

ORIGINAL ARTICLE

Foetal and infant growth patterns, airway resistance and school-age asthma

AGNES M.M. SONNENSCHIN-VAN DER VOORT,^{1,2,3} ROMY GAILLARD,^{1,3,4} JOHAN C. de JONGSTE,² ALBERT HOFMAN,³ VINCENT W.V. JADDOE^{1,3,4} AND LIESBETH DUIJTS^{2,3,5}

¹The Generation R Study Group, ²Department of Pediatrics, Division of Respiratory Medicine, ³Department of Epidemiology, ⁴Department of Pediatrics, and ⁵Department of Pediatrics, Division of Neonatology, Erasmus University Medical Center, Rotterdam, The Netherlands

ABSTRACT

Background and objective: Preterm birth, low birth weight and rapid infant weight gain are associated with increased risks of asthma symptoms in childhood. The underlying mechanism may include persistently higher airway resistance (Rint). The aim of our study was to examine the associations of longitudinally measured foetal and infant growth characteristics with Rint and asthma outcomes in school-age children.

Methods: This study was embedded in a population-based prospective cohort study in Rotterdam, The Netherlands. Foetal growth was estimated by ultrasound in the second and third trimesters. Infant growth was measured at birth, 3, 6 and 12 months. At age 6 years, Rint was measured, and information about wheezing and asthma was obtained by questionnaires. The number of subjects per analysis differed per available outcome (3954–5066 subjects).

Results: Longitudinal growth analyses showed that school-age children with increased Rint had lower foetal length growth and weight gain, and lower infant length growth. Children with persistent wheezing until age 6 years and physician-diagnosed asthma had a higher Rint compared with children who never wheezed or without asthma (difference *z*-scores Rint: 0.58 (0.19, 0.97) and 0.55 (0.15, 0.95), respectively).

Conclusion: Rint in school-age children is influenced by foetal growth restriction and is associated with asthma outcomes.

SUMMARY AT A GLANCE

In school-age children, increased airway resistance is influenced by foetal and infant growth patterns, and increased airway resistance is associated with increased risk for asthma symptoms. Respiratory symptoms seem not influenced by foetal growth patterns but tended to be influenced by infant growth patterns.

Key words: asthma, cohort study, foetal growth, infant growth, wheezing.

Abbreviations: 95% CI, 95% confidence interval; MD, mean difference; OR, odds ratio; Rint, airway resistance; SD, standard deviation; SDS, standard deviation score.

INTRODUCTION

Preterm birth (<37 weeks), low birth weight (<2500 grams) and high infant weight gain (≥10th percentile weight change between birth and 1 year) seem to be associated with an increased risk of respiratory morbidity and lower pulmonary function in childhood and adulthood.^{1–3} The mechanisms underlying these associations are not known. Clearly, low birth weight is not the causal factor *per se* leading to respiratory morbidity.^{1–5} Low birth weight is the result of various adverse foetal growth exposures and growth patterns and the starting point of infant growth. We have recently shown that not low birth weight, but accelerated infant growth, independent from foetal growth patterns, is associated with wheezing and asthma-related symptoms until the age of 4 years.⁵ The mechanisms underlying these associations may include suboptimal development of the respiratory system. Such developmental adaptations may result in direct mechanical effects due to impaired lung growth with smaller airways and airway diameters leading to a higher airway resistance (Rint).^{2,6–8} We hypothesized that smaller foetal growth and higher infant growth, small for gestational age and preterm

Correspondence: Liesbeth Duijts, Erasmus University Medical Center Rotterdam, Sp-3435, PO Box 2060, 3000 CB Rotterdam, The Netherlands. Email: l.duijts@erasmusmc.nl

Disclosure Statement: AMMSvdV is the recipient of a European Respiratory Society Fellowship (STRTF 93–2012) and received a grant from the Ter Meulen Fund, Royal Netherlands Academy of Arts and Sciences (TMF2012/228). VVVJ received an additional grant from the Netherlands Organization for Health Research and Development (ZonMw-VIDI). LD received funding from the European Respiratory Society and the European Community's Seventh Framework Programme FP7/2007–2013—Marie Curie Actions under grant agreement RESPIRE, PCOFUND-GA-2008–229571, and Lung Foundation Netherlands (no 3.2.12.089; 2012).

Received 24 March 2015; invited to revise 26 April and 1 September 2015; revised 2 August and 14 October 2015; accepted 8 November 2015 (Associate Editor: Lisa Wood).

Article first published online: 23 December 2015

birth lead to persistent increased Rint, and subsequently increased risk of childhood asthma.

Therefore, our main focus was to examine in a population-based prospective cohort study whether Rint in school-age children was associated with longitudinally measured foetal and infant growth patterns. Secondly, we examined whether foetal and infant growth was associated with wheezing patterns, overall wheezing and physician-diagnosed asthma at school-age. Thirdly, we examined whether growth characteristics at birth were associated with respiratory outcomes. Finally, we examined whether Rint was associated with wheezing patterns and physician-diagnosed asthma at school-age.

METHODS

This study of 6259 subjects was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children⁹ (Fig. 1).

Growth characteristics

Foetal growth characteristics were measured in the second and third trimester.¹⁰ We used femur length as

proxy for foetal length, and estimated foetal weight was calculated using the Hadlock formula.^{11,12} Length and weight at birth were obtained from community midwife and hospital registries. Infant growth characteristics were measured at the ages of 3, 6 and 12 months. All growth characteristics were converted into standard deviation scores (SDS) using foetal^{10,12} and infant (GrowthAnalyzer 3.0, Dutch Growth Research Foundation, Rotterdam, The Netherlands) reference growth charts.

Respiratory outcomes at 6 years

Rint was measured in kPa/L (MicroRint, MicroMedical, Rochester, Kent, UK) during tidal expiration according to European Respiratory Society/American Thoracic Society guidelines. We calculated sex- and height-adjusted z-scores.¹³ We corrected for a stepwise variation of the median during the study period due to technical issues that required replacement of the Rint device. Wheezing was based on annual parental reports with available information on at least two time points. Wheezing patterns in four categories (never wheezing; early wheezing; late wheezing; persistent wheezing) were based on a previously used definitions.^{14,15} Parents reported ever physician-diagnosed asthma (no, yes) at 6 years.

