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Breastfeeding and asthma outcomes at the age of 6 years.

The Generation R Study.

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ABSTRACT

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Breastfeeding and asthma outcomes at the age of 6 years. The Generation R Study.

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Background Breastfeeding is associated with a lower risk of asthma symptoms in early childhood, but its effect at older ages is unclear. We examined the associations ofduration and exclusiveness of breastfeeding, with asthma outcomes in children aged 6 years, and whether these associations were explained by atopic or infectious mechanisms.

Methods We performed a population-based prospective cohort study among 5,675 children. Information about breastfeeding was collected by questionnaires. At age 6 years, we measured interrupter resistance (Rint) and Fractional exhaled Nitric Oxide (FeNO). Information about wheezing patterns (early (≤3 years only), late (>3 years only), persistent (≤3 and >3 years)), and current asthma at 6 years was derived from repeated questionnaires.

Results Compared to children who were ever breastfed, those who were never breastfed had lower FeNO levels (sympercent (95% CI): -16.0 (-24.5, -7.5)), and increased risks of late and persistent wheezing (OR(95% CI): 1.69 (1.06, 2.69) and 1.44 (1.00, 2.07), respectively). Shorter duration of breastfeeding was associated with early wheezing and current asthma (1.40 (1.14, 1.73) and 2.19 (1.29, 3.71), respectively). Less exclusive breastfeeding was associated with early wheezing (1.28 (1.08, 1.53)). Breastfeeding duration and exclusiveness were not associated with FeNO or Rint. The associations were not explained by inhalant allergies, partly by lower respiratory tract infections in early life, and to a lesser extent by lower respiratory tract infections in later life.

Conclusions Breastfeeding patterns may influence wheezing and asthma in childhood, which seems partly explained by infectious mechanisms.

Keywords: breastfeeding, Rint, FeNO, wheezing, asthma

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INTRODUCTION

Breastfeeding may influence the development of childhood asthma (1). Prolonged and exclusive breastfeeding has been associated with a decreased risk of asthma symptoms in early childhood with a possible diminishing effect over time (2-5). Underlying mechanisms might involve secretory IgA, cytokines, and long-chain fatty acids in breast milk that stimulate the immune system (6) and change the balance between pro- and anti-inflammatory mechanisms (7). This might lead to altered airway inflammation or airway resistance. Previous studies suggest a potential mediating role of inhalant allergies and respiratory tract infections (8).

In a recent meta-analysis using data of 775,718 children from 117 observational studies (5), breastfeeding was associated with a decreased risk of asthma, regardless of asthma definition or the age at which asthma was measured (0–2 years, 3–6 years, or 7 years and older). The authors observed a large heterogeneity, which might partly be explained by the high variability of the asthmatic phenotypes used by the individual studies (9). Furthermore, in a randomized trial amongst 13,889 children followed up until age 6.5 years receiving an experimental breastfeeding intervention, no differences in allergic symptoms or asthma prevalence were observed (3). More detailed asthma phenotyping and use of objective measurements, such as lung function tests, might improve the understanding of the protective effect of breastfeeding. Furthermore, observing dose-response relationships based on breastfeeding duration or exclusivity would support the causality of the association of breastfeeding with childhood asthma.

Therefore, we aimed to examine among 5,675 children participating in a population-based prospective cohort study the associations of breastfeeding duration and exclusiveness with airway resistance, airway inflammation, and the risks of wheezing and asthma in children up to age 6 years, and to explore whether these associations were mediated by atopic or infectious mechanisms.

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METHODS

Design and cohort This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands. A detailed description of the study design has been published previously (10). The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC-2007-413-NL21545.078). Written informed consent was obtained from all participants. A total of 5,675 children were included for the current analyses (**Supplementary Figure 1**).

Breastfeeding duration and exclusiveness In the Netherlands, use of breastfeeding until age 6 months is encouraged and supported. Detailed information about breastfeeding was collected using questionnaires administered at 2, 6 and 12 months after birth. Children were classified as 'never breastfed' and 'ever breastfed'. Duration of breastfeeding was categorized into 4 groups: '<2 months', '2-4 months', 4-6 months and '≥6 months'. Exclusivity of breastfeeding was defined by at which age infant formula, other drinks or food was introduced. Exclusivity of breastfeeding was categorized into 'non-exclusive breastfeeding for 4 months', and 'exclusive breastfeeding for 4 months'.

Asthma outcomes Children visited the research center at a mean age of 6.1 (SD 0.4) years. Lung function tests were performed according to international guidelines (11, 12). Airway resistance (Rint) was measured in kPa/L (MicroRint, MicroMedical, Rochester, Kent, UK) during tidal expiration, and sex- and height-adjusted z-scores were calculated (12). We corrected for a stepwise variation due to technical issues that required replacement of the Rint device. Fractional exhaled Nitric Oxide (FeNO) was used as a measure of eosinophilic airway inflammation and measured online (NIOX chemiluminescence analyzer; Aerocrine AB, Solna, Sweden). Questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) provided information on wheezing (no, yes) at ages 1 to 4 and 6 years (13). Wheezing patterns were characterized by time of onset and subsequent

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absence or persistence into 'never', 'early' (wheezing ≤3 years only), 'late' (wheezing >3 to 6 years only), or 'persistent wheezing (wheezing ≤3 years and >3 to 6 years) in children with information on wheezing for at least two time points (13). We defined 'current asthma' (no, yes) based on information on ever physician-diagnosed asthma (no, yes) and presence of wheezing in the past 12 months obtained at age 6 years.

