

**Mode of delivery and childhood asthma, fractional exhaled nitric oxide
and interrupter resistance. The Generation R Study.**

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ABSTRACT

Background Previous studies suggest that caesarean section may influence the risk of childhood asthma. We examined the associations of different modes of delivery with childhood wheezing patterns, asthma, fractional exhaled nitric oxide (FeNO), and airway interrupter resistance (Rint) up to school-age.

Methods This study among 5,885 children was embedded in a population-based prospective cohort study. Information on mode of delivery was obtained from midwives and hospital registries. Wheezing patterns from birth onwards and ever physician-diagnosed asthma at age 6 years were assessed by questionnaires. FeNO and Rint were measured at age 6 years. We used multivariate polynomial, logistic and linear regression models.

Results Compared with vaginal delivery, caesarean section was associated with increased risks of early and persistent wheezing up to school-age (odds ratios (95% Confidence Interval): 1.36 (1.06, 1.75) and 1.73 (1.24, 2.40), respectively). The effect sizes of elective and emergency caesarean section with wheezing outcomes were similar. Only elective caesarean section was associated with a higher FeNO level (sympercent (95% CI): 12.7

(0.6, 24.8)). We did not observe associations of mode of delivery with asthma or Rint. Also, vacuum or forceps assisted vaginal delivery were not associated with any asthma or related outcome.

Conclusions Both elective and emergency caesarean sections are associated with increased risks of early and persistent wheezing up to school-age. This might be explained by increased airway inflammation reflected by higher FeNO levels.

Keywords asthma, cohort study, fractional exhaled nitric oxide (FeNO), interrupter resistance (Rint), mode of delivery, wheezing.

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INTRODUCTION

Asthma is the most common non-communicable disease in childhood with a prevalence among school-age children of 5-10% (1). Fetal and perinatal factors such as preterm birth and low birth weight lead to increased risks of childhood asthma (2). Also, birth by caesarean section may influence the risk of childhood asthma (3, 4). The underlying mechanisms by which caesarean section leads to asthma are unclear. Mode of delivery is thought to influence the development of the neonatal immune system through several pathways (5). First, the hygiene hypothesis proposes that improper exposure to microbes in early life contributes to a greater risk of developing atopic diseases. Newborns delivered vaginally are colonized by bacteria from the mother's vaginal and perianal region, whereas those born by caesarean section are predominantly colonized by environmental microbes. This might lead to an altered activation and maturation of their immune system (6-11), and subsequently an increased risk of asthma (12). Alternatively, a stress-induced mode of delivery such as a device-assisted delivery or emergency caesarean section could lead to an altered perinatal stress response which might also impact the developing immune system (13), and also an

increased risk of asthma in later life (7). Third, mode of delivery might alter the epigenetic regulation of gene expression. It has been suggested that early adverse gene methylation may be involved in silencing the pathway that regulates the balance between T-helper type 1 and 2 cells, which could contribute to a greater risk of developing immune related atopic diseases (5). All proposed mechanisms might lead to lung adaptations including increased airway inflammation, obstruction-related higher airway resistance, and subsequently increased risk of asthma. Previous individual studies that assessed associations of caesarean section with asthma in childhood showed inconsistent findings. A recent meta-analysis reported that elective and emergency caesarean sections are associated with a 1.21 fold and 1.23 fold risk, respectively, for asthma in children (14). Associations with device-assisted delivery were highly heterogenic. Results might be biased by a suboptimal anatomical or immunological lung development, due to either the lack of mechanical forces exerted during vaginal delivery with subsequent surges of stress-induced hormones, or to the confounding effects of younger gestational age and lower birth weight. The effect of specific modes of delivery on airway inflammation and airway resistance has not been explored yet.

Therefore, we assessed in a population-based prospective cohort study among 6,128 children the associations of specific modes of delivery with wheezing patterns, asthma, airway inflammation and airway resistance, respectively, measured as fractional exhaled nitric oxide (FeNO) and by interrupter technique (Rint), in children up to school-age.

MATERIALS AND METHODS

General design This study was embedded in the Generation R Study, a population-based prospective cohort study designed to study the growth, development and health during fetal life, childhood and adulthood (15, 16). The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC2007-413). A detailed design of the Generation R Study is described elsewhere (15). In total 9,749 live births were included. At age 6 years, 8,305 children participated in the follow-up study. Written informed consent

was obtained from all participants by their parents. Children with missing data on mode of delivery (n = 1,094) or wheezing patterns, asthma, FeNO and Rint were excluded (n = 1,083) leaving a total of 6,128 children included for the current analyses (Supplementary Figure 1).

Mode of delivery Information on mode of delivery was obtained from midwife and hospital registries. Mode of delivery was categorized as 1) vaginal delivery, 2) forceps or vacuum assisted delivery, and 3) caesarean section delivery, which was subsequently categorized into 3a) elective caesarean and 3b) emergency caesarean section delivery.

Childhood asthma and related outcomes Information about wheezing in the previous 12 months was collected at ages 1, 2, 3, 4 and 6 years with questionnaires based on the International Study on Asthma and Allergy in Childhood (ISAAC) (17). As previously described (18), we defined 4 categories of wheezing patterns: 1) never wheezing, no wheezing episode at any age; 2) early wheezing: at least 1 wheezing episode during the first 3 years of life but no wheezing episode at 4 and 6 years of age; 3) late wheezing: no wheezing episode during the first 3 years of life but at least 1 wheezing episode at 4 or 6 years of age; and 4) persistent wheezing: at least 1 wheezing episode in the first 3 years of life and 1 wheezing episode at 4 or 6 years of age. At the age of 6 years, information on ever physician-diagnosed asthma was reported by a questionnaire. Airway inflammation and airway resistance were measured during the visit at our research center by Fractional exhaled Nitric Oxide (FeNO) (NIOX chemiluminescence analyser, Aerocrine AB, Solna, Sweden) and interrupter technique (Rint) during tidal breathing (Micro Rint, MicroMedical, Rochester, Kent, UK), respectively, according to American and European Thoracic Society guidelines (19).