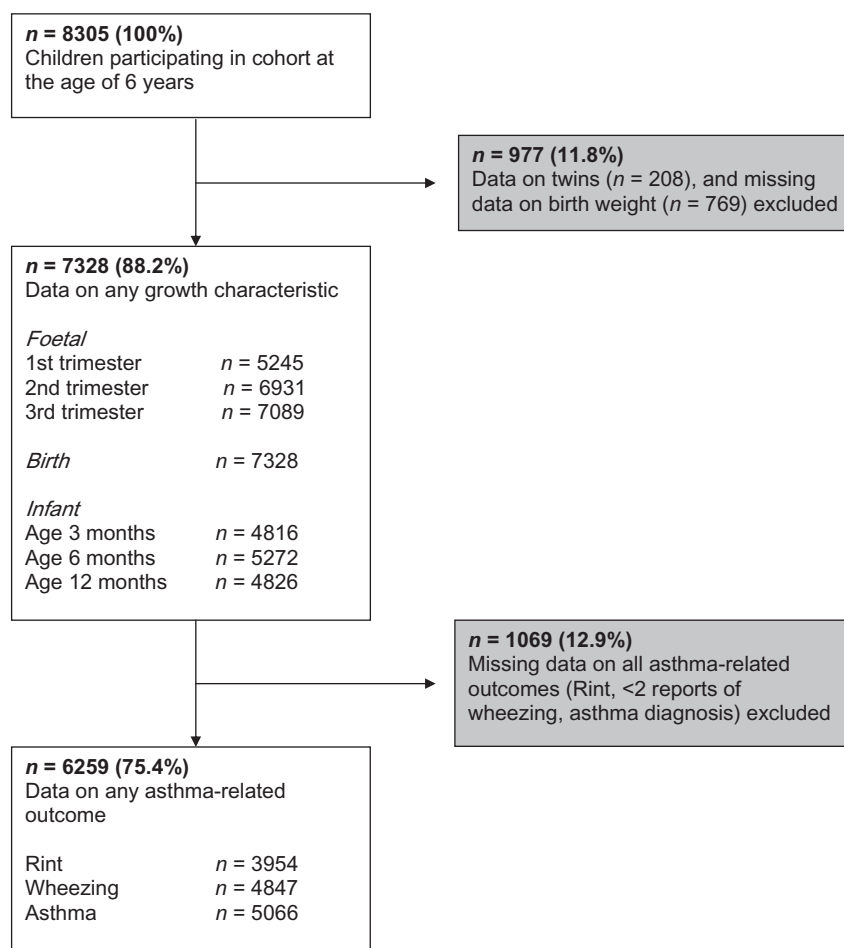


Figure 1 Flowchart.

Covariates

Information on maternal age, anthropometrics, socioeconomic status, history of asthma and atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders, and gestational diabetes and child's sex, gestational age, birth weight, ethnicity, pet keeping, breastfeeding, inhalant allergy and lower respiratory tract infections were collected by questionnaires or midwife and hospital registries.^{9,15,16}

Statistical analysis

Firstly, longitudinal repeated measurement models were performed using mixed-models, to assess differences in foetal and infant growth between children with and without high Rint (>85 vs <85 percentile), wheezing patterns and asthma. Secondly, we additionally performed conditional analyses to assess the independent effects of foetal and infant growth characteristics on respiratory outcomes; this allowed identification of critical periods of foetal and infant growth in relation to respiratory outcomes.¹⁷ Generalized estimating equations were used to assess the effect of growth with overall wheezing. Thirdly, we used adjusted logistic and linear regression analyses to examine the associations of growth characteristics at birth with respiratory outcomes. Finally, linear regression analyses were used to assess the differences in Rint between those with and without wheezing or asthma. All models were adjusted for potential confounders. Rint analyses were additionally adjusted for time period of the measurement. Missing data were imputed with multiple imputations using chained equations creating 25 new datasets. For extended methods see Supplementary Appendix 1.

RESULTS

Detailed characteristics of children and their mothers are presented in Table 1. Observed and imputed characteristics are presented in supplementary information (Supplementary Table S1).

Foetal and infant growth

Results from adjusted longitudinal foetal and infant growth analyses are given in Figure 2 (Supplementary Table S2). We observed that compared with school-age children with normal Rint (lower 85%), those with increased Rint (upper 15%) had a lower foetal length and weight growth (-0.008 (-0.016 , 0.000), and -0.007 (-0.014 , 0.000) standard deviation (SD) length or weight per week, respectively), but not a lower infant length or weight growth (Fig. 2a–d). No differences in longitudinal foetal and infant length or weight growth were observed between the different wheezing patterns or asthma groups (Supplementary Figure S1 A–H and Table S3). Sex and ethnicity were added as product terms with growth in the model, to explore potential effect modification on the association of growth with Rint, but no effect modification was observed (P -values for interaction >0.05).

We additionally performed conditional analyses, which reflect all foetal and infant growth characteristics as separate and independent measures in one model (Fig. 3, see Supplementary Table S4). Higher third trimester foetal length and higher second and third trimester weight were associated with decreased Rint at school-age (z -score difference -0.11 (-0.21 , -0.01), -0.13 (-0.23 , -0.04) and -0.12 (-0.22 , -0.02), respectively) (Fig. 3a,b). These analyses also showed that only weight at 3 months of age was associated with persistent wheezing (odds ratio (OR) 1.17 (1.04 , 1.31)) (Figure S2 B). Similar results were observed for associations of foetal and infant growth characteristics with overall wheezing until the age of 6 years (Supplementary Table S5), but not with asthma (Figure S2 C, D). Additional adjustment of these analyses for either upper or lower respiratory tract infections or inhalant allergy did not materially change the effect estimates (data not shown). Effect estimates for the associations of growth with respiratory outcomes, without taking account for the same growth measure at other ages, are given in the supplementary materials (Supplementary Table S6). We observed no effect modification between foetal (2nd trimester—birth) and infant (birth—12 months) length or weight growth for the associations with Rint, wheezing patterns or asthma (all P -values for interaction >0.05).

Birth outcomes

Rint z -scores were not different between children born preterm and born at term (Table 2). Birth weight analyses across the full range showed that per 500 grams decrease in birth weight Rint values increased (z -score difference (95% confidence interval) 0.16 (0.25 , 0.07)) (Table 2). We observed no association of low birth weight (<2500 grams) with Rint, compared with normal birth weight. However, after taking gestational age into account, a low birth weight for gestational age was associated with increased Rint (z -score difference 0.78 (0.33 , 1.22) z -score) (Table 2). Associations of preterm birth and low birth weight with wheezing and asthma are given in Supplementary Table S7.

Rint and asthma outcomes

In school-age children with persistent wheezing, we observed an increased Rint (z -score difference 0.58 (0.19 , 0.97) z -score) compared with children who never wheezed (Table 3). Also, children with a report of physician-diagnosed asthma had an increased Rint (z -score difference 0.55 (0.15 , 0.95) z -score), compared with those without asthma.

DISCUSSION

For this study, we hypothesized that smaller foetal growth and higher infant growth lead to respiratory morbidity. We observed that school-age children with an increased Rint had lower foetal length and weight growth and lower infant length growth, whereas

Table 1 Characteristics of children and their mothers

	All children in the study <i>n</i> = 6259	Rint available <i>n</i> = 3954	Wheezing or asthma available <i>n</i> = 5578
Maternal characteristics			
Age (years)	30.5 (5.0)	30.3 (5.1)	30.9 (4.8)
Body mass index			
<20 kg/cm ²	8.6 (536)	8.2 (324)	8.6 (482)
20–25.0 kg/cm ²	54.0 (3382)	54.1 (2141)	55.0 (3067)
25–30.0 kg/cm ²	26.1 (1634)	26.4 (1043)	25.8 (1439)
≥30 kg/cm ²	11.1 (697)	11.3 (446)	10.6 (589)
Educational level (%)			
Primary or secondary education	52.6 (3292)	54.6 (2158)	48.7 (2719)
Higher education	47.4 (2967)	45.4 (1796)	51.3 (2859)
History of asthma or atopy (%)			
No	62.2 (3891)	62.0 (2452)	62.4 (3482)
Yes	37.8 (2368)	38.0 (1502)	37.6 (2096)
Smoking during pregnancy (%)			
No	83.4 (5220)	82.5 (3262)	84.9 (4738)
Yes	16.6 (1039)	17.5 (692)	15.1 (840)
Parity (%)			
0	57.4 (3592)	56.9 (2249)	58.1 (3239)
≥1	42.6 (2667)	43.1 (1705)	41.9 (2339)
Maternal psychological distress (%)			
No	90.6 (5670)	89.7 (3548)	91.9 (5126)
Yes	9.4 (589)	10.3 (406)	8.1 (452)
Gestational hypertensive disorders (%)			
No	93.8 (5874)	93.8 (3709)	93.8 (5230)
Yes	6.2 (385)	6.2 (245)	6.2 (348)
Gestational diabetes (%)			
No	98.7 (6180)	98.7 (3902)	98.8 (5513)
Yes	1.3 (79)	1.3 (52)	1.2 (65)
Child characteristics			
Female sex (%)	50.1 (3136)	51.0 (2016)	50.2 (2799)
Gestational age at birth (weeks)	40.1 (35.9–42.3)	40.1 (35.8, 42.3)	40.1 (35.9, 42.3)
Birth weight (grams)	3435 (556)	3430 (552)	3450 (552)
Ethnicity (%)			
European	66.2 (4143)	64.1 (2533)	70.0 (3905)
Non-European	33.8 (2116)	35.9 (1421)	30.0 (1673)
Breastfeeding habits (%)			
Never	8.7 (545)	8.5 (335)	8.6 (478)
Ever	91.3 (5714)	91.5 (3619)	91.4 (5100)
Pet keeping (%)			
No	66.9 (4185)	67.3 (2663)	66.1 (3686)
Yes	33.1 (2074)	32.7 (1291)	33.9 (1892)
Inhalant allergy at 6 years (%)			
No	92.5 (5787)	92.2 (3647)	93.1 (5192)
Yes	7.5 (472)	7.8 (307)	6.9 (386)
Lower respiratory tract infections at 6 years (%)			
No	94.2 (5899)	93.6 (3701)	94.9 (5295)
Yes	5.8 (360)	6.4 (253)	5.1 (283)