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 questionnaires completed by the mother at enrolment. Information about active maternal smoking was obtained by postal questionnaires during the first, second and third trimester of pregnancy and combined into smoking during pregnancy (no, yes). Maternal psychological distress was defined using the global severity index (GSI), a measure of current level or depth of the symptoms, which denotes overall psychological distress (14, 15). Midwife and hospital registries at birth provided information on sex, gestational age and birth weight. Ethnicity of the child was based on country of birth of the parents. Information on inhalant allergies (pollen, house dust mite, pets) and lower respiratory tract infections (pneumonia, bronchitis) was obtained by questionnaire at age 6 years. More detailed information on covariates is provided in the **Supplementary Materials**.

Data analysis We used multivariate regression models to examine the associations between duration and exclusiveness of breastfeeding with Rint, FeNO, wheezing patterns, and current asthma at 6 years .Detailed information on covariates is given in the supplementary material. Missing data of covariates and wheezing were imputed to reduce bias and improve efficiency. The final models were adjusted for maternal BMI, educational level, parity, smoking, and child's sex, birth >37 weeks of gestation, birth weight and ethnicity. We additionally adjusted our models for inhalant allergies and respiratory tract infections as they are hypothesized to be within the causal pathway, and calculated the percentage change of the effect estimate by the formulas: 100x(effect estimate$_{mediator}$-effect estimate$_{model1}$)/(effect

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estimate$_{model1}$) for Rint and FeNO, and 100x(effect estimate$_{mediator}$-effect estimate$_{model1}$)/(effect estimate$_{model1}$-1) for wheezing patterns and current asthma. A 95% confidence interval for the percentage change of the effect estimate was calculated using a bootstrap method with 1,000 resamplings (16). FeNO levels were natural log-transformed to obtain normality and presented as sympercent difference (sym%), which represents the regression coefficient of ^{e}log transformed FeNO*100%, and can be interpret as percentage change (17). All measures of association are presented with their 95% confidence intervals (95% CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 (IBM Corp., Armonk, NY, USA), and R version 3.0.0 (The R foundation for Statistical Computing).

RESULTS

Detailed characteristics of children and their mothers are presented in **Table 1 and Supplementary Table 1**. 92.2% of the children were ever breastfed, 20.1% was breastfed <2 months, 16.5% for 2-4 months, 9.4% for 4-6 months and 24.7% for ≥6 months. Mean Rint was 0.84 (SD 0.29) kPa/l/s and median FeNO was 9.2 (range 0.10– 19) ppb. Of the children, 54.1% were categorized as never, 28.0% as early, 4.8% as late, and 13.0% as persistent wheeze. Current asthma was reported for 3.2%. Non-responders and participants without follow-up data had younger, lower educated mothers with a higher pre-pregnancy BMI and higher prevalence of smoking and psychological distress. Children were more often non-from European ethnicity, born younger, had a lower birth weight, and more often had respiratory tract infections than those included in the study (**Supplementary Table 2**).

Breastfeeding and asthma outcomes In crude analyses, breastfeeding was associated with FeNO, wheezing patterns and asthma, but not with Rint (**Supplementary Tables 3a** and **3b**). Results did not materially change after adjustment for confounders (**Tables 2** and **3**). Compared with children who were ever breastfed, those never breastfed had lower FeNO levels (sym% (95% CI): -16.0 (-24.5, -7.5) (**Table 2**). The duration and exclusivity of

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breastfeeding was not associated with FeNO. Never breastfed children had increased risks of late and persistent wheezing (Odds Ratio (OR) (95% CI): 1.69 (1.06, 2.69) and 1.44 (1.00, 2.07), respectively) (**Table 3**). Among breastfed children, those breastfed for <2 months had increased risks of early wheeze and current asthma (OR (95% CI): 1.40 (1.14, 1.73) and 2.19 (1.29, 3.71), respectively) compared with those breastfed for ≥6 months. Longer duration of breastfeeding was associated with early wheezing and current asthma (OR (95% CI): 0.97 (0.94, 1.00) and 0.92 (0.87, 0.98), respectively) (**Tables 2 and 3**). Similarly, non-exclusively breastfed children had an increased risk of early wheezing (OR (95% CI): 1.28 (1.08, 1.53)), compared with those breastfed exclusively for 4 months. Additional adjustment for inhalant allergies did not materially change the effect estimates (**Supplementary Table 4a**). After additional adjustment for early respiratory tract infections the effect estimates most prominently and significantly attenuated for children breastfed <2 months with early and persistent wheezing and current asthma, and for children non-exclusively breastfed for 4 months with early and persistent wheezing (range %change -8.8 to -66.4)). After additional adjustment for late respiratory tract infections, only the effect estimate for children breastfed <2 months with persistent wheezing attenuated (%change (95% CI): -33.5 (-82.8, -17.6)) (**Supplementary Table 4b**).