Covariates We obtained information on maternal age, pre-pregnancy body mass index (BMI), educational level, parity, history of asthma or atopy, and pet keeping by a questionnaire completed by the mother at enrollment. Maternal psychological distress was

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assessed in the second trimester of pregnancy and defined by the global severity index (GSI), a measure of current level or depth of the symptoms (20). We collected maternal smoking during pregnancy by multiple questionnaires during pregnancy. Information on gestational diabetes, pre-eclampsia and pregnancy induced hypertension was obtained from medical records. Information on child's sex, ethnicity, gestational age at birth and birth weight was obtained from midwives and hospital registers. Postal questionnaires at 6 and 12 months provided information about breastfeeding. Information about inhalant allergies (pollen, house dust mite, pets), ever doctor diagnosed eczema, and lower respiratory tract infections (pneumonia, bronchitis, bronchiolitis or pertussis) was obtained by a questionnaire at age 6 years.

Statistical analysis We used polynomial, logistic and linear regression models to examine the associations of mode of delivery with wheezing patterns, asthma, and differences in FeNO and Rint, respectively. We adjusted the models for maternal age, pre-pregnancy BMI, educational level, history of asthma or atopy, parity, pet keeping, psychological distress during pregnancy, smoking during pregnancy, gestational diabetes, pre-eclampsia, pregnancy induced hypertension, and child's sex, ethnicity, gestational age and size at birth. Covariates were included in our models using multiple measures (21), such as selection based on literature, if they were associated with both the determinant and the outcome or if they changed the effect estimates with $\geq 10\%$. Although each method has its strength and limitation, the use of all three methods increases the identification of all potential confounders for the associations of mode of delivery with wheezing, asthma, FeNO and Rint. To test for intermediates we additionally adjusted our analyses for breastfeeding, inhalant allergies, eczema and lower respiratory tract infections. For all covariates, the percentage of missing values was $<20\%$, except for pet keeping (20.4%), maternal psychological distress during pregnancy (24.0%) and eczema (26.9%) (Supplementary Table 2). Analyses restricted to study participants with complete data might induce bias and imprecision (22, 23). Therefore, we imputed missing data in the covariates using Markov Chain Monte Carlo methods to

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select the most likely value for a missing response. Ten imputed data sets were created and analysed together. No major differences in the magnitude or direction of the effect estimates were observed between analyses with imputed missing data and complete cases only. We only present the results based on imputed data sets. All measures of association are presented as odds ratios (OR), or change in sympercents FeNO (s%) which represents the regression coefficient of $e^{\log \text{ transformed FeNO} \times 100\%}$, and can be interpreted as percentage change without transforming a variable back to its original scale, (24) and Z-scores (Rint) with their 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version 21.0 for Windows (SPSS Inc).

RESULTS

Of all included children, 72.9% was born by a vaginal delivery, 14.2% by a forceps or vacuum assisted delivery, 5.2% by an elective caesarean section and 7.8% by an emergency caesarean section delivery. Table 1 summarizes the characteristics of the study population, stratified for mode of delivery. Most prominently, we observed that mothers of the caesarean section groups, compared with the vaginal delivery group, had a higher mean age, pre-pregnancy BMI, lower education, and lower prevalence of multi-parity, and pregnancy induced hypertension and history of asthma and atopy, pre-eclampsia, and pregnancy induced hypertension, and children of non-European ethnicity, a lower median gestational age, lower mean birth weight, and less breastfeeding (p -values < 0.001 5). Non-responders and participants without follow-up data at age 6 years had mothers who were younger, had a higher pre-pregnancy BMI, were lower educated, had a higher prevalence of multi-parity and psychological distress, more frequently smoking parents, and children more often were of non-European ethnicity, had a lower birth weight, and were less breastfed than those participants with follow-up data (p -values < 0.001) (Supplementary Table 1).

Figure 1 shows the prevalences of wheezing patterns and asthma according to modes of delivery, and these were not different between children born by a vacuum or forceps assisted delivery and children born by a vaginal delivery. The prevalences of early and persistent

wheezing of children born by a caesarean section were higher than of children born by a vaginal delivery (34.4% and 19.0% vs. 29.1% and 15.3% respectively) (Figure 1A). We observed no differences in prevalence of asthma between children born by a caesarean section and children born by a vaginal delivery (Figure 1B). When covariates were taken into account, caesarean section was associated with an increased risk of early and persistent wheezing (OR (95% CI): 1.36 (1.06, 1.75) and 1.73 (1.24, 2.40), respectively) but not with late wheezing or asthma (Table 2). We observed no materially different effect sizes of elective (OR (95% CI): 1.43 (0.99, 2.05) and 1.56 (0.99, 2.46), respectively), and emergency caesarean section (OR (95% CI): 1.31 (0.95, 1.80) and 1.56 (1.05, 2.31), respectively) with early and persistent wheezing. Of the different modes of delivery, only elective caesarean section was associated with a higher FeNO level (s% (95% CI): 12.7 (0.6, 24.8)) (Table 3). We observed no associations of any mode of delivery with Rint (Table 3). Additional adjustment for intermediates did not materially change the effect estimates (Supplementary Tables 3 and 4).

DISCUSSION

In this large-scale population-based prospective cohort study we observed that caesarean section was associated with increased risks of early and persistent wheezing in children up to school age. We observed similar effect sizes of elective and emergency caesarean section with early and persistent wheezing. Only elective caesarean section was associated with a higher FeNO level. No consistent associations of modes of delivery with asthma or Rint were observed. Additional adjustment for potential intermediates did not materially change the effect estimates.