Values are means (SD), medians (2.5–97.5th percentile) or percentages (absolute numbers) based on imputed data.

Rint, airway resistance SD; standard deviation.

school-age children with higher infant weight growth had increased risks of wheezing. Also, lower gestational age adjusted birth weight was associated with a higher Rint in childhood. Rint was not different between children born preterm and at term. Furthermore, children with persistent wheezing and physician-diagnosed asthma had increased Rint.

The associations of foetal growth with respiratory outcomes have been studied until pre-school age but showed inconsistent results.^{5,18,19} In a previous study, we observed that children with restricted foetal and infant weight gain had a decreased risk of preschool wheezing, whereas those with higher infant weight gain, independent of their foetal weight gain, had an

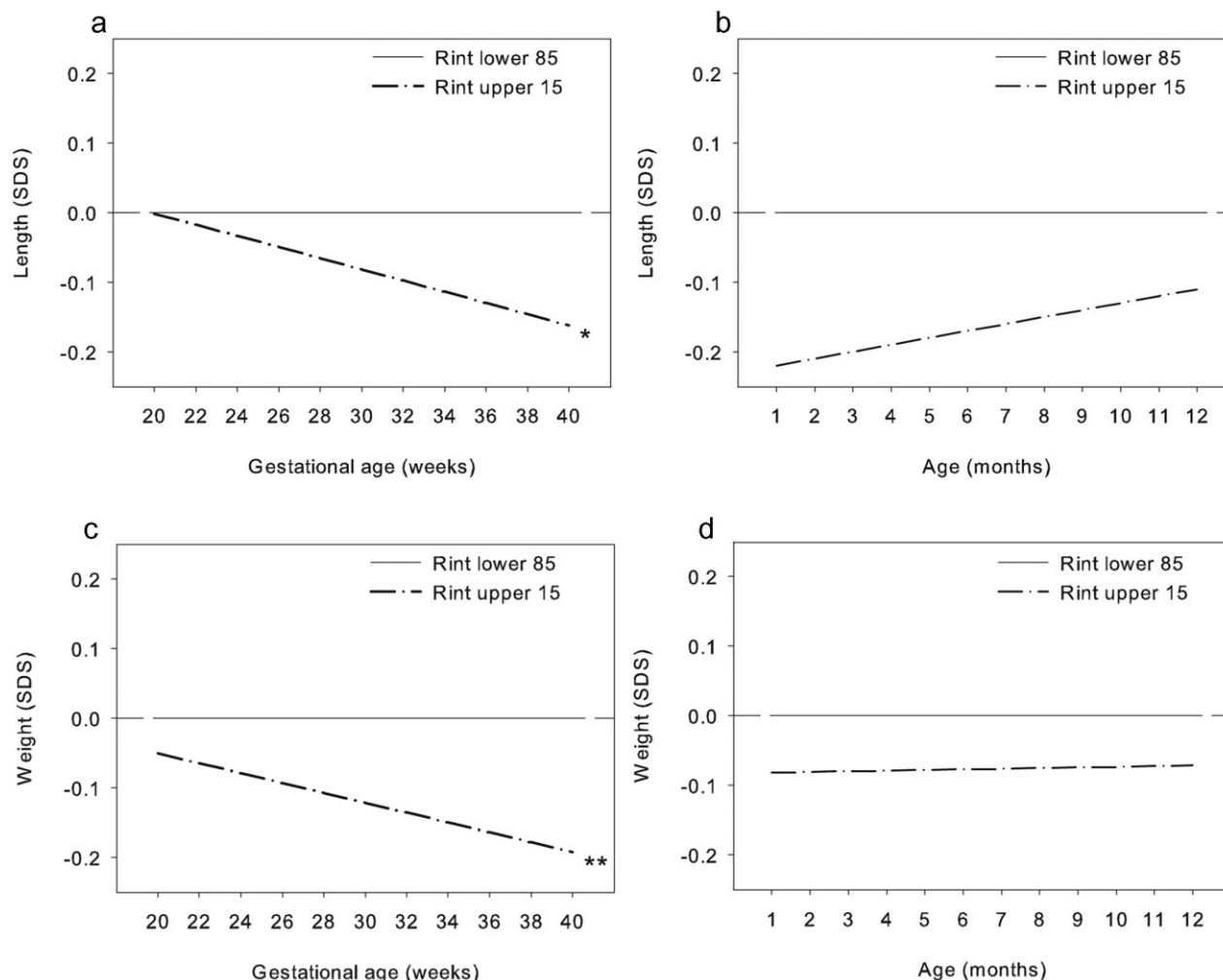


Figure 2 Differences in foetal and infant growth (length, weight) between Rint groups at the age of 6 years.

Lines are differences in length or weight growth in foetal life or infancy between the respiratory outcome groups Rint (low; high). Values were obtained from longitudinal repeated measurement models. * $P < 0.05$ and ** $P < 0.01$, given for the difference in growth over time. Specific estimates and P -values are given in Supplementary Table S2. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, gestational age, ethnicity, breastfeeding and pet keeping. Rint analyses were additionally adjusted for time period of the measurement.

Rint, airway resistance; SDS, standard deviation score.

increased risk of preschool wheezing.⁵ Based on the conditional regression models of the current study, we observed that children with high second trimester weight gain had a decreased risk of wheezing until age 6 years. However, similar to our previous study and now independent of all other growth periods, children with higher infant weight gain had an increased risk of wheezing until age 6 years.

Only one previous study has assessed the associations of foetal growth with asthma at an older age,²⁰ and this showed that increased foetal growth was associated with a reduced risk of asthma and a higher lung volume at the age of 10 years. In addition to this study, we now report that a lower foetal length and weight growth and lower infant length growth were associated with an increased Rint at school-age. Also, increased Rint was associated with increased risks of persistent

wheezing and asthma. Airways become larger with height,²¹ and therefore a decreased Rint might be expected in taller children. Our Rint measures were independent of height at time of measurement and size of the children therefore does not explain our consistent associations of foetal and infant length with Rint. Animal studies suggest that the mechanisms underlying the associations of foetal length and weight restriction with increased Rint are not atopy related but due to a decreased number of alveoli, thicker interalveolar septa and a greater volume density of lung tissue.^{7,22,23} Based on autopsy studies and magnetic resonance imaging- and ultrasound-based measurements during pregnancy, it is known that during the second trimester the airways grow wider, and a growth spurt of the distal pulmonary vasculature and of the first alveoli occurs. During the first year after

