema

		Asthma 6-7 y* (n = 1862)		Wheeze‡ (n = 2534)§		Eczema‡ (n = 2533)§	
	n/N	cOR (95% CI)	aOR† (95% CI)	cOR (95% CI)	aOR† (95% CI)	cOR (95% CI)	aOR† (95% CI)
Unstratified analyses							
Vaginal in the hospital (reference)	67/792	1	1	1	1	1	1
Vaginal at home	44/854	**0.59 (0.40-0.88)‖**	**0.59 (0.40-0.89)‖**	0.86 (0.73-1.01)	0.88 (0.74-1.04)	**0.84 (0.71-0.99)‖**	**0.84 (0.71-1.00)‖**
Cesarean section	17/216	0.95 (0.54-1.65)	0.89 (0.50-1.56)	1.08 (0.86-1.37)	1.05 (0.82-1.33)	0.89 (0.69-1.15)	0.89 (0.68-1.15)
Stratified analyses							
Parental atopy							
Vaginal in the hospital (reference)	51/448	1	1		1	1	1
Vaginal home	29/515	**0.47 (0.29-0.75)‖**	**0.47 (0.29-0.77)‖**	0.85 (0.69-1.04)	0.89 (0.73-1.10)	0.87 (0.71-1.06)	0.86 (0.70-1.06)
Cesarean section	12/130	0.78 (0.40-1.52)	0.80 (0.41-1.57)	1.02 (0.76-1.35)	1.02 (0.77-1.36)	0.77 (0.56-1.07)	0.80 (0.57-1.11)
No parental atopy							
Vaginal in the hospital (reference)	16/340	1	1	1	1	1	1
Vaginal at home	15/338	0.95 (0.46-1.96)	1.03 (0.49-2.14)	0.84 (0.63-1.12)	0.85 (0.63-1.15)	0.79 (0.60-1.04)	0.80 (0.60-1.06)
Cesarean section	5/81	1.35 (0.48-3.81)	1.00 (0.34-2.92)	1.16 (0.77-1.76)	1.07 (0.70-1.65)	1.18 (0.79-1.78)	1.04 (0.68-1.59)

Boldfaced text indicates statistically significant associations at $P < .05$.

cOR, Crude odds ratio.

*Logistic regression analyses.

†Analyses adjusted for recruitment group, sex, birth weight, breast-feeding, maternal age, maternal smoking during pregnancy, maternal education level, and siblings with atopy (and parental atopy in case of unstratified analyses).

‡Generalized estimating equations (measurements at 7, 12, and 24 months; 4-5 years [only wheeze]; and 6-7 years).

§Outcome data are available for at least 1 time point in generalized estimating equation analyses.

‖$P < .05$.

TABLE V. Association of mode and place of delivery with sensitization to food and inhalant allergens

	Sensitization to food allergens* (n = 1087)†		Sensitization to inhalant allergens* (n = 867)†	
	cOR (95% CI)	aOR‡ (95% CI)	cOR (95% CI)	aOR‡ (95% CI)
Unstratified analyses				
Vaginal in the hospital (reference)	1	1	1	1
Vaginal at home	**0.61 (0.45-0.82)§**	**0.61 (0.45-0.83)§**	1.01 (0.69-1.48)	0.92 (0.62-1.38)
Cesarean section	1.12 (0.72-1.75)	1.11 (0.70-1.77)	1.23 (0.70-2.14)	1.31 (0.73-2.35)
Stratified analyses				
Parental atopy				
Vaginal in the hospital (reference)	1	1	1	1
Vaginal at home	**0.52 (0.36-0.76)§**	**0.52 (0.35-0.77)§**	0.79 (0.50-1.26)	0.73 (0.44-1.19)
Cesarean section	1.14 (0.66-1.97)	1.17 (0.66-2.07)	0.96 (0.48-1.91)	1.16 (0.57-2.36)
No parental atopy				
Vaginal in the hospital (reference)	1	1	1	1
Vaginal at home	0.76 (0.47-1.25)	0.83 (0.51-1.36)	1.41 (0.71-2.82)	1.46 (0.71-3.02)
Cesarean section	1.02 (0.47-2.21)	0.98 (0.42-2.26)	1.73 (0.66-4.67)	1.80 (0.57-5.67)

Boldfaced text indicates statistically significant associations at $P < .05$.
cOR, Crude odds ratio.
*Generalized estimating equations (measurements at 12 months [only food allergens], 24 months, and 6-7 years).
†Outcome data are available for at least 1 time point in generalized estimating equation analyses.
‡Analyses adjusted for recruitment group, sex, birth weight, breast-feeding, maternal age, maternal smoking during pregnancy, maternal education level, and siblings with atopy (and parental atopy in case of unstratified analyses).
§$P < .05$.