Comparison of main findings with other studies Previous published studies examining associations of mode of delivery with asthma and asthma-related symptoms showed inconsistent results (3, 4, 25-27). A recent meta-analysis assessed the associations between elective and emergency caesarean section with childhood asthma in 15 retrospective and 11

prospective, mainly European, cohort studies (14). Children born by a caesarean section were associated with an increased risk of asthma (OR 1.16 (95% CI 1.14, 1.19), compared with children born vaginally. When specified for type of caesarean section, elective and emergency, the effect estimates were slightly greater (OR 1.21 (1.17, 1.25) and 1.23 (1.19–1.26), respectively). Also, children born by vacuum or forceps assisted delivery were associated with an increased risk of asthma (OR 1.07 (1.04–1.11)), although heterogeneity was high (I²: 54.9%). Sibling and registry based studies observed similar results (28, 29). For wheezing outcomes and asthma, although the latter lacked significance most probably due to low absolute numbers of asthma cases, our reported effect estimates are similar in size and direction as the reported effect sizes from this meta-analysis, even after additional adjustment for more detailed socio-economic, lifestyle and growth factors including gestational age and weight at birth, and maternal diseases during pregnancy. We observed no association of vacuum-or forceps assisted delivery with wheezing patterns or asthma which results are consistent with a recent published meta-analysis (27). To the best of our knowledge, there have been no previous studies that examined the association of mode of delivery with FeNO and Rint as outcome measures. We only observed an association of elective caesarean section with increased levels of FeNO, but not with Rint. This suggests that the underlying mechanisms of associations of mode of delivery with asthma symptoms might be explained by changes in airway inflammation and not by changes in airway resistance due to alterations in airway calibre.

Interpretation main results We observed that both elective and emergency caesarean section were associated with increased risk of wheezing outcomes, and that elective caesarean section was associated with higher FeNO levels. The mechanisms underlying the association between mode of delivery and asthma or related outcomes are unclear. First, it has been hypothesized that modified colonization of the microbiome in newborns delivered by caesarean sections could distort the development of the immune system (5). Intestinal and pulmonic bacterial colonization primes the newborn immune system, balance Th1 and 2

cells, and secretion of cytokines (30). Several human studies provide evidence that a lack of exposure to microbes in early life is associated with the development of asthma later in life (31-33). Most studies have focused on the associations between environmental and gastrointestinal microbiome with immune function in infancy (34, 35). Theoretically, alterations in immune function of the respiratory tract are linked to the immunomodulatory activity of the gut microbiome, through a “common mucosal response” (36). Relationships between the airway microbiome and asthmatic phenotypes have also been observed in patients with asthma (37). Second, foetal stress during delivery might lead to changes in the development of the newborn immune system, and subsequently increased risk of atopic diseases (13). Our results showed similar sizes of effect estimates for both elective and emergency caesarean section with wheezing outcomes and seem to support both hypotheses. Also, our observed association of elective caesarean section with higher FeNO levels support the hypothesis of an altered immunological system due to different exposure to microbial vaginal flora leading to increased inflammation of the airways. We did not observe associations of vacuum or forceps assisted deliveries, less stressful events, and asthma or related outcomes. A third hypothesis states that mode of delivery might alter the epigenetic regulation of gene expression. It has been suggested that early adverse gene methylation may be involved in silencing the pathway that regulates the balance between T-helper type 1 and 2 cells, which could contribute to a greater risk of developing immune diseases (5). We did not observe any association between mode of delivery and Rint, even after adjustment for gestational age and weight at birth, which suggests that the associations between mode of delivery and asthma are stronger regulated by immunological changes than anatomical changes. The potential underlying role of epigenetic changes due to caesarean section for the development of atopic diseases such as asthma is still. Last, It has been suggested that mechanical forces during vaginal delivery might induce a catecholamine surge (38), higher levels of cortisol (39), and higher levels of pulmonary surfactant (40) of the child, which might stimulate postnatal lung adaptation and development. In that perspective,

a vaginal delivery might exert a “protective” effect for further lung development and clinical respiratory complaints in childhood.

Strengths and weaknesses The strengths of our study were its prospective population-based design with a large number of subjects being studied from early life onwards, the availability of detailed data on mode of delivery, wheezing patterns, asthma, FeNO and Rint, and the adjustment for a large number of confounders. However, some limitations need to be discussed. First, characteristics of non-responders at baseline and those lost to follow-up differed from those included in the study. This could have led to biased results if associations of mode of delivery with Rint, FeNO, wheezing patterns and asthma would be different between those included and not included. This seems unlikely but cannot totally be excluded. Secondly, wheezing and asthma in our study were self-reported which might have led to misclassification due to under- or over-reporting. However, this method is widely accepted in epidemiological studies and seems adequately to reflect prevalences of wheezing patterns and asthma until the school age (41). We observed an association of mode of delivery with early and late wheezing, while we did not observe an association with ever asthma. Diagnosis of asthma in young children is difficult and mainly based on symptoms (42). Ever asthma might have comprised different phenotypes and this could have diluted the effect estimates. Furthermore, definitions of categories of wheezing might be arbitrary. We were not able to define wheezing based on viral respiratory tract infections, allergy or other triggers, or wheezing severity based on number of episodes or medication use. Also, we might have lacked power for the associations of caesarean section with late wheezing. Future studies for replication of our findings are needed. Third, due to the low number of cases in several dichotomised outcomes such as late wheezing ($n = 17$), it is possible we lacked power to observe any associations for these subgroups. Fourth, the presence of residual confounders such as maternal use of antibiotics during pregnancy or influence of genetic variance cannot be excluded (43, 44).

In conclusion, our results suggest that elective and emergency caesarean section are associated with increased risks of early and persistent wheezing, and elective emergency section with higher FeNO levels in school age children. We did not observe evidence for associations of mode of delivery with asthma or Rint. Also, no associations of vacuum or forceps assisted delivery with asthma or related outcomes were observed. Further studies are needed to replicate our findings specifically with FeNO and Rint as outcomes, and to further explore plausible underlying pathways.

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REFERENCES

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006 Aug 26;368(9537):733-43.
2. Duijts L, Reiss IK, Brusselle G, de Jongste JC. Early origins of chronic obstructive lung diseases across the life course. *Eur J Epidemiol*. 2014 Dec;29(12):871-85.
3. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy*. 2008 Apr;38(4):629-33.
4. Tollanes MC, Moster D, Daltveit AK, Irgens LM. Cesarean section and risk of severe childhood asthma: a population-based cohort study. *J Pediatr*. 2008 Jul;153(1):112-6.