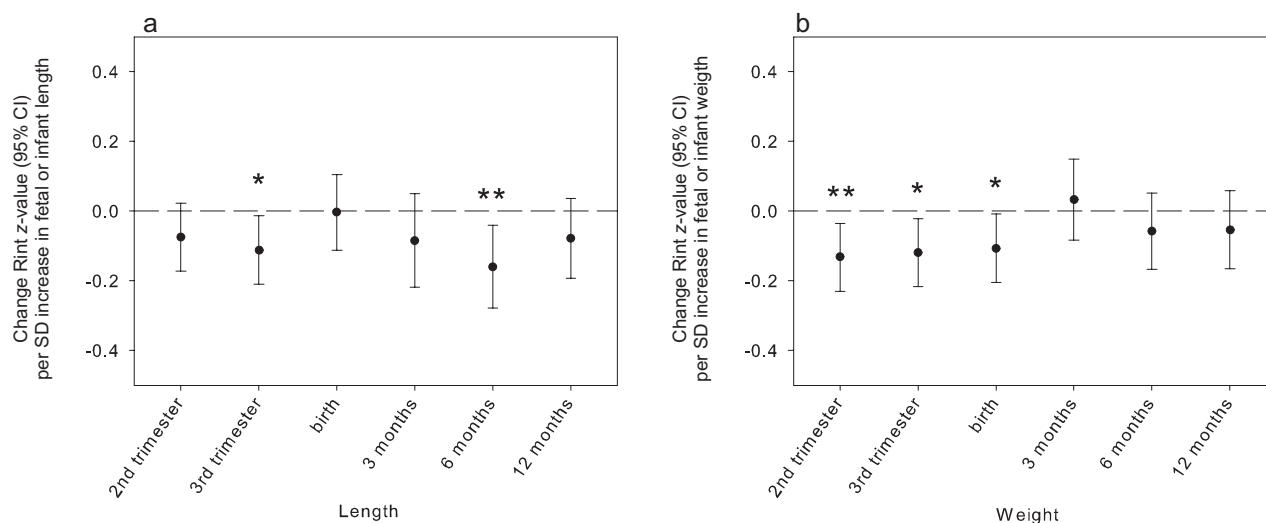


Figure 3 Associations of independent foetal and infant growth characteristics per SD with Rint at the age of 6 years. Values are Rint's z-score change (95% confidence interval) from conditional analysis in which associations are independent of the associations at other time points and reflect the risk per standard deviation of residual increase in length or weight. Specific estimates and *P*-values are given in Supplementary Table S4. **P* < 0.05 and ***P* < 0.01. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, gestational age, ethnicity, breastfeeding and pet keeping. Rint analyses were additionally adjusted for time period of the measurement. 95% CI, 95% confidence interval; Rint, airway resistance; SD, standard deviation.

birth most alveoli have been formed.^{8,24,25} Children with increased Rint had lower foetal length and weight growth, whereas children with specific wheezing patterns or asthma did not. Rint being too low to induce respiratory symptoms, or other underlying mechanisms for the association of early growth with respiratory symptoms might explain this.

Infant weight gain was associated with increased risks of wheezing and asthma and lower lung function in later life.^{26,27} However, we did not observe associations of infant weight gain with Rint. Therefore, the association of infant growth with wheezing, as previously observed in the same study population, cannot be explained by high Rint. Higher infant weight growth may lead to altered levels of inflammatory adipokines secreted from adipose tissue and potentially result in altered airway inflammation and responsiveness.²⁸ Higher infant weight growth might also have a direct mechanical effect on lung function other than increased Rint.²⁹

Thus, our results suggest different potential underlying pathways for foetal and infant length and weight growth and their associations with Rint and wheezing or asthma. Potential U-shaped associations need to be further explored.

A recent meta-analysis observed that preterm born children (<37 weeks of pregnancy) have a 1.40-fold increased risk of childhood asthma.³⁰ In line with this study, we observed an inverse association of gestational age with early and persistent wheezing, and asthma until age 6 years. Previous studies showed lower lung volumes and reduced airway calibre in preterm-born children, which persist in later life.³¹ We did not observe an association with Rint. The small

proportion of preterm born children in our cohort might have resulted in insufficient power to detect small effect estimates. The pathways leading from preterm birth to childhood asthma might not include Rint, but lung volume or immunomodulatory effects.^{6,32} Birth weight adjusted for gestational age was associated with a lower Rint only. Birth weight, adjusted for gestational age, seems not consistently associated with increased risks of wheezing and asthma in childhood.^{1,5,33}

This study was embedded in a population-based prospective cohort study with a large number of subjects being studied from early foetal life onwards. We performed detailed and frequent measurements on foetal and infant anthropometrics and respiratory outcomes. Some methodological issues need to be discussed. First, from the children participating in the general cohort at age 6 years, 75% were included in this sub-study. It is possible that biased effect estimates could occur if participants lost to follow-up, or non-responders, had different associations between foetal and infant growth with respiratory outcomes compared with those included in the study. This is unlikely but difficult to study. We observed differences in characteristics between children with data on Rint and wheezing or asthma. Although we adjusted our analyses for these differences in characteristics, these and other unmeasured covariates could limit the comparability of the associations between early growth and Rint or wheezing and asthma. Secondly, to estimate gestational age of the foetus in early pregnancy, we used crown-rump-length. During early gestation, identical growth velocities have been observed for embryos and fetuses. However, there might be

Table 2 Birth characteristics and airway resistance at the age of 6 years

	Rint (z-score) Difference (95% CI) n = 3954
Gestational age	
Preterm birth (<37 weeks) n = 293	0.18 (−0.26, 0.62)
Term birth (37–42 weeks) n = 5445	Reference
Post term birth (>42 weeks) n = 300	0.07 (−0.36, 0.50)
<i>Trend (per week)</i>	−0.03 (−0.08, 0.03)
Absolute birth weight	
Low birth weight (<2500 grams) n = 268	0.46 (−0.01, 0.92)
Normal birth weight (2500–4500 grams) n = 5634	Reference
High birth weight (>4500 grams) n = 136	−0.43 (−1.09, 0.23)
<i>Trend (weight per 500 grams)</i>	−0.16 (−0.25, −0.07)**
Birth weight for gestational age	
Small size for gestational age n = 314	0.78 (0.33, 1.22)**
Appropriate size for gestational age n = 5631	Reference
Large size for gestational age n = 314	−0.59 (−1.04, −1.14)**
<i>Trend (per SD)</i>	−0.18 (−0.28, −0.08)**

Values are Rint's z-score difference and, if continuously measured, reflect the difference of Rint per 500 grams birth weight or week of gestational age or per SD of size for gestational age increase (95% confidence interval). * $P < 0.05$, ** $P < 0.01$. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes, and for child's gender, ethnicity, breastfeeding habits and pet keeping. The analyses were additionally adjusted for time period of the Rint measurement. Small size for gestational age was defined as the lowest 5% (SD = −1.74) and large size for gestational age was defined as the highest 5% (SD = 1.59) of size for gestational age.

95% CI, 95% confidence interval; Rint, airway resistance; SD, standard deviation.

differences in size between fetuses.³⁴ Consequently, using crown-rump-length as a measure of pregnancy duration reduces the variation in early growth towards zero. This might have led to an underestimation of the effect estimates. Thirdly, we did not perform spirometry at this age, because children participating in this population-based cohort were not familiar with this procedure, and it is known that lung function values according to ATS criteria are feasible and acceptable for approximately 50% of the children performing spirometry for the first time.³⁵ The Rint technique is much more feasible in this age group, with success rates >90%, and enables detection of small differences in Rint with good within- and

Table 3 Differences in Rint z-scores between those with and without wheezing, or asthma

	Mean Rint z-score (SD)	Difference Rint z-score
Never wheezing n = 1537	0.90 (3.04)	Reference
Early wheezing n = 781	1.15 (2.97)	0.25 (−0.03, 0.54)
Late wheezing n = 131	0.80 (3.08)	−0.06 (−0.66, 0.54)
Persistent wheezing n = 375	1.47 (3.37)	0.58 (0.19, 0.97)**
No asthma n = 2793	0.98 (2.90)	Reference
Asthma n = 236	1.54 (3.08)	0.55 (0.15, 0.95)**

Values are mean differences (95% confidence interval) from linear regression analysis. * $P < 0.05$; ** $P < 0.01$. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes, and for child's gender, gestational age at birth, ethnicity, breastfeeding habits and pet keeping. Rint analyses were additionally adjusted for time period of the measurement.