TABLE VI. Mediation of *C difficile* in the association between mode and place of delivery and atopic outcomes

Dependent	Independent§	Group	a Path* Coefficient	SE	b Path† Coefficient	SE	Mediated effect‡ ab	95% CI
Asthma	Vaginal home delivery	**All**	**−0.149**	**0.052**	**0.147**	**0.067**	**−0.022‖**	**−0.052 to −0.001**
		Parental atopy +	−0.101	0.064	0.160	0.076	−0.016	−0.048 to 0.004
		Parental atopy −	−0.259	0.092	0.006	0.182	−0.001	−0.105 to 0.101
	Cesarean section	**All**	**0.137**	**0.045**	**0.150**	**0.069**	**0.021‖**	**0.001 to 0.048**
		Parental atopy +	**0.164**	**0.056**	**0.167**	**0.079**	**0.027‖**	**0.001 to 0.065**
		Parental atopy −	0.078	0.074	0.006	0.182	0.000	−0.041 to 0.043
Wheeze	Vaginal home delivery	All	−0.126	0.045	0.058	0.034	−0.007	−0.019 to 0.001
		Parental atopy +	−0.078	0.057	0.029	0.040	−0.002	−0.012 to 0.005
		Parental atopy −	−0.217	0.077	0.114	0.063	−0.025	−0.064 to 0.002
	Cesarean section	All	0.121	0.039	0.058	0.034	0.007	−0.001 to 0.018
		Parental atopy +	0.138	0.050	0.029	0.040	0.004	−0.007 to 0.017
		Parental atopy −	0.087	0.064	0.114	0.063	0.010	−0.005 to 0.034
Eczema	Vaginal home delivery	**All**	**−0.126**	**0.045**	**0.087**	**0.034**	**−0.011‖**	**−0.025 to −0.001**
		Parental atopy +	−0.078	0.057	0.093	0.040	−0.007	−0.023 to 0.003
		Parental atopy −	−0.217	0.077	0.053	0.057	−0.011	−0.042; to 0.013
	Cesarean section	**All**	**0.121**	**0.039**	**0.087**	**0.034**	**0.011‖**	**0.002 to 0.023**
		Parental atopy +	**0.138**	**0.050**	**0.094**	**0.041**	**0.013‖**	**0.001 to 0.030**
		Parental atopy −	0.087	0.064	0.053	0.057	0.005	−0.006 to 0.022
IgE, food	Vaginal home delivery	All	−0.126	0.045	0.062	0.045	−0.008	−0.023 to 0.003
		Parental atopy +	−0.078	0.057	0.100	0.057	−0.008	−0.027; to 0.004
		Parental atopy −	−0.217	0.077	−0.011	0.074	0.002	−0.032 to 0.038
	Cesarean section	All	0.121	0.039	0.063	0.045	0.008	−0.003 to 0.022
		Parental atopy +	0.138	0.050	0.100	0.057	0.014	−0.002 to 0.036
		Parental atopy −	0.087	0.064	−0.011	0.074	−0.001	−0.019 to 0.016
IgE, air	Vaginal home delivery	All	−0.126	0.045	0.063	0.045	−0.008	−0.023 to 0.003
		Parental atopy +	−0.078	0.057	0.078	0.068	−0.006	−0.025 to 0.006
		Parental atopy −	−0.217	0.077	0.016	0.107	−0.003	−0.055 to 0.046
	Cesarean section	All	0.121	0.039	0.063	0.045	0.008	−0.003 to 0.022
		Parental atopy +	0.138	0.050	0.078	0.068	0.011	−0.008 to 0.035
		Parental atopy −	0.087	0.064	0.016	0.108	0.001	−0.023 to 0.028

Boldfaced text indicates statistically significant mediation.
*Standardized coefficient of the association between mode of delivery and *C difficile*.
†Standardized coefficient of the association between *C difficile* and study outcomes (note that the *b* path is also adjusted for mode of delivery).
‡Product of the standardized coefficients of the *a* and *b* paths (mediated effect) and asymmetric 95% CIs.
§Versus vaginal hospital delivery.
‖$P < .05$.

mode and allergies. Studies that extensively profile the whole neonatal GM composition are needed to further unravel the role of the GM composition in the relation between birth mode and allergic diseases. Such studies could add additional mediating mechanisms by other microorganisms and provide new leads for the primary prevention of allergies.