5. Cho CE, Norman M. Cesarean section and development of the immune system in the offspring. *Am J Obstet Gynecol*. 2013 Apr;208(4):249-54.
6. Xu B, Pekkanen J, Hartikainen AL, Jarvelin MR. Cesarean section and risk of asthma and allergy in adulthood. *J Allergy Clin Immunol*. 2001 Apr;107(4):732-3.
7. Xu B, Pekkanen J, Jarvelin MR. Obstetric complications and asthma in childhood. *J Asthma*. 2000;37(7):589-94.
8. Kero J, Gissler M, Gronlund MM, Kero P, Koskinen P, Hemminki E, et al. Mode of delivery and asthma -- is there a connection? *Pediatr Res*. 2002 Jul;52(1):6-11.
9. McKeever TM, Lewis SA, Smith C, Hubbard R. Mode of delivery and risk of developing allergic disease. *J Allergy Clin Immunol*. 2002 May;109(5):800-2.
10. Hakansson S, Kallen K. Cesarean section increases the risk of hospital care in childhood for asthma and gastroenteritis. *Clin Exp Allergy*. 2003 Jun;33(6):757-64.
11. Bager P, Melbye M, Rostgaard K, Benn CS, Westergaard T. Mode of delivery and risk of allergic rhinitis and asthma. *J Allergy Clin Immunol*. 2003 Jan;111(1):51-6.
12. Bendiks M, Kopp MV. The relationship between advances in understanding the microbiome and the maturing hygiene hypothesis. *Curr Allergy Asthma Rep*. 2013 Oct;13(5):487-94.
13. Duijts L, Bakker-Jonges LE, Labout JA, Jaddoe VW, Hofman A, Steegers EA, et al. Perinatal stress influences lymphocyte subset counts in neonates. The generation R study. *Pediatr Res*. 2008 Mar;63(3):292-8.
14. Huang L, Chen Q, Zhao Y, Wang W, Fang F, Bao Y. Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. *J Asthma*. 2015 Feb;52(1):16-25.
15. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. *Eur J Epidemiol*. 2012 Sep;27(9):739-56.
16. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, et al. The Generation R Study: Biobank update 2015. *Eur J Epidemiol*. 2014 Dec;29(12):911-27.

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17. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J*. 1995 Mar;8(3):483-91.
 18. Guxens M, Sonnenschein-van der Voort AM, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, et al. Parental psychological distress during pregnancy and wheezing in preschool children: the Generation R Study. *J Allergy Clin Immunol*. 2014 Jan;133(1):59-67 e1-12.
 19. American Thoracic S, European Respiratory S. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005 Apr 15;171(8):912-30.
 20. De Beurs E. "Brief Symptom Inventory, handleiding addendum." Leiden, The Netherlands: PITS BV. 2009.
 21. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol*. 2002 Jan 15;155(2):176-84.
 22. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;338:b2393.
 23. Spratt M, Carpenter J, Sterne JA, Carlin JB, Heron J, Henderson J, et al. Strategies for multiple imputation in longitudinal studies. *Am J Epidemiol*. 2010 Aug 15;172(4):478-87.
 24. Cole TJ, Kryakin YV. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. *Stat Med*. 2002 Aug 15;21(15):2287-90.
 25. Rusconi F, Galassi C, Forastiere F, Bellasio M, De Sario M, Ciccone G, et al. Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. *Am J Respir Crit Care Med*. 2007 Jan 1;175(1):16-21.

26. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol*. 2011 Nov;128(5):948-55 e1-3.
27. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. *Clin Exp Allergy*. 2008 Apr;38(4):634-42.
28. Braback L, Ekeus C, Lowe AJ, Hjern A. Confounding with familial determinants affects the association between mode of delivery and childhood asthma medication - a national cohort study. *Allergy Asthma Clin Immunol*. 2013;9(1):14.
29. Magnus MC, Haberg SE, Stigum H, Nafstad P, London SJ, Vangen S, et al. Delivery by Cesarean section and early childhood respiratory symptoms and disorders: the Norwegian mother and child cohort study. *Am J Epidemiol*. 2011 Dec 1;174(11):1275-85.
30. Ly NP, Ruiz-Perez B, Onderdonk AB, Tzianabos AO, Litonjua AA, Liang C, et al. Mode of delivery and cord blood cytokines: a birth cohort study. *Clin Mol Allergy*. 2006;4:13.
31. Huang YJ, Boushey HA. The microbiome in asthma. *J Allergy Clin Immunol*. 2015 Jan;135(1):25-30.
32. Huang YJ. The respiratory microbiome and innate immunity in asthma. *Curr Opin Pulm Med*. 2015 Jan;21(1):27-32.
33. Arrieta MC, Finlay B. The intestinal microbiota and allergic asthma. *J Infect*. 2014 Nov;69 Suppl 1:S53-5.
34. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep*. 2012 May;13(5):440-7.
35. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol*. 2012 Oct;9(10):565-76.
36. Czerkinsky C, Prince SJ, Michalek SM, Jackson S, Russell MW, Moldoveanu Z, et al. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a

common mucosal immune system in humans. *Proc Natl Acad Sci U S A*. 1987

Apr;84(8):2449-53.

37. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnelykke K, et al.

Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med*. 2007

Oct 11;357(15):1487-95.

38. Wang L, Zhang W, Zhao Y. The study of maternal and fetal plasma catecholamines

levels during pregnancy and delivery. *J Perinat Med*. 1999;27(3):195-8.

39. Murphy BE. Human fetal serum cortisol levels at delivery: a review. *Endocr Rev*. 1983

Spring;4(2):150-4.

40. Madar J, Richmond S, Hey E. Surfactant-deficient respiratory distress after elective

delivery at 'term'. *Acta Paediatr*. 1999 Nov;88(11):1244-8.

41. Jenkins MA, Clarke JR, Carlin JB, Robertson CF, Hopper JL, Dalton MF, et al.

Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician

assessment in the diagnosis of asthma. *Int J Epidemiol*. 1996 Jun;25(3):609-16.

42. Edwards CA, Osman LM, Godden DJ, Douglas JG. Wheezy bronchitis in childhood: a

distinct clinical entity with lifelong significance? *Chest*. 2003 Jul;124(1):18-24.

43. Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for

infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med*.

2014 Aug;2(8):631-7.

44. Martino DJ, Prescott SL. Silent mysteries: epigenetic paradigms could hold the key to

conquering the epidemic of allergy and immune disease. *Allergy*. 2010 Jan;65(1):7-15.

Table 1. Characteristics of mothers and their children.