Rint, airway resistance; SD, standard deviation.

between-occasion reproducibility.³⁶ The biological validity of increased Rint has been extensively demonstrated, and Rint is associated with clinically relevant end-points including bronchodilator reversibility, methacholine responsiveness,³⁷ asthma diagnosis or wheezing and can distinguish between groups of symptomatic and healthy young children.³⁶ Although the observed mean differences in Rint were small, and not clinically relevant for the individual child, they might be important from an aetiological perspective and may help to target prevention programmes towards high-risk groups. Mean Rint values for children with no wheezing or asthma were slightly elevated that might limit the generalizability of the observed effect estimates.³⁶ Last, we adjusted for a large number of confounders. However, as in any observational study, residual confounding due to unmeasured or insufficiently measured confounders might still be an issue.

In conclusion, Rint in school-age children is influenced by foetal length and weight restriction and associated with increased risk of persistent wheezing and asthma. The pathways from preterm birth to asthma outcomes may include other mechanisms than differences in Rint. Further studies are needed to replicate our findings and to assess the underlying mechanisms of foetal and infant growth on airway and lung development and subsequent lung function and risk for asthma in later life.

Acknowledgements

The Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus Uni-

versity Rotterdam and the Netherlands Organization for Health Research and Development. The researchers are independent from the funders. The study sponsors had no role in the study design, data analysis, interpretation of data or writing of this report.

REFERENCES

- Kindlund K, Thomsen SF, Stensballe LG, Skytthe A, Kyvik KO, Backer V, Bisgaard H. Birth weight and risk of asthma in 3–9-year-old twins: exploring the fetal origins hypothesis. *Thorax* 2010; **65**: 146–9.
- Kotecha SJ, Watkins WJ, Heron J, Henderson J, Dunstan FD, Kotecha S. Spirometric lung function in school-age children: effect of intrauterine growth retardation and catch-up growth. *Am. J. Respir. Crit. Care Med.* 2010; **181**: 969–74.
- Duijts L, Reiss IK, Brusselle G, de Jongste JC. Early origins of chronic obstructive lung diseases across the life course. *Eur. J. Epidemiol.* 2014; **29**: 871–85.
- Shaheen SO, Sterne JA, Tucker JS, Florey CD. Birth weight, childhood lower respiratory tract infection, and adult lung function. *Thorax* 1998; **53**: 549–53.
- Sonnenschein-van der Voort AM, Jaddoe VW, Raat H, Moll HA, Hofman A, de Jongste JC, Duijts L. Fetal and infant growth and asthma symptoms in preschool children: the Generation R Study. *Am. J. Respir. Crit. Care Med.* 2012; **185**: 731–7.
- Schulzke SM, Hall GL, Nathan EA, Simmer K, Nolan G, Pillow JJ. Lung volume and ventilation in homogeneity in preterm infants at 15–18 months corrected age. *J. Pediatr.* 2010; **156**: 542–9, e2.
- Wignarajah D, Cock ML, Pinkerton KE, Harding R. Influence of intrauterine growth restriction on airway development in fetal and postnatal sheep. *Pediatr. Res.* 2002; **51**: 681–8.
- Kasprian G, Balassy C, Brugger PC, Prayer D. MRI of normal and pathological fetal lung development. *Eur. J. Radiol.* 2006; **57**: 261–70.
- Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H *et al.* The Generation R Study: design and cohort update 2012. *Eur. J. Epidemiol.* 2012; **27**: 739–56.
- Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet. Gynecol.* 2008; **31**: 388–96.
- Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology* 1984; **150**: 535–40.
- Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). *Acta Paediatr. Scand.* 1991; **80**: 756–62.
- Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the Asthma UK Initiative. *Eur. Respir. J.* 2010; **36**: 157–63.
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N. Engl. J. Med.* 1995; **332**: 133–8.
- Guxens M, Sonnenschein-van der Voort AM, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, Jaddoe VW, Duijts L. Parental psychological distress during pregnancy and wheezing in preschool children: the Generation R Study. *J. Allergy Clin. Immunol.* 2014; **133**: 59–67, e1–12.
- den Dekker HT, Sonnenschein-van der Voort AM, de Jongste JC, Reiss IK, Hofman A, Jaddoe VW, Duijts L. Tobacco smoke exposure, airway resistance, and asthma in school-age children: the Generation R Study. *Chest* 2015; **148**: 607–17.
- Keijzer-Veen MG, Euser AM, van Montfoort N, Dekker FW, Vandenbroucke JP, Van Houwelingen HC. A regression model with unexplained residuals was preferred in the analysis of the fetal origins of adult diseases hypothesis. *J. Clin. Epidemiol.* 2005; **58**: 1320–4.
- Pike KC, Crozier SR, Lucas JS, Inskip HM, Robinson S, The Southampton Women's Survey Study G, Roberts G, Godfrey KM. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. *Thorax* 2010; **65**: 1099–106.
- Turner SW, Campbell D, Smith N, Craig LC, McNeill G, Forbes SH, Harbour PJ, Seaton A, Helms PJ, Devereux GS. Associations between fetal size, maternal [alpha]-tocopherol and childhood asthma. *Thorax* 2010; **65**: 391–7.
- Turner S, Prabhu N, Danielan P, McNeill G, Craig L, Allan K, Cutts R, Helms P, Seaton A, Devereux G. First- and second-trimester fetal size and asthma outcomes at age 10 years. *Am. J. Respir. Crit. Care Med.* 2011; **184**: 407–13.
- Cole TJ, Stanojevic S, Stocks J, Coates AL, Hankinson JL, Wade AM. Age- and size-related reference ranges: a case study of spirometry through childhood and adulthood. *Stat. Med.* 2009; **28**: 880–98.
- Maritz GS, Cock ML, Louey S, Joyce BJ, Albuquerque CA, Harding R. Effects of fetal growth restriction on lung development before and after birth: a morphometric analysis. *Pediatr. Pulmonol.* 2001; **32**: 201–10.
- Joyce BJ, Louey S, Davey MG, Cock ML, Hooper SB, Harding R. Compromised respiratory function in postnatal lambs after placental insufficiency and intrauterine growth restriction. *Pediatr. Res.* 2001; **50**: 641–9.
- Emery JL, Mithal A. The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. *Arch. Dis. Child.* 1960; **35**: 544–7.
- Kajekar R. Environmental factors and developmental outcomes in the lung. *Pharmacol. Ther.* 2007; **114**: 129–45.
- Anderson EL, Fraser A, Martin RM, Kramer MS, Oken E, Patel R, Tilling K. Associations of postnatal growth with asthma and atopy: the PROBIT Study. *Pediatr. Allergy Immunol.* 2013; **24**: 122–30.
- Lucas JS, Inskip HM, Godfrey KM, Foreman CT, Warner JO, Gregson RK, Clough JB. Small size at birth and greater postnatal weight gain: relationships to diminished infant lung function. *Am. J. Respir. Crit. Care Med.* 2004; **170**: 534–40.
- Shore SA, Terry RD, Flynt L, Xu A, Hug C. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J. Allergy Clin. Immunol.* 2006; **118**: 389–95.
- Beuther DA, Weiss ST, Sutherland ER. Obesity and asthma. *Am. J. Respir. Crit. Care Med.* 2006; **174**: 112–19.
- Sonnenschein-van der Voort AM, Arends LR, de Jongste JC, Annesi-Maesano I, Arshad SH, Barros H, Basterrechea M, Bisgaard H, Chatzi L, Corpeleijn E *et al.* Preterm birth, infant weight gain, and childhood asthma risk: a meta-analysis of 147,000 European children. *J. Allergy Clin. Immunol.* 2014; **133**: 1317–29.
- Kotecha SJ, Watkins WJ, Paranjothy S, Dunstan FD, Henderson AJ, Kotecha S. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. *Thorax* 2012; **67**: 54–61.
- Matias V, San Feliciano L, Fernandez JE, Lapena S, Garrido E, Ardura J, Soga MJ, Aragon MP, Remesal A, Benito F *et al.* Host and environmental factors influencing respiratory secretion of pro-wheezing biomarkers in preterm children. *Pediatr. Allergy Immunol.* 2012; **23**: 441–7.
- Caudri D, Wijga A, Gehring U, Smit HA, Brunekreef B, Kerkhof M, Hoekstra M, Gerritsen J, de Jongste JC. Respiratory symptoms in the first 7 years of life and birth weight at term: the PIAMA Birth Cohort. *Am. J. Respir. Crit. Care Med.* 2007; **175**: 1078–85.
- Blaas HG, Eik-Nes SH, Bremnes JB. The growth of the human embryo. A longitudinal biometric assessment from 7 to 12 weeks of gestation. *Ultrasound Obstet. Gynecol.* 1998; **12**: 346–54.