DISCUSSION

We observed that children who were never breastfed had lower FeNO levels and increased risks of late and persistent wheezing. Those who were shorter breastfed had increased risks of early wheezing and current asthma. Less exclusive breastfeeding was associated with an increased risk of early wheezing. The associations were partly explained by lower respiratory tract infections in early life, and to a lesser extent by lower respiratory tract infections in later life. Inhalant allergies did not explain the associations. Breastfeeding was not associated with Rint.

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Comparison of main findings with other studies Recently, 117 studies that examined the associations between breastfeeding and asthma were meta-analysed (5). The effect of breastfeeding on asthma was most pronounced in children aged 0–2 years and decreased with age, but seemed still evident at school age. The size and the directions of our effect estimates were similar when we used the same definition of breastfeeding duration (ever vs. never, ≥3-4 months vs <3-4 months; and ≥6 months vs < 6 months) (data not shown). It has also been reported that children who were breastfed longer (18-21) or more exclusive (19) had a higher forced expiratory volume in 1 second (FEV_1) at ages 8-18 years, although not all studies observed positive effects (22). We observed no association between breastfeeding and Rint. Besides different lung function test, differences in results might be explained by different definitions of duration of exclusiveness of breastfeeding, the age at which lung function measurements were performed, adjustment for confounders and sample sizes. Further studies on the associations between breastfeeding and lung function are needed. Only one study examined the association of breastfeeding duration with FeNO levels, and among asthmatic children only. Children who were never breastfed or breastfed for <6 months had no difference in FeNO level at age 8 years, compared with children who were breastfed ≥6 months (23). We observed that children who were never breastfed had lower FeNO levels, compared to children who were ever breastfed. The duration and exclusiveness of breastfeeding was not associated with FeNO. Further studies are needed to replicate our findings before any strong conclusion might be drawn.

Previous studies suggested a mediating effect of allergies and respiratory tract infections (5, 19, 20, 24-26). We applied thorough mediation analyses, and observed that the associations were not explained by inhalant allergies. Lower respiratory tract infections in early life, and to a lesser extent lower respiratory tract infections in later life, did partly explain the associations. Other potential underlying mechanisms such as the impact of breastmilk on the microbiome need to be explored.

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Interpretation of results Underlying mechanisms for the associations of breastfeeding and asthma might include secretory factors in breast milk such as IgA, cytokines, and long-chain fatty acids (6) which stimulates the development of the infants immune system. Also, breastmilk stimulates the intestinal microbiota, which influences the developing immune system and activates T-regulatory cells (27). Opposite of expected, we observed that children who were never breastfed had lower FeNO levels than children who were longer or more exclusively breastfed, and thus might have less eosinophillic airway inflammation. Based on previous findings, we speculate that children who were never, shorter or less exclusively breastfed more often had respiratory tract infections in early life (28, 29). This is supported by our results that shorter or less exclusive breastfeeding was associated with increased risks of early wheezing, which is more commonly induced by respiratory tract infections (13). Respiratory tract infections usually lead to high amounts of neutrophillic granulocytes in the airways (30). The presence of numerous neutrophillic granulocytes might suppress the production of eosinophils (31), and lead to less eosinophillic airway inflammation. However, asthma phenotypes based on cell type might not be consistent over time (32). Furthermore, we observed that results changed less when we additionally adjusted for respiratory tract infections in later life, as compared to when respiratory tract infections in early life. This implies that the associations between breastfeeding and asthma-related outcomes are partly explained by the protective effect of breastfeeding on lower respiratory tract infections in early life. Finally, as the child develops, more factors influence respiratory morbidity, making it difficult to identify the specific role of breastfeeding. In later childhood, associations of atopic mechanisms with persistent wheezing and asthma seem stronger than the associations of infectious mechanisms with persistent wheezing and asthma (13, 33). The dose-dependent effect of breastfeeding on asthma in atopic children remains under debate as earlier studies observed more (26) or no (34, 35) protective effects of breastfeeding on asthma in school-aged children. In the current study, the associations between breastfeeding and asthma-related outcomes were not mediated by inhalant allergies, which might be limited by the unavailability of objective allergy measures.

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Strengths and limitations This study was embedded in a population-based prospective cohort study with detailed data on breastfeeding status, lung function and asthma outcomes. However, some limitations do apply. 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 breastfeeding status with asthma-related outcomes would be different between those included and not included. Second, we did not perform spirometry. It is known that spirometry is feasible and acceptable for approximately 50% of children performing spirometry for the first time (36). Rint is more feasible at this age, and can detect small differences in airway resistance with good within- and between-occasion reproducibility. The biological validity of increased airway resistance has been extensively demonstrated and airway resistance is associated with clinically relevant endpoints (37). Also, Rint can distinguish between groups of symptomatic and healthy young children (38). Third, exploring mediation using the method proposed by Baron and Kenny is limited by assumptions of causality, absence of mediator-outcome confounding and absence of exposure-mediator interaction (39). Objective measures of inhalant allergies and respiratory tract infections were not available. Although questionnaires are efficient tools in epidemiological studies (40, 41), lack of objective measures could have affected our results. Further studies with longitudinally and objective measured data on inhalant allergies and respiratory tract infections are needed to disentangle the direction of causality and possible mediating effects. Current asthma was defined as ever physician-diagnosed asthma (5.9%) and presence of wheezing symptoms in the past 12 months at age 6 years (9.0%), which led to a relatively low prevalence. This might have been an underestimation of true asthma cases, as asthmatic children with proper treatment might not have had any wheezing symptoms. Furthermore, the Generation R study is a multi-ethnic population–based birth cohort. Of the population for the current analysis, those of non-European ethnicity were mainly of Turkish (6.4%), Surinamese (6.2%), Moroccan (4.5%) or Dutch Antilles (2.2%) origin with current asthma prevalences of 3.7%, 4.8%, 3.4%, and 11.1%, respectively. Europeans (76.6%) had a current asthma prevalence of 3.1%. Last, as in any observational

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study, residual confounding due to unmeasured or insufficiently measured confounders might be an issue.