	Mode of delivery (n = 6,128)				P-value*
	Vaginal Delivery	Vacuum or forceps assisted delivery	Elective caesarean section delivery	Emergency caesarean section delivery	
	n = 4,465	n = 868	n = 320	n = 475	
Maternal characteristics					
Age (years)	30.5 (5.1)	30.5 (4.7)	32.2 (4.4)	31.5 (5.1)	0.01
Body mass index (kg/m ²) [#]	23.7 (18.8- 35.8)	23.6 (18.6- 34.3)	24.4 (19.4- 37.4)	24.5 (19.0- 37.9)	<0.001
Education, lower (%)	52.6 (2,174)	44.0 (357)	50.6 (145)	49.6 (214)	<0.001
Parity, ≥1 (%)	51.4 (2,268)	10.9 (95)	36.6 (117)	23.7 (112)	<0.001
History of asthma or atopy, yes (%)	38.6 (1,387)	43.4 (301)	44.7 (117)	41.3 (155)	0.04
Pet keeping, yes (%)	32.4 (1,159)	37.8 (266)	34.3 (81)	36.4 (134)	0.02
Maternal psychological distress, yes (%)	8.7 (295)	6.8 (47)	5.8 (13)	9.8 (35)	0.009
Smoking during pregnancy, yes (%)	25.8 (1,015)	22.9 (173)	25.2 (67)	23.7 (100)	0.07
Gestational diabetes, yes (%)	1.0 (44)	0.7 (6)	1.6 (5)	1.5 (7)	0.04
Pregnancy induced hypertension, yes (%)	3.5 (143)	6.6 (51)	5.4 (13)	8.3 (33)	<0.001
Pre-eclampsia, yes (%)	1.1 (43)	3.0 (22)	7.4 (18)	6.2 (24)	<0.001
Child characteristics					
Sex, female (%)	50.7 (2,263)	45.2 (392)	54.1 (173)	44.4 (211)	0.001
Ethnicity, non-European (%)	34.4 (1,451)	23.7 (195)	24.7 (72)	30.2 (135)	<0.001

Gestational age at birth (weeks) [#]	40.1 (36.0 – 42.3)	40.4 (36.4 – 42.6)	38.7 (29.3 – 42.0)	40.4 (34.4 – 42.6)	<0.001
Birth weight (grams)	3,438 (529)	3,479 (520)	3,125 (824)	3,483 (668)	<0.001
Breastfeeding, yes (%)	92.0 (3,276)	95.9 (717)	88.1 (230)	91.4 (362)	<0.001
Inhalant allergies, yes (%)	6.5 (236)	6.9 (51)	6.4 (17)	4.4 (17)	0.24
Eczema, yes (%)	19.7 (616)	18.6 (124)	20.9 (53)	21.0 (74)	0.652
Lower respiratory infections, yes (%)	4.8 (179)	4.1 (31)	4.9 (13)	4.6 (18)	0.09

Values are means (SD), [#]medians (2.5-97.5th percentile) or percentages (absolute numbers) based on imputed data. *P-values based on unadjusted logistic or linear regression models with mode of delivery as the independent variable and maternal and child characteristics as the dependent variables.

Table 2. Associations of mode of delivery with wheezing patterns and asthma in children aged 6 years.

	Early wheezing	Late wheezing	Persistent wheezing	Ever Asthma
	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
Vaginal delivery n = 4,465	n = 901 Reference n = 621	n = 128 Reference n = 91	n = 466 Reference n = 327	n = 4,415 Reference n = 216 / 3,150
Vacuum or forceps assisted delivery n = 868	0.97 (0.76, 1.24) n= 135	0.87 (0.51, 1.48) n= 20	0.99 (0.71, 1.39) n= 59	0.90 (0.61, 1.33) n = 36 / 665
Caesarean Section overall n = 795	1.36 (1.06, 1.75)* n = 145	1.06 (0.59, 1.90) n = 17	1.73 (1.24, 2.40)** n = 80	1.09 (0.76, 1.55) n = 45 / 600
Elective caesarean section n = 320	1.43 (0.99, 2.05) n= 66	0.81 (0.31, 2.11) n= 5	1.56 (0.99, 2.46) n= 35	0.89 (0.52, 1.52) n = 18 / 249
Emergency caesarean section n = 475	1.31 (0.95, 1.80) n= 79	1.08 (0.55, 2.09) n= 12	1.56 (1.05, 2.31)* n= 45	1.26 (0.81, 1.95) n = 27 / 351

*p < 0.05 and **p < 0.01. Values are odds ratios (95% confidence interval) from multivariate polynomial regression and logistic regression models with 'vaginal delivery' (n = 4,465) and 'never wheezing' (n = 1,523) or 'never asthma' (n = 4,118) as reference groups. "n = " represents number of cases per total group. Models were adjusted for maternal age, pre-pregnancy BMI, educational level, parity, maternal history of asthma and atopy, pet keeping, psychological distress during pregnancy, smoking during pregnancy, gestational diabetes, pre-eclampsia, pregnancy induced hypertension, and child's sex, ethnicity, gestational age and size at birth.