- 35 Gaffin JM, Shotola NL, Martin TR, Phipatanakul W. Clinically useful spirometry in preschool-aged children: evaluation of the 2007 American Thoracic Society Guidelines. *J. Asthma* 2010; **47**: 762–7.
- 36 Merkus PJ, Mijnsbergen JY, Hop WC, de Jongste JC. Interrupter resistance in preschool children: measurement characteristics and reference values. *Am. J. Respir. Crit. Care Med.* 2001; **163**: 1350–5.
- 37 Koopman M, Brackel HJ, Vaessen-Verberne AA, Hop WC, van der Ent CK. Evaluation of interrupter resistance in methacholine challenge testing in children. *Pediatr. Pulmonol.* 2011; **46**: 266–71.

Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's website.

Appendix 1 Methods.

Figure S1 Differences in foetal and infant growth (length, weight) between wheezing and asthma groups at the age of 6 years.

Figure S2 Associations of independent foetal and infant growth characteristics per SD with wheezing and asthma at the age of 6 years.

Table S1 Characteristics of mothers and their children.

Table S2 Differences in growth between Rint groups at the age of 6 years (presented in Fig. 2).

Table S3 Differences in growth between wheezing patterns and asthma groups at the age of 6 years (presented in Fig. 2E).

Table S4 Associations of foetal and infant growth characteristics per SD from conditional growth models with respiratory outcomes (presented in Fig. 3).

Table S5 Associations of foetal and infant growth characteristics per SD with overall wheezing.

Table S6 Associations of foetal and infant growth characteristics per SD with respiratory outcomes.

Table S7 Birth characteristics and respiratory outcomes at the age of 6 years.

SUPPLEMENTARY INFORMATION

Fetal and infant growth patterns, airway resistance and school-age asthma.

Agnes M.M. Sonnenschein-van der Voort, MSc, PhD^{1,2,3}, Romy Gaillard, MSc, PhD^{1,3,4}, Johan C. de Jongste, MD, PhD², Albert Hofman, MD, PhD³, Vincent W.V. Jaddoe, MD, PhD^{1,3,4} Liesbeth Duijts, MD, PhD^{2,3,5}

¹The Generation R Study Group, ²Department of Pediatrics, Division of Respiratory Medicine,

³Department of Epidemiology, ⁴Department of Pediatrics, ⁵Department of Pediatrics, Division of Neonatology, Erasmus Medical Center, Rotterdam, The Netherlands

APPENDIX 1 - METHODS

Design and setting

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children in Rotterdam, The Netherlands¹. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants. The design of the study has been previously described in detail¹. In summary, mothers with an expected delivery date between April 2002 and January 2006 and who were resident in the study area were eligible. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care, handed out the information package and asked these mothers to make an appointment for their first ultrasound examination. Enrolment was aimed in first trimester, but was allowed until birth of the child¹. Of all children included in the Generation R study, 85.2% (n = 8,305) participated in the follow up studies at the age of 6 years for which parents gave written informed consent. Twins (n=208), and

children with no information on birth weight for gestational age (n=769) were excluded (Figure 1). Children with missing data on all respiratory outcomes (Rint, less than two reports of wheezing (no, yes), or physician-diagnosed asthma) were excluded (n=1,069) leaving a total of 6,259 children included for the current analyses. Children were born after a median pregnancy duration of 40.1 weeks (95% range: 35.9 – 42.3) and a mean birth weight of 3,435 grams (SD 556 grams). The average Rint was 0.94 kPa/L/s (SD 0.25). Of the children, 42.2% were classified as never wheezing, 21.9% as early wheezing, 3.6% as late wheezing, and 9.7% as persistent wheezing. A physician diagnosis of asthma was reported for 7.7% of the children. Data on Rint was less frequently available (63.2%) than data on wheezing (77.4%) and asthma (80.9%). Subjects with Rint seemed lower educated, smoked more, had more psychological distress, and were more often of non-European ethnicity (see table 1).

Growth characteristics

Fetal growth characteristics

Fetal ultrasound examinations were carried out in a dedicated research center in each trimester of pregnancy. The ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). These examinations were used for both establishing gestational age and assessing fetal growth characteristics². Because using the last menstrual period has several limitations, such as the large number of mothers who do not know the exact date of their last menstrual period or have irregular menstrual cycles, gestational age was established by first trimester fetal ultrasound. In the second and third trimesters of pregnancy, we measured head circumference (HC), abdominal

circumference (AC), and femur length (FL) to the nearest millimeter using standardized ultrasound procedures^{2, 3}. Estimated fetal weight (EFW) was subsequently calculated by using the Hadlock formula ($\log_{10} \text{ EFW} = 1.5662 - 0.0108 (\text{HC}) + 0.0468 (\text{AC}) + 0.171 (\text{FL}) + 0.00034 (\text{HC})^2 - 0.003685 (\text{AC} \cdot \text{FL})$)^{4, 5}. Standard deviation scores (SD) for all fetal growth characteristics were constructed^{2, 5}.

At birth, information on gestational age, length and weight of the infants was obtained from community midwife and hospital registries. Birth length was only available in 3,931 individuals, since this was not routinely measured in obstetric practices in The Netherlands. Gestational age adjusted standard deviation scores for weight at birth was constructed using reference growth standards⁵. Small and large size for gestational age were defined as the lower 5% ($\text{SD} \leq -1.74$), and the upper 5% ($\text{SD} \geq 1.59$) of birth weight standard deviation score, respectively.

Infant growth characteristics Infant growth was measured at the Community Health Centers according to a standard schedule and procedures by a well-trained staff at the ages of 3 months (range: 3.00-3.96 months), 6 months (range: 5.01-9.96 months), and 12 months (range: 10.00-12.97 months). Length was determined in supine position to the nearest millimeter using a neonatometer. Weight was measured using a mechanical personal scale (SECA). Standard deviation scores for postnatal length, and weight were obtained using reference growth charts (Growth Analyzer 3.0, Dutch Growth Research Foundation).