In conclusion, never breastfeeding was associated with lower FeNO levels, and increased risks of persistent wheezing. A shorter duration or non-exclusiveness of breastfeeding was associated with an increased risk of wheezing and asthma, providing evidence for a dose-response relationship. Results were independent of atopic mechanisms, but were partly explained by infectious mechanisms in early life. Further studies using detailed information on allergies and respiratory tract infections throughout life are needed to explore the underlying pathophysiological mechanisms.

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HT, AS and LD contributed to the conception and design, acquisition of data, analyses and interpretation of the data, drafted the article, revised it critically for important intellectual content, and gave final approval of the version to be published. VJ and JJ contributed to the conception and design, acquisition of data, revised the drafted manuscript critically for important intellectual content, and gave final approval of the version to be published. HT and LD had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1. Characteristics of mothers and their children.

	Imputed data (n = 5,675)
Maternal characteristics	
Age (years)	31.1 (4.9)
Body mass index (kg/m^2)	
<20	8.6 (487)
20-25.0	55.0 (3,121)
25-30.0	26.5 (1,504)
≥30	9.9 (562)
Higher educational level (%)	53.0 (3,009)
Multi-parous (%)	42.9 (2,431)
History of asthma or atopy (%)	37.8 (2,142)
Pet keeping (%)	32.9 (1,897)
Smoking during pregnancy (%)	14.3 (812)
Psychological distress during pregnancy (%)	7.8 (444)
Child characteristics	
Female sex (%)	50.1 (2,845)
Gestational age at birth (weeks)	40.1 (26.7, 42.9)
Birth weight (grams)	3,459 (545)
European ethnicity (%)	70.8 (4,018)
Inhalant allergy (%)	6.9 (391)
Lower respiratory tract infections at 6 years (%)	5.1 (290)
Breastfeeding ever (%)	92.2 (5,231)
Breastfeeding duration (%)	
Never	9.9 (444)
<2 months	20.1 (1,140)
2-4 months	16.5 (939)
4-6 months	9.4 (531)
≥6 months	24.7 (1,404)
Breastfeeding exclusiveness (%)	
Never	9.6 (444)
Non-exclusive for 4 months	65.0 (2,993)
Exclusive for 4 months	25.4 (1,171)

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Rint (kPa/L/s)	0.84 (0.29)
FeNO (ppb)	9.2 (0.1, 119)
Wheezing	
Never	54.1 (3,072)
Early	28.0 (1,590)
Late	4.8 (274)
Persistent	13.0 (739)
Current asthma (%)	3.2 (132)

Values are means (SD), medians (range) or percentages (absolute numbers). Data on breastfeeding duration and exclusiveness, Rint, FeNO and current asthma were not imputed.

Table 2. Associations of breastfeeding with childhood Rint and FeNO

	Rint	**FeNO**
	Z-score difference	**Sympercent difference**
	n = 3,422	n = 3,150
Breastfeeding (n = 5,675)		
Never (n = 444)	-0.11 (-0.51, 0.29) n = 248	**-16.0 (-24.5, -7.5)**** n = 241
Ever (n = 5,231)	Reference n = 3,174	Reference n = 2,906
Duration of breastfeeding (n = 4,023)		
0.1 - 2 months (n = 1,138)	-0.7 (-0.38, 0.25) n = 677	-1.1 (-7.8, 5.6) n = 621
2 - 4 months (n = 941)	0.07 (-0.25, 0.40) n = 556	-4.5 (-11.3, 2.4) n = 535
4 - 6 months (n = 540)	0.20 (-0.19, 0.59) n = 320	0.20 (-4.3, -12.5, 3.9) n = 304
≥6 months (n = 1,404)	Reference n = 848	Reference n = 776
Duration (per month) (n = 4,023)	0.01 (-0.03, 0.04) n = 2,401	0.2 (-0.5, 0.9) n = 2,236
Exclusivity of breastfeeding (n = 4,164)		
Non-exclusive for 4 months (n = 2,993)	-0.10 (-0.37, 0.17) n = 1,784	-2.8 (-8.7, 3.1) n = 1,672
Exclusive for 4 months (n = 1,171)	Reference n = 718	Reference n = 663

Values are z-scores differences (95% confidence intervals) or sympercent differences (95% confidence intervals). "n =" represents number of total group. Models were adjusted for maternal body mass index, educational level, parity, smoking, and child's sex, gestational age at birth, weight at birth and ethnicity. **p < 0.01.

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Table 3. Associations of breastfeeding with wheezing patterns and current asthma.