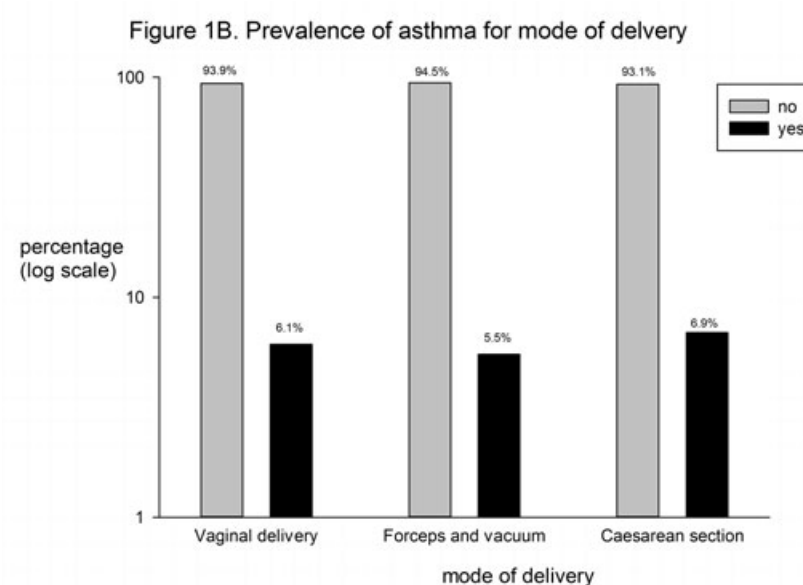
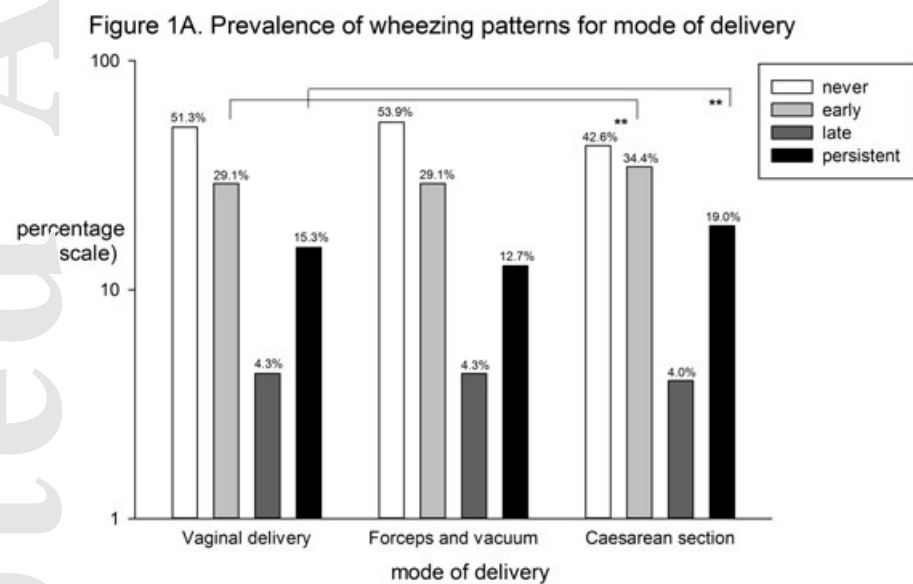
Table 3. Associations of mode of delivery with fractional exhaled nitric oxide (FeNO) and Rint in children aged 6 years.

	FeNO s% (95% CI)	Rint Z-score (95% CI)
	n = 3,522	n = 3,902
Vaginal delivery n = 4,465	Reference n = 2,549	Reference n = 2,815
Vacuum or forceps assisted delivery n = 868	3.4 (-4.3, 11.1) n = 515	0.00 (-0.36, 0.36) n = 545
Caesarean Section n = 795	0.7 (-4.5, 11.4) n = 458	0.01 (-0.37, 0.38) n = 506
Elective caesarean section n = 320	12.7 (0.6, 24.8)* n = 181	0.05 (-0.52, 0.61) n = 199
Emergency caesarean section n = 475	-6.6 (-16.6, 3.4) n = 277	0.06 (-0.41, 0.53) n = 307

*p < 0.05. Values are sympercents (s%) or z-score changes in FeNO or Rint (95% confidence interval) from linear regression models. "n =" represents number of total group. Models were adjusted for maternal age, pre-pregnancy BMI, educational level, parity, maternal history of asthma and atopy, pet keeping, psychological distress during pregnancy, smoking during pregnancy, gestational diabetes, pre-eclampsia, pregnancy induced hypertension, and child's sex, ethnicity, gestational age and size at birth.

Figure 1. Prevalences of wheezing patterns (A) and ever asthma (B) in children aged 6 years for categories of mode of delivery.

* $p < 0.05$, ** $p < 0.01$. Differences in prevalences of wheezing patterns in strata of mode of delivery were tested using a unadjusted polynomial regression model, with “vaginal delivery” and “associated wheezing category” as reference standard.



**Mode of delivery and childhood asthma, fractional exhaled nitric oxide
and interrupter resistance. The Generation R Study.**

Supplementary material

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Supplementary Table 1	Characteristics of population for analysis and lost to follow-up
Supplementary Table 2	Non-imputed characteristics of mothers and their children
Supplementary Table 3	Associations of mode of delivery with wheezing patterns and asthma in children aged 6 years, additionally adjusted for intermediates
Supplementary Table 4	Associations of mode of delivery with fractional exhaled nitric oxide (FeNO) and Rint in children aged 6 years, additionally adjusted for intermediates
Supplementary Figure 1	Flowchart of participants

Supplementary Table 1. Characteristics of population for analysis and lost to follow-up.

	Study population (n = 8,305)		P-value
	Included n = 6,128	Not included n = 2,177	
Maternal characteristics			
Age (years)	30.7 (5.1)	29.2 (5.5)	<0.001
Missing	0	0.1 (1)	
Pre-pregnancy BMI (kg/m ²) #	23.8 (18.8- 36.0)	24.1 (34.5)	<0.001
Missing	7.9 (482)	16.9 (368)	
Education (%)			
Primary, or secondary	51.1 (2,890)	66.3 (1,200)	<0.001
Higher	48.9 (2,764)	33.7 (611)	
Missing	7.7 (474)	16.8 (366)	
Parity (%)			
0	57.3 (3,484)	49.5 (963)	<0.001
≥1	42.7 (2,592)	50.5 (984)	
Missing	0.8 (52)	10.6 (230)	
History of asthma or atopy (%)			
No	60.2 (2,968)	63.8 (1,031)	0.01
Yes	39.8 (1,960)	36.2 (585)	
Missing	19.6 (1,200)	25.8 (561)	
Pet keeping until 1 st year (%)			
No	66.4 (3,244)	67.9 (1,012)	0.15
Yes	33.6 (1,640)	32.1 (478)	
Missing	20.3 (1,244)	31.6 (687)	
Maternal psychological distress (%)			
No	91.6 (4,268)	86.2 (1,071)	<0.001
Yes	8.4 (390)	13.8 (171)	
Missing	24.0 (1,470)	42.9 (935)	
Smoking during pregnancy (%)			
No	74.8 (4,022)	71.7 (1,269)	<0.001
Yes	25.2 (1,355)	28.4 (502)	
Missing	12.3 (751)	18.6 (406)	
Gestational diabetes			
No	99.0 (5,998)	98.5 (1,811)	0.08
Yes	1.0 (62)	1.5 (27)	
Missing	1.1 (68)	15.6 (339)	
Pre-eclampsia			
No	98.0 (5,236)	97.1 (1,586)	0.01
Yes	2.0 (107)	3.1 (50)	
Missing	12.8 (785)	24.9 (543)	
Pregnancy induced hypertension (%)			
No	95.6 (5,236)	97.1 (1,586)	0.01
Yes	4.4 (240)	2.9 (48)	
Missing	10.6 (652)	24.9 (543)	
Child characteristics			
Sex (%)			
Male	50.4 (3,089)	50.7 (1,103)	0.42
Female	49.6 (3,039)	49.3 (1,073)	
Missing	0	0.0 (1)	
Ethnicity (%)			
European	68.0 (3,930)	57.5 (1,081)	<0.001
Non-European	32.0 (1,853)	42.5 (798)	
Missing	5.6 (345)	13.7 (298)	
Gestational age at birth (weeks) #	40.1 (25.29)	39.9 (16.7)	0.04
Missing	0.1 (3)	3.1 (67)	
Birth weight (grams)	3,430 (563)	3,308 (606)	<0.001
Missing	0.1 (2)	1.3 (28)	
Breastfeeding until 1 st year (%)			
No	7.6 (379)	10.5 (130)	<0.001