We thank all of the families that participated in the KOALA study.

Key messages

- **This is the first study to demonstrate that infant gut microbiota composition is not only associated with early atopic manifestations, such as eczema and wheeze, but also with asthma.**

- **Gut microbiota composition and mode and place of delivery are both associated with the development of atopic manifestations, including asthma, mainly in children with a positive family history of atopy.**

- **The mediating role of *C difficile* in the association between type of birth and atopic manifestations strengthens the evidence for a causal pathway.**

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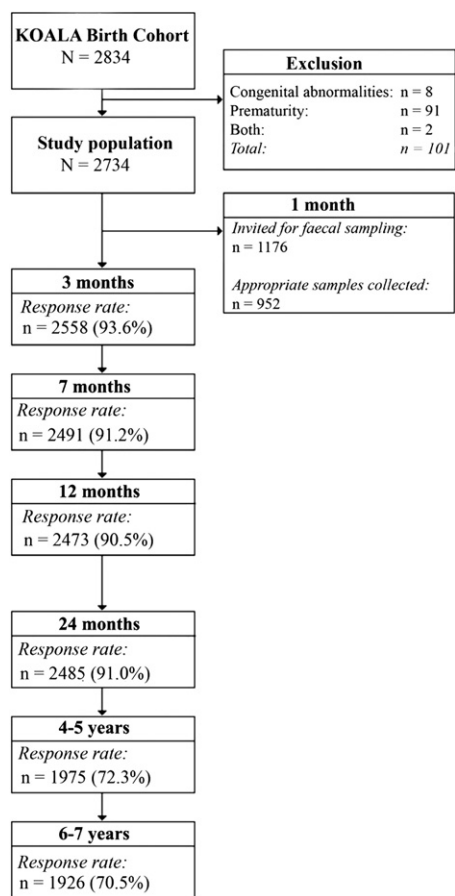32. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Ann Rev Psychol 2007;58:593-614.

KOALA Birth Cohort
N = 2834

Exclusion

Congenital abnormalities:	n = 8
Prematurity:	n = 91
Both:	n = 2
Total:	*n = 101*

Study population
N = 2734

1 month

Invited for faecal sampling:
n = 1176

Appropriate samples collected:
n = 952

3 months
Response rate:
n = 2558 (93.6%)

7 months
Response rate:
n = 2491 (91.2%)

12 months
Response rate:
n = 2473 (90.5%)

24 months
Response rate:
n = 2485 (91.0%)

4-5 years
Response rate:
n = 1975 (72.3%)

6-7 years
Response rate:
n = 1926 (70.5%)

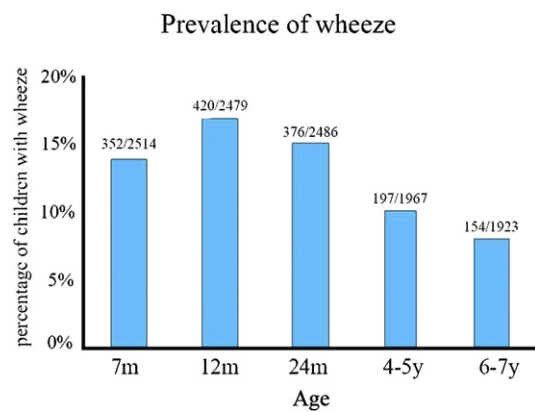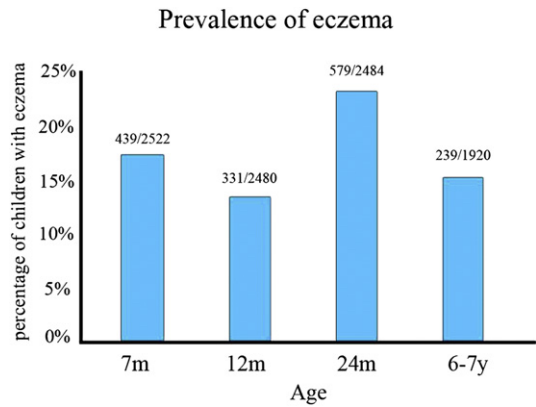**FIG E1.** Population flow chart.

Prevalence of wheeze

FIG E2. Prevalence of wheeze.

FIG E3. Prevalence of eczema.