	Wheezing patterns			Current asthma
	Early Wheezing	**Late Wheezing**	**Persistent Wheezing**	
	Odds Ratio	**Odds Ratio**	**Odds Ratio**	**Odds Ratio**
	n = 1,590	n = 274	n = 739	n = 4,093
Breastfeeding (n = 5,675)				
Never (n = 444)	1.31 (0.98, 1.75) n = 141	**1.69 (1.06, 2.69)*** n = 28	**1.44 (1.00, 2.07)*** n = 76	1.57 (0.90, 2.74) n = 17 / 317
Ever (n = 5,231)	Reference n = 1,449	Reference n = 246	Reference n = 663	Reference n = 115 / 3,776
Duration of breastfeeding (n = 4,023)				
0.1 - 2 months (n = 1,138)	**1.40 (1.14, 1.73)**** n = 344	1.13 (0.72, 1.77) n = 62	1.24 (0.94, 1.65) n = 156	**2.19 (1.29, 3.71)**** n = 41 / 806
2 - 4 months (n = 941)	1.20 (0.97, 1.48) n = 266	0.80 (0.49, 1.32) n = 37	1.14 (0.86, 1.52) n = 119	1.27 (0.69, 2.31) n = 20 / 695
4 - 6 months (n = 540)	1.14 (0.89, 1.46) n = 153	0.55 (0.26, 1.14) n = 15	0.90 (0.63, 1.30) n = 53	0.86 (0.39, 1.93) n = 8 / 420
≥6 months (n = 1,404)	Reference n = 355	Reference n = 65	Reference n = 157	Reference n = 25 / 1,130
Duration (per month) (n = 4,023)	**0.96 (0.94, 0.98)**** n = 1,118	0.99 (0.95, 1.04) n = 179	**0.97 (0.94, 1.00)*** n = 485	**0.92 (0.87, 0.98)*** n = 94 / 3,051
Exclusivity of breastfeeding (n = 4,164)				
Non-exclusive for 4 months (n = 2,993)	**1.28 (1.08, 1.53)**** n = 858	1.23 (0.81, 1.86) n = 146	1.23 (0.97, 1.56) n = 391	1.48 (0.89, 2.47) n = 76 / 2,200
Exclusive for 4 months (n = 1,171)	Reference n = 295	Reference n = 45	Reference 126	Reference n = 20 / 941

Values are odds ratios (95% confidence intervals). "n =" represents number of cases (wheezing patterns) and number of cases per total group (current asthma). Models were adjusted for maternal body mass index, educational level, parity, smoking, and child's sex, gestational age at birth, weight at birth and ethnicity. *p < 0.05, **p < 0.01

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Breastfeeding and asthma outcomes at the age of 6 years.

The Generation R Study.

SUPPLEMENTAL MATERIAL

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METHODS

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 questionnaires completed by the mother at enrolment. Information about active maternal smoking was obtained by postal questionnaires during all trimester of pregnancy and combined into smoking during pregnancy (no, yes). Maternal psychological distress was defined using the global severity index (GSI), a measure of current level or depth of the symptoms, which denotes overall psychological distress (15, 16). Midwife and hospital registries at birth provided information on sex, gestational age and birth weight. Ethnicity of the child was based on country of birth of the parents. Information on inhalant allergies (pollen, house dust mite, pets) was obtained by questionnaire at age 6 years based on ISAAC core questions (13). Response rate for this questionnaire was 68%. Quantitative data on sensitization was not available for the participants in this age group. Information on physician-attended lower respiratory tract infections was obtained by questionnaires at the ages of 6 months and 1 to 4 and 6 years. Questions about physician-attended lower respiratory tract infections included bronchitis, bronchiolitis and pneumonia in the past 6 or 12 months (no, yes and physician-attended, yes but not physician-attended). Lower respiratory tract infections were defined in 2 categories: 1) lower respiratory tract infections in early life: at least 1 physician attended lower respiratory tract infection during the first 3 years of life vs no lower respiratory tract infections or only lower respiratory tract infections in later life (at 4 or 6 years of age), and 2) lower respiratory tract infections in later life: at least 1 physician attended lower respiratory tract infection during at 4 or 6 years of age vs no lower respiratory tract infections or only lower respiratory tract infections in early life. Confounders were included in our statistical models based on literature, if they were associated with the determinant and the outcome and were not in the causal pathway, or if they changed the effect estimates with ≥10%. We used a directed acyclic graph to determine multicollinearity between confounders to prevent over-adjustment (14).

The percentages of missing covariate values were ≤10% except for maternal smoking during pregnancy (10.9%). Twenty-five datasets were created by imputation. No differences in results were observed between original and imputed data. We only present results based on imputed data.