Respiratory outcomes at 6 years

Airway resistance (Rint) was measured in kPa/L (MicroRint, MicroMedical, Rochester, Kent, UK) during tidal expiration, with occlusion of the airway at peak

expiratory flow, according to ERS and ATS guidelines⁶. Median values for at least 5 acceptable Rint measurements were calculated and these were used to calculate sex and height adjusted z-scores⁷. We corrected for a stepwise variation of the median during the study period due to technical issues that required replacement of the Rint⁸ and statistical analyses were adjusted for the time period of the measurement. Wheezing and physician-diagnosed asthma were assessed using questions adapted from the International Study on Asthma and Allergy in Childhood (ISAAC)⁹. Wheezing patterns in four categories (never wheezing, early wheezing, late wheezing, and persistent wheezing) were based on a previously used preschool wheezing patterns definition¹⁰ and extended into wheezing patterns until the age of 6 years: (1) no wheezing, no recorded wheezing at any age; (2) early wheezing, at least 1 wheezing symptom during the first 3 years of life but no wheezing at 4 and 6 years of age; (3) late wheezing, no wheezing episodes during the first 3 years of age but wheezing at 4 or 6 years of age; and (4) persistent wheezing, at least 1 wheezing episode in the first 3 years of life and wheezing at 4 or 6 years of age. Wheezing was reported annually for the first four years and at 6 years of age. We imputed missing reports only in those children of whom information on wheezing at a minimum of two timepoints was available.

Covariates

Information on maternal age, anthropometrics, socio-economic status, history of asthma and atopy, parity, child's ethnicity and pet keeping were obtained by questionnaire, completed by the mother at enrollment. Socio-economic status was assessed using the highest educational level achieved by the mother. Information about active maternal smoking during pregnancy was obtained by postal

questionnaires sent in first, second and third trimester of pregnancy and combined into smoking (no, yes)¹. Although assessing smoking habits by questionnaires is valid in epidemiological studies, misclassification may occur due to underreporting.^{11,}
¹² However, the use of biomarkers of tobacco smoke exposure in urine, saliva or blood, or nicotine in indoor air seemed not superior to self-report.^{11, 12}. We used parity as a proxy for siblings and the correlation between those variables was good (kappa = 0.896). Maternal psychological distress is suggested to influence growth and the risk of asthma through an altered hypothalamic-pituitary-adrenal axis, immunomodulatory factors, or epigenetic programming¹⁰. Maternal psychological distress was defined using a global severity index (GSI) at second trimester, a measure of current level or depth of the symptoms, and denotes overall psychological distress¹⁰. High scores represented an increased occurrence of overall distress which was based on Dutch cut-offs¹³. Gestational hypertensive disorders, and diabetes might influence the association between growth and respiratory morbidity via altered placental and vascular function and development or via gestational age at birth¹⁴. Maternal gestational hypertensive disorders (gestational hypertension, preeclampsia, eclampsia, and HELLP-syndrome (Hemolysis Elevated Liver enzymes and Low Platelets)), gestational diabetes and gestational age and sex of the children were obtained from midwife and hospital registries at birth. Postal questionnaires at 6 and 12 months provided information about breastfeeding and daycare attendance¹. Information on lower respiratory tract infections (pneumonia, bronchitis) and inhalation allergy (pollen, house dust mite, pets) was obtained by questionnaires at the age of 6 years.

Statistical analysis

First, the associations of respiratory outcomes (high Rint values (top 15%), wheezing patterns, or asthma) with repeatedly measured fetal and infant length and weight were analyzed using unbalanced longitudinal repeated measurement regression models. This allowed us to study the age independent effects (difference constant over time) as well as age dependent effects (difference non constant over time) of fetal and infant growth in relation the asthma outcomes. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete growth data. Respiratory outcomes were included in these models as intercept and as an interaction term with age. These models can be written as: fetal growth = $\beta_0 + \beta_1 \text{ respiratory outcome} + \beta_2 \text{ gestational age} + \beta_3 \text{ respiratory outcome} \times \text{gestational age} + \beta_i \text{ confounders}$. Similarly, infant growth = $\beta_0 + \beta_1 \text{ respiratory outcome} + \beta_2 \text{ age in months} + \beta_3 \text{ respiratory outcome} \times \text{age in months} + \beta_i \text{ confounders}$. In these models, ' $\beta_0 + \beta_1 \text{ respiratory outcome}$ ' reflects the length or weight growth per respiratory outcome group and ' $\beta_2 \text{ age}$ ' reflects the slope of growth per week of gestational age or month, and ' $\beta_3 \text{ respiratory outcome} \times \text{age}$ ' the difference in change in length or weight growth per week of gestational age or month for respiratory outcome groups.

Secondly, growth measurements at different time points are strongly correlated.

Taking into account the correlations between these different growth measurements, we used conditional linear and logistic regression models to assess the independent associations of fetal and infant growth characteristics with respiratory outcomes. We constructed growth variables that are statistically independent from growth characteristics at other time points, using standardized residuals from linear regression models. In these linear regression models, a growth measurement at a

given time point was regressed on all prior growth measurements. The excess in growth at given time point as predicted by prior growth measurements is similar as the standardized residuals resulting from this regression model. The standardized residuals obtained from this model are entirely uncorrelated with prior growth measurements. This enables inclusion of all growth measures together in a regression model without problems that may occur due to the correlation between different growth measures. Additionally, we used generalized estimating equations (GEEs) to examine the longitudinal effects of fetal and infant growth with the risk of overall wheezing at the ages of 1, 2, 3, 4, and 6 years. These models take into account the correlations between repeated measurements within the same subject. We used an unstructured matrix. Thirdly, we used adjusted logistic and linear regression analysis to examine the associations of growth characteristics at birth with airway resistance measurements (Rint), the risk of wheezing patterns, and of asthma diagnosis. Finally, adjusted linear regression analyses were used to assess the differences in Rint between those with and without wheezing, or asthma. All models were adjusted for potential confounders including maternal age, body mass index, education, history of asthma or atopy, smoking habits, parity, maternal psychological distress, gestational hypertensive problems, and gestational diabetes, and child's sex, gestational age at birth, ethnicity, breastfeeding status, and pet keeping. Variables were included as confounders when they were associated with both the determinant and the outcome, suggested to be based on previous literature, or changed the effect estimate >10% when adding the variable to the unadjusted model. Lower respiratory tract infections at the age of 6 years and inhalation allergy were potential intermediates. The percentages of missing values within the population for analysis were lower or near to 10%, except for maternal history of

asthma or atopy (18.2%), psychological distress (17.9%), and child's breastfeeding (18.5%), and pet keeping (13.8%). The high percentage missings of these confounders, except for psychological distress, is explained by a reduction of data collection between the age of 2 months and 4 years in a subgroup of the current study¹. Missing data of the confounders and wheezing patterns were imputed with multiple imputations. We imputed missing growth data only for the conditional models. Twenty-five new datasets were created by imputation based on all determinants, covariates and outcomes in the model plus maternal ethnicity, paternal age, educational level and history of asthma or atopy, smoking habits, and child's upper and lower respiratory tract infections until the age of 6 years, shortness of breath until the age of 4 years, and physician diagnosis of eczema until the age of 6 years¹⁵. All datasets were analyzed separately after which results were combined. No differences in results were observed between analyses with imputed missing data or complete cases only. The effect estimates of the unadjusted analyses did not materially change when we added confounders in our main models. We only present the results based on imputed datasets. Statistical analyses were performed using the Statistical Package of Social Sciences version 20.0 for Windows (IBM Corp., Armonk, NY, USA.), and for the repeated measurement models we used SAS 9.2 (SAS institute, Cary, NC, USA).