Yes	92.4 (4,585)	89.5 (1,112)	
Missing	19.0 (1,164)	42.9 (935)	
Inhalant allergies age 6 years (%)			
No	93.6 (4,721)	93.2 (932)	0.33
Yes	6.4 (321)	6.8 (68)	
Missing	17.7 (1,086)	54.1 (1,177)	
Eczema ever until age 6 years (%)			
No	80.3 (3,532)	80.9 (550)	0.38
Yes	19.7 (867)	19.1 (130)	
Missing	28.2 (1,729)	68.8 (1,497)	
Lower respiratory infections			
No	95.3 (4,888)	93.9 (951)	0.04
Yes	4.7 (241)	6.1 (62)	
Missing	16.3 (999)	53.5 (1,164)	

Values are means (SD), #medians (2.5-97.5th percentile) or percentages (absolute

numbers). Body mass index (BMI).

Supplementary Table 2. Non-imputed characteristics of mothers and their children.

	Study population (n = 6,128)			
	Vaginal delivery	Vacuum or forceps assisted delivery	Elective caesarean section delivery	Emergency caesarean section delivery
	n = 4,465	n = 868	n = 320	n = 475
Maternal characteristics				
Age (years)	30.5 (5.1)	30.5 (4.7)	32.1 (4.5)	31.4 (5.2)
Missing	0	0	0	0
Pre-pregnancy BMI (kg/m ²) #	23.7 (18.8 – 35.8)	23.6 (18.4 – 34.9)	24.4 (19.2 – 38.0)	24.5 (19.0 – 39.3)
Missing	7.0 (314)	7.5 (65)	16.3 (52)	10.7 (51)
Education (%)				
Primary, or secondary	52.6 (2,174)	44.0 (357)	50.6 (145)	49.6 (214)
Higher	47.3 (1,951)	56.0 (454)	49.4 (141)	50.4 (218)
Missing	7.6 (340)	6.6 (57)	10.6 (34)	9.1 (43)
Parity (%)				
0	48.6 (2,147)	89.1 (773)	63.4 (203)	76.3 (361)
≥1	51.4 (2,268)	10.9 (95)	36.6 (117)	23.7 (112)
Missing	1.1 (50)	0	0	0.4 (2)
History of asthma or atopy (%)				
No	61.4 (2,210)	56.6 (393)	55.3 (145)	58.7 (220)
Yes	38.6 (1,387)	43.4 (301)	44.7 (117)	41.3 (155)
Missing	19.4 (868)	20.0 (174)	18.1 (58)	21.1 (100)
Pet keeping until 1 st year (%)				
No	67.6 (2,418)	62.2 (437)	65.7 (155)	63.6 (234)
Yes	32.4 (1,159)	37.8 (266)	34.3 (81)	36.4 (134)
Missing	19.9 (888)	19.0 (165)	26.3 (84)	22.5 (107)
Maternal psychological distress (%)				
No	91.3 (3,091)	93.2 (642)	94.2 (212)	90.2 (323)
Yes	8.7 (295)	6.8 (47)	5.8 (13)	9.8 (35)
Missing	24.2 (1,079)	20.6 (179)	29.7 (95)	24.6 (117)
Smoking during pregnancy (%)				
No	74.2 (2,1917)	77.1 (581)	75.1 (202)	76.3 (322)
Yes	25.8 (1,015)	22.9 (173)	25.2 (67)	23.7 (100)
Missing	11.9 (533)	13.1 (114)	15.9 (51)	11.2 (53)