Table S1. Characteristics of mothers and their children.

	n = 5,675	
	Observed	**Imputed**
Maternal characteristics		
Age (years)	31.1 (4.9)	31.1 (4.9)
Body mass index (kg/m^2)		
<20	9.1 (468)	8.6 (487)
20-25.0	55.1 (2,839)	55.0 (3,121)
25-30.0	25.0 (1,289)	26.5 (1,504)
≥30	10.8 (555)	9.9 (562)
Missing	*9.2 (524)*	-
Educational level (%)		
Primary or secondary	46.0 (2,452)	47.0 (2,666)
Higher	54.0 (2,876)	53.0 (3,009)
Missing	*6.1 (347)*	-
Parity (%)		
0	57.2 (3,183)	57.1 (3,244)
≥1	42.8 (2,378)	42.9 (2,431)
Missing	*2.0 (114)*	-
History of asthma or atopy (%)		
No	60.7 (2,827)	62.2 (3,533)
Yes	39.3 (1,833)	37.8 (2,142)
Missing	*17.9 (1,015)*	-
Pet keeping (%)		
No	66.5 (2,985)	67.1 (3,778)
Yes	33.5 (1,505)	32.9 (1,897)
Missing	*20.9 (1,185)*	-
Smoking during pregnancy (%)		
No	85.7 (4,337)	85.7 (4,863)
Yes	14.3 (722)	14.3 (812)
Missing	*10.9 (616)*	-
Psychological distress (%)		
No	92.5 (4,075)	92.2 (5,231)
Yes	7.5 (332)	7.8 (444)
Missing	*22.3 (1,268)*	-
Child characteristics		
Female sex (%)	50.1 (2,845)	50.1 (2,845)
Gestational age at birth (weeks)	40.1 (26.7, 42.9)	40.1 (26.7, 42.9)
Birth weight (grams)	3,459 (545)	3,459 (545)
Ethnicity (%)		

European	71.4 (3,888)	70.8 (4,018)
Non-European	28.6 (1,557)	29.2 (1,657)
Missing	*4.1 (230)*	-
Inhalant allergy (%)		
No	93.9 (4,423)	93.1 (5,283)
Yes	6.1 (286)	6.9 (391)
Missing	*17.0 (966)*	-
Lower respiratory tract infections at 6 years (%)		
No	95.5 (4,569)	94.9 (5,385)
Yes	4.5 (213)	5.1 (290)
Missing	*15.7 (893)*	-
Breastfeeding ever (%)		
No	7.8 (444)	7.8 (444)
Yes	92.2 (5,231)	92.2 (5,231)
Missing	-	-
Breastfeeding duration (%)		
Never	9.9 (444)	9.9 (444)
<2 months	20.1 (1,140)	20.1 (1,140)
2-4 months	16.5 (939)	16.5 (939)
4-6 months	9.4 (531)	9.4 (531)
≥6 months	24.7 (1,404)	24.7 (1,404)
Missing		
Breastfeeding exclusiveness (%)		
Never	9.6 (444)	9.6 (444)
Non-exclusive for 4 months	65.0 (2,993)	65.0 (2,993)
Exclusive for 4 months	25.4 (1,171)	25.4 (1,171)
Missing	*18.8 (1,067)*	*18.8 (1,067)*
Rint (kPa/L/s)	0.84 (0.29)	0.84 (0.29)
Missing	*39.6 (2,245)*	*39.6 (2,245)*
FeNO (ppb)	9.2 (0.1, 119)	9.2 (0.1, 119)
Missing	*44.5 (2,525)*	*44.5 (2,525)*
Wheezing		
Never	54.2 (1,884)	54.1 (3,072)
Early	27.4 (952)	28.0 (1,590)
Late	4.0 (140)	4.8 (274)
Persistent	8.8 (498)	13.0 (739)
Missing	38.8 (2,201)	-
Current asthma (%)		
No	96.8 (3,961)	96.8 (3,961)
Yes	3.2 (132)	3.2 (132)

Missing	*27.9 (1,582)*	*27.9 (1,582)*

Values are means (SD), medians (range) or percentages (absolute numbers). Data on breastfeeding duration and exclusiveness, Rint, FeNO and current asthma were not imputed.

Table S2. Non-response and lost to follow-up analysis of mothers and their children.

	Included N = 5,675	Non-response or lost to follow-up N = 2,630	P-value
Maternal characteristics			
Age (years)	31.1 (4.9)	28.4 (5.7)	<0.01
Body mass index (kg/m^2)			
<20	9.1 (468)	8.3 (310)	<0.01
20-25.0	55.1 (2,839)	47.1 (1,768)	
25-30.0	25.0 (1,289)	28.4 (1,067)	
≥30	10.8 (555)	16.2 (608)	
Missing	*9.2 (524)*	*11.2 (473)*	
Educational level (%)			
Primary, or secondary	46.0 (2,452)	74.9 (2,496)	<0.01
Higher	54.0 (2,876)	25.1 (838)	
Missing	*6.1 (347)*	*21.1 (892)*	
Parity (%)			
0	57.2 (3,183)	51.9 (2,056)	<0.01
≥1	42.8 (2,378)	48.1 (1,906)	
Missing	*2.0 (114)*	*6.2 (264)*	
History of asthma or atopy (%)			
No	60.7 (2,827)	62.3 (1,839)	0.15
Yes	39.3 (1,833)	37.7 (1,113)	
Missing	*17.9 (1,015)*	*30.1 (1,274)*	
Pet keeping (%)			
No	66.5 (2,985)	68.9 (2,067)	0.03
Yes	33.5 (1,505)	31.1 (933)	
Missing	*20.9 (1,185)*	*29.0 (1,226)*	
Smoking during pregnancy (%)			
No	85.7 (4,337)	76.3 (2,506)	<0.01
Yes	14.3 (722)	23.7 (779)	
Missing	*10.9 (616)*	*22.3 (941)*	
Psychological distress (%)			
No	92.5 (4,075)	84.3 (1,952)	<0.01
Yes	7.5 (332)	15.7 (363)	
Missing	*22.3 (1,268)*	*45.2 (1,911)*	
Child characteristics			
Female sex (%)	50.1 (2,845)	46.5 (1,964)	0.06
Gestational age at birth (weeks)	40.1 (36.0, 42.3)	39.9 (33.3, 42.3)	<0.01
Birth weight (grams)	3,459 (545)	3,281 (626)	<0.01
Ethnicity (%)			