Gestational diabetes				
No	99.0 (4,365)	99.3 (862)	98.4 (306)	98.5 (465)
Yes	1.0 (44)	0.7 (6)	1.6 (5)	1.5 (7)
<i>Missing</i>	<i>1.3 (56)</i>	<i>0</i>	<i>2.8 (9)</i>	<i>0.6 (3)</i>
Pre-eclampsia				
No	98.9 (3,928)	97.0 (718)	92.6 (226)	93.8 (364)
Yes	1.1 (43)	3.0 (22)	7.4 (18)	6.2 (24)
<i>Missing</i>	<i>11.1 (494)</i>	<i>14.7 (128)</i>	<i>23.8 (76)</i>	<i>18.3 (87)</i>
Pregnancy induced hypertension (%)				
No	96.5 (3,928)	93.4 (718)	94.6 (226)	91.7 (364)
Yes	3.5 (143)	6.6 (51)	5.4 (13)	8.3 (33)
<i>Missing</i>	<i>8.8 (394)</i>	<i>11.4 (99)</i>	<i>25.3 (81)</i>	<i>16.4 (78)</i>
Child characteristics				
Sex (%)				
Male	49.3 (2,202)	54.8 (476)	45.9 (147)	55.6 (264)
Female	50.7 (2,263)	45.2 (392)	54.1 (173)	44.4 (211)
<i>Missing</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>
Ethnicity (%)				
European	65.6 (2,772)	76.3 (627)	75.3 (219)	69.8 (312)
Non-European	34.4 (1,451)	23.7 (195)	24.7 (72)	30.2 (135)
<i>Missing</i>	<i>5.4 (242)</i>	<i>5.3 (46)</i>	<i>9.1 (29)</i>	<i>5.9 (28)</i>
Gestational age at birth (weeks) #	40.1 (36.0 – 42.3)	40.4 (36.4 – 42.6)	38.7 (29.3 – 42.0)	40.4 (34.4 – 42.6)
<i>Missing</i>	<i>0.1 (2)</i>	<i>0</i>	<i>0.3 (1)</i>	<i>0</i>
Birth weight (grams)	3,438 (529)	3,479 (520)	3,125 (824)	3,483 (668)
<i>Missing</i>	<i>0</i>	<i>0</i>	<i>0.3 (1)</i>	<i>0</i>
Breastfeeding until 1 st year (%)				
No	8.0 (283)	4.1 (31)	11.9 (31)	8.6 (34)
Yes	92.0 (3,276)	95.9 (717)	88.1 (230)	91.4 (362)
<i>Missing</i>	<i>20.3 (906)</i>	<i>13.8 (120)</i>	<i>18.4 (59)</i>	<i>16.6 (79)</i>
Inhalant allergies age 6 years (%)				
No	93.5 (3,416)	93.1 (684)	93.6 (249)	95.6 (372)
Yes	6.5 (236)	6.9 (51)	6.4 (17)	4.4 (17)
<i>Missing</i>	<i>18.2 (813)</i>	<i>15.3 (133)</i>	<i>16.9 (54)</i>	<i>18.1 (86)</i>
Eczema ever until age 6 years (%)				
No	80.3 (2,509)	81.4 (544)	79.1 (201)	79.0 (278)
Yes	19.7 (616)	18.6 (124)	20.9 (53)	21.0 (74)

<i>Missing</i>	30.0 (1,340)	23.0 (200)	20.6 (66)	25.9 (123)
Lower respiratory infections age 6 years (%)				
No	95.2 (3,538)	95.9 (721)	95.1 (255)	95.4 (374)
Yes	4.8 (179)	4.1 (31)	4.9 (13)	4.6 (18)
<i>Missing</i>	16.8 (748)	13.4 (116)	16.3 (52)	17.5 (83)

Values are means (SD), # medians (2.5-97.5th percentile) or percentages (absolute numbers). Body mass index (BMI).

Supplementary Table 3. Associations of mode of delivery with wheezing patterns and asthma in children aged 6 years, additionally adjusted for intermediates.

	Early wheezing	Late wheezing	Persistent wheezing	Ever Asthma
	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
Vaginal delivery n = 4,465	n = 901 Reference n = 621	n = 128 Reference n = 91	n = 466 Reference n = 327	n = 4,415 Reference n = 216 / 3,150
Vacuum or forceps assisted delivery n = 868	0.96 (0.75, 1.23) n = 135	0.96 (0.56, 1.67) n = 20	1.12 (0.76, 1.59) n = 59	0.83 (0.58, 1.34) n = 36 / 665
Caesarean Section overall n = 795	1.37 (1.06, 1.77)* n = 145	1.06 (0.59, 1.86) n = 17	1.74 (1.25, 2.42)** n = 80	1.15 (0.78, 1.68) n = 45 / 600
Elective caesarean section n = 320	1.42 (0.99, 2.06) n = 66	0.76 (0.28, 2.04) n = 5	1.56 (0.96, 2.51) n = 35	0.83 (0.47, 1.49) n = 18 / 249
Emergency caesarean section n = 475	1.33 (0.96, 1.83) n = 79	1.26 (0.64, 2.50) n = 12	1.90 (1.26, 2.87)** n = 45	1.44 (0.91, 2.29) n = 27 / 351

*P < 0.05 and **p < 0.01. Values are odds ratios (95% confidence interval) from multivariate polynomial regression and logistic regression models with 'vaginal delivery' (n = 4,465) and 'never wheezing' (n = 1,523) or 'never asthma' (n = 4,118) as reference groups. "n =" represents number of cases per total group. Models were adjusted for maternal age, pre-pregnancy BMI, educational level, parity, maternal history of asthma and atopy, pet keeping, psychological distress during pregnancy, smoking during pregnancy, gestational diabetes, pre-eclampsia,

pregnancy induced hypertension, and child's sex, ethnicity, gestational age and size at birth, breastfeeding, inhalant allergies, eczema and lower respiratory tract infections.

Supplemental Table 4. Associations of mode of delivery with fractional exhaled nitric oxide (FeNO) and Rint in children aged 6 years, additionally adjusted for intermediates.

	FeNO s% (95% CI)	Rint Z-score (95% CI)
	n = 3,522	n = 3,902
Vaginal delivery n = 4,465	Reference n = 2,549	Reference n = 2,815
Vacuum or forceps assisted delivery n = 868	2.2 (-3.9, 8.3) n = 515	-0.03 (-0.32, 0.26) n = 545
Caesarean Section n = 795	1.7 (-4.6, 8.1) n = 458	-0.07 (-0.37, 0.22) n = 506
Elective caesarean section n = 320	10.8 (1.1, 20.5)* n = 181	-0.07 (-0.52, 0.37) n = 199
Emergency caesarean section n = 475	-4.0 (-11.9, 3.8) n = 277	-0.07 (-0.44, 0.29) n = 307

*p < 0.05. Values are sympercents (s%) or z-score changes in FeNO or Rint (95% confidence interval) from linear regression models. "n = " represents number of total group. Models were adjusted for maternal age, pre-pregnancy BMI, educational level, parity, maternal history of asthma and atopy, pet keeping, psychological distress during pregnancy, smoking during pregnancy, gestational diabetes, pre-eclampsia, pregnancy induced hypertension, and child's sex, ethnicity, gestational age and size at birth, breastfeeding, inhalant allergies, eczema and lower respiratory tract infections.

Supplemental Figure 1. Flowchart of participants.