European	71.4 (3,888)	49.1 (1,703)	<0.01
Non-European	28.6 (1,557)	50.9 (1,767)	
Missing	*4.1 (230)*	*17.9 (756)*	
Breastfeeding ever (%)			
No	7.8 (444)	10.4 (115)	<0.01
Yes	92.2 (5,231)	89.6 (994)	
Missing	-	*73.8 (3,117)*	
Breastfeeding duration (%)			
<3 months	39.9 (1,605)	61.1 (350)	<0.01
3-6 months	24.9 (1,003)	19.5 (112)	
≥6 months	35.2 (1,415)	19.5 (111)	
Missing	*21.3 (1,208)*	*83.7 (3,538)*	
Breastfeeding exclusiveness (%)			
Non-exclusive for 4 months	71.9 (2,993)	84.0 (524)	<0.01
Exclusive for 4 months	28.1 (1,171)	16.0 (100)	
Missing	*18.8 (1,067)*	*82.5 (3,487)*	
Inhalant allergy (%)			
No	93.9 (4,423)	92.3 (1,230)	0.03
Yes	6.1 (286)	7.7 (103)	
Missing	*17.0 (966)*	*68.5 (2,893)*	
Lower respiratory tract infections at 6 years (%)			
No	95.5 (4,569)	93.4 (1,270)	<0.01
Yes	4.5 (213)	6.6 (90)	
Missing	*15.7 (893)*	*67.8 (2,866)*	

Values are means (SD), medians (2.5-97.5th percentile) or percentages (absolute numbers). Differences between groups were evaluated using chi-squared tests for categorical values, one-way ANOVA for continues variables, and Mann-Whitney for non-normal distributed variables.

Supplemental Table 3a. Crude associations of breastfeeding with childhood Rint and FeNO

	Rint	**FeNO**
	Z-score difference	**Sympercent difference**
	n = 3,422	n = 3,150
Breastfeeding (n = 5,675)		
Never (n = 444)	-0.11 (-0.50, 0.29)	**-16.4 (-24.7, -8.2)****
Ever (n = 5,231)	Reference	Reference
Duration of breastfeeding (n = 4,023)		
0.1 - 2 months (n = 1,138)	-0.11 (-0.42, 0.20)	-0.9 (-7.4, 5.6)
2 - 4 months (n = 941)	0.08 (-0.24, 0.41)	-4.2 (-11.0, 2.6)
4 - 6 months (n = 540)	0.24 (-0.15, 0.63)	-5.0 (-13.1, 3.2)
≥6 months (n = 1,404)	Reference	Reference
p-trend	0.50	0.69
Exclusivity of breastfeeding (n = 4,164)		
Non-exclusive for 4 months (n = 2,993)	-9.7 (-36.1, 16.7)	-1.9 (-7.6, 3.9)
Exclusive for 4 months (n = 1,171)	Reference	Reference

Values are z-scores differences (95% confidence intervals) or sympercent differences (95% confidence intervals). “n =” represents number of total group. **p < 0.01.

Supplemental Table 3b. Crude associations of breastfeeding with wheezing patterns and current asthma.

	Wheezing patterns			Current asthma
	Early Wheezing	**Late Wheezing**	**Persistent Wheezing**	
	Odds Ratio	**Odds Ratio**	**Odds Ratio**	**Odds Ratio**
	n = 1,590	n = 274	n = 739	n = 4,093
Breastfeeding (n = 5,675)				
Never (n = 444)	**1.40 (1.06, 1.84)***	**1.66 (1.07, 2.60)***	**1.65 (1.16, 2.33)****	1.80 (1.07, 3.04)
Ever (n = 5,231)	Reference	Reference	Reference	Reference
Duration of breastfeeding (n = 4,023)				
0.1 - 2 months (n = 1,138)	**1.38 (1.14, 1.68)****	1.35 (0.88, 2.09)	**1.42 (1.09, 1.86)***	**2.37 (1.43, 3.93)****
2 - 4 months (n = 941)	1.20 (0.98, 1.47)	0.91 (0.56, 1.47)	1.21 (0.91, 1.60)	1.31 (0.72, 2.38)
4 - 6 months (n = 540)	1.15 (0.90, 1.46)	0.59 (0.29, 1.21)	0.90 (0.63, 1.28)	0.84 (0.38, 1.88)
≥6 months (n = 1,404)	Reference	Reference	Reference	Reference
p-trend	<0.01	0.18	<0.01	<0.01
Exclusivity of breastfeeding (n = 4,164)				
Non-exclusive for 4 months (n = 2,993)	**1.29 (1.09, 1.52)****	1.44 (0.96, 2.17)	**1.37 (1.09, 1.73)****	**1.65 (1.00, 2.71)***
Exclusive for 4 months (n = 1,171)	Reference	Reference	Reference	Reference

Values are odds ratios (95% confidence intervals). “n =” represents number of cases (wheezing patterns) and number of cases per total group (current asthma). *p < 0.05, **p < 0.01

Table S4a. Associations of breastfeeding with asthma-related outcomes, additionally adjusted for early respiratory tract infections.

	Rint	**FeNO**	**Wheezing patterns[#]**			**Current asthma**
			Early	**Late**	**Persistent**	
	% change	**% change**	**% change**	**% change**	**% change**	**% change**
Breastfeeding						
Never	10.1 (-110, 109)	1.7 (-2.7, 9.1)	**-51.5 (-219, -5.1)***	**-8.8 (-22.8, -1.1)***	**-60.8 (-402, -4.4)***	-30.2 (-406, 310)
Ever	Reference	Reference	Reference	Reference	Reference	Reference
Duration of breastfeeding						
0.1 - 2 months	7.3 (-78.9, 92.7)	0.2 (-62.3, 64.7)	**-30.2 (-42.6, -21.6)****	-46.2 (-195, 1793)	**-66.4 (-233, -33.6)****	**-23.4 (-59.5 -7.3)****
2 - 4 months	-13.1 (-77, 69.3)	0.1 (-24.0, 24.7)	**-42.5 (-66.5, -25.2)****	9.3 (-210, 73.0)	-91.2 (-229, 5909)	-37.4 (-339, 263)
4 - 6 months	-1.0 (-21.6, 20.3)	-0.2 (-18.4, 15.7)	-11.2 (-49.1, 12.5)	0.33 (-1.1, 3.2)	20.1 (-131, 109)	38.1 (-282, 303)
≥6 months	Reference	Reference	Reference	Reference	Reference	Reference
Exclusivity of breastfeeding						
Non-exclusive for 4 months	-4.4 (-57.4, 59.8)	2.6 (-31.2, 36.7)	**-30.2 (-39.9, -24.8)****	-15.3 (-85.9, 36.8)	**-58.9 (-90.6, -38.1)***	-34.3 (-261, 134)
Exclusive for 4 months	Reference	Reference	Reference	Reference	Reference	Reference

Values are percentage change (95% confidence intervals) of the effect estimates after additional adjustment for lower respiratory tract infections, compared to the models without adjustment for lower respiratory tract infections. Percentage change is calculated using the formula (100 x ($\beta_{mediator}$ - $\beta_{model\ 1}$)/($\beta_{model\ 1}$)), with corresponding 95% confidence interval obtained by bootstrap procedures. Models were adjusted for maternal body mass index, educational level, parity, smoking, and child's sex, gestational age at birth, weight at birth and ethnicity, and additionally for lower respiratory tract infections in the first 3 years of life.

Table S4b. Associations of breastfeeding with asthma-related outcomes, additionally adjusted for respiratory tract infections between age 4 and 6 years.

	Rint	**FeNO**	**Wheezing patterns**[#]			**Current asthma**
			Early	**Late**	**Persistent**	
	% change	**% change**	**% change**	**% change**	**% change**	**% change**
Breastfeeding						
Never	0.0 (-64.7, 64.7)	4.0 (-2.6, 20.4)	NA	-15.7 (-44.3, 2726)	**-35.4 (-53.5, -0.0)***	-34.7 (-461, 345)
Ever	Reference	Reference	Reference	Reference	Reference	Reference
Duration of breastfeeding						
0.1 - 2 months	-3.7 (-41.7, 39.3)	17.3 (-149, 157)	NA	-71.9 (-716, 1728)	**-33.5 (-82.8, -17.6)***	-20.6 (-62.9, 9.1)
2 - 4 months	5.5 (-36.0, 41.4)	8.7 (-69.8, 81.5)	NA	23.9 (-418, 147)	-64.9 (-187, 5756)	-51.4 (-462, 444)
4 - 6 months	-1.2 (-22.0, 18.5)	-1.9 (-51.4, 45.8)	NA	0.2 (-4.9, 5.9)	-12.5 (-72.3, 433)	-37.0 (-311, 345)
≥6 months	Reference	Reference	Reference	Reference	Reference	Reference
Exclusivity of breastfeeding						
Non-exclusive for 4 months	-1.2 (-24.4, 22.8)	10.1 (-76.6, 95.8)	NA	-3.7 (-54.7, 61.1)	-11.5 (-50.0, 14.7)	-41.1 (-279, 199)
Exclusive for 4 months	Reference	Reference	Reference	Reference	Reference	Reference

Values are percentage change (95% confidence intervals) of the effect estimates after additional adjustment for lower respiratory tract infections, compared to the models without adjustment for lower respiratory tract infections. Percentage change is calculated using the formula (100 x ($\beta_{mediator}$ - $\beta_{model\ 1}$)/($\beta_{model\ 1}$)), with corresponding 95% confidence interval obtained by bootstrap procedures. Models were adjusted for maternal body mass index, educational level, parity, smoking, and child's sex, gestational age at birth, weight at birth and ethnicity, and additionally for lower respiratory tract infections at age 4 and 5 years.

Figure S1. Flow chart of participants in the study.