References

- 1 Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Ijzendoorn MH, de Jongste JC, van der Lugt A, Mackenbach JP, Moll HA, Raat H, Rivadeneira F, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study: design and cohort update 2012. *Eur J Epidemiol.* 2012; **27**: 739-56.
- 2 Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, Moll HA, Jaddoe VW, Witteman JC. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. *Ultrasound Obstet Gynecol.* 2008; **31**: 388-96.
- 3 Verburg BO, Mulder PG, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. Intra- and interobserver reproducibility study of early fetal growth parameters. *Prenat Diagn.* 2008; **28**: 323-31.
- 4 Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology.* 1984; **150**: 535-40.
- 5 Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). *Acta Paediatr Scand.* 1991; **80**: 756-62.
- 6 Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, Bisgaard H, Davis GM, Ducharme FM, Eigen H, Gappa M, Gaultier C, Gustafsson PM, Hall GL, Hantos Z, Healy MJ, Jones MH, Klug B, Lodrup Carlsen KC, McKenzie SA, Marchal F, Mayer OH, Merkus PJ, Morris MG, Oostveen E, Pillow JJ, Seddon PC, Silverman M, Sly PD, Stocks J, Tepper RS, Vilozni D, Wilson NM. An official American Thoracic

Society/European Respiratory Society statement: pulmonary function testing in preschool children. *Am J Respir Crit Care Med.* 2007; **175**: 1304-45.

7 Merkus PJ, Stocks J, Beydon N, Lombardi E, Jones M, McKenzie SA, Kivastik J, Arets BG, Stanojevic S. Reference ranges for interrupter resistance technique: the Asthma UK Initiative. *Eur Respir J.* 2010; **36**: 157-63.

8 van der Valk RJ, Kieffe-de Jong JC, Sonnenschein-van der Voort AM, Duijts L, Hafkamp-de Groen E, Moll HA, Tiemeier H, Steegers EA, Hofman A, Jaddoe VW, de Jongste JC. Neonatal folate, homocysteine, vitamin B12 levels and methylenetetrahydrofolate reductase variants in childhood asthma and eczema. *Allergy.* 2013; **68**: 788-95.

9 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J.* 1995; **8**: 483-91.

10 Guxens M, Sonnenschein-van der Voort AM, Tiemeier H, Hofman A, Sunyer J, de Jongste JC, Jaddoe VW, Duijts L. Parental psychological distress during pregnancy and wheezing in preschool children: The Generation R Study. *The Journal of allergy and clinical immunology.* 2013.

11 Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. *Am J Public Health.* 1994; **84**: 1086-93.

12 Wang X, Tager IB, Van Vunakis H, Speizer FE, Hanrahan JP. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. *Int J Epidemiol.* 1997; **26**: 978-88.

13 De Beurs E. Brief Symptoms Inventory, handleiding addendum. Leiden, The Netherlands. PITS BV. 2009.

- 14 Rusconi F, Galassi C, Forastiere F, Bellasio M, De Sario M, Ciccone G, Brunetti L, Chellini E, Corbo G, La Grutta S, Lombardi E, Piffer S, Talassi F, Biggeri A, Pearce N. Maternal complications and procedures in pregnancy and at birth and wheezing phenotypes in children. *Am J Respir Crit Care Med*. 2007; **175**: 16-21.
- 15 Spratt M, Carpenter J, Sterne JA, Carlin JB, Heron J, Henderson J, Tilling K. Strategies for multiple imputation in longitudinal studies. *Am J Epidemiol*. 2010; **172**: 478-87.

Table S1 Characteristics of mothers and their children

	Observed	Imputed
Maternal characteristics		
Age (years)	30.5 (5.0)	30.5 (5.0)
Body mass index		
<20 kg/cm ²	8.6 (535)	8.6 (536)
20-25.0 kg/cm ²	54.1 (3,358)	54.0 (3,382)
25-30.0 kg/cm ²	26.1 (1,621)	26.1 (1,634)
≥30 kg/cm ²	11.2 (696)	11.1 (697)
Missing	0.8 (49)	-
Education (%)		
Primary, or secondary	51.3 (3,055)	52.6 (3,292)
Higher	48.7 (2,881)	47.4 (2,967)
Missing	5.5 (343)	-
History of asthma or atopy (%)		
No	60.5 (3,098)	62.2 (3,891)
Yes	39.5 (2,024)	37.8 (2,368)
Missing	18.2 (1,137)	-
Smoking during pregnancy (%)		
No	83.4 (4,671)	83.4 (5,220)
Yes	16.6 (927)	16.6 (1,039)
Missing	10.6 (661)	-
Parity (%)		
0	57.4 (3,571)	57.4 (3,592)
≥1	42.6 (2,761)	42.6 (2,667)
Missing	0.6 (37)	-
Maternal psychological distress (%)		
No	91.6 (4,709)	90.6 (5,670)
Yes	8.4 (431)	9.4 (589)
Missing	17.9 (1,119)	-
Gestational hypertensive disorders (%)		
No	93.9 (5,768)	93.8 (5,874)
Yes	6.1 (374)	6.2 (385)
Missing	1.9 (117)	-
Gestational diabetes (%)		
No	99.1 (6,005)	98.7 (6,180)
Yes	0.9 (57)	1.3 (79)
Missing	3.1 (197)	-
Child characteristics		
Female sex (%)	50.1 (3,136)	50.1 (3,136)

Gestational age at birth (weeks)	40.1 (35.9-42.3)	40.1 (35.9-42.3)
Birth weight (grams)	3,435 (556)	3,435 (556)
Ethnicity (%)		
European	67.0 (4,042)	66.2 (4,143)
Non-European	33.0 (1,991)	33.8 (2,116)
Missing	3.6 (226)	-
Breastfeeding (%)		
Never	7.6 (388)	8.7 (545)
Ever	92.4 (4,713)	91.3 (5,714)
Missing	18.5 (1,158)	-
Pet keeping (%)		
No	66.5 (3,589)	66.9 (4,185)
Yes	33.5 (1,804)	33.1 (2,074)
Missing	13.8 (866)	-
Inhalant allergy at 6 years (%)		
No	93.4 (4,754)	92.5 (5,787)
Yes	6.6 (336)	7.5 (472)
Missing	18.7 (1,169)	-
Lower respiratory tract infections at 6 years (%)		
No	95.3 (4,940)	94.2 (5,899)
Yes	4.7 (241)	5.8 (360)
Missing	17.2 (1,078)	-

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

Table S2 Differences in growth between Rint groups at the age of 6 years (presented in Figure 2)

	Difference in fetal length growth				Difference in infant length growth			
	Intercept (SD length)	P- value	Slope (SD length/ week)	P- value	Intercept (SD length)	P- value	Slope (SD length/ month)	P- value
Respiratory outcome								
Low Rint	Reference		Reference		Reference		Reference	
High Rint	0.160 (-0.091, 0.411)	0.212	-0.008 (-0.016, 0.000)	0.054	-0.230 (-0.272, -0.09)	0.002	0.010 (-0.003, 0.023)	0.120
	Difference in fetal weight growth				Difference in infant weight growth			
	Intercept (SD weight)	P- value	Slope (SD weight/ week)	P- value	Intercept (SD weight)	P- value	Slope (SD weight/ month)	P- value
Respiratory outcome								
Low Rint	Reference		Reference		Reference		Reference	
High Rint	0.092 (-0.135, 0.318)	0.426	-0.007 (-0.014, -0.000)	0.040	-0.083 (-0.221, 0.055)	0.240	0.001 (-0.011, 0.013)	0.873

Values are intercepts and slopes from repeated measurement regression models, which were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for child's sex, gestational age at birth, ethnicity, breastfeeding, and pet keeping. Rint analyses were additionally adjusted for time period of the measurement.

Table S3 Differences in growth between wheezing patterns and asthma groups at the age of 6 years (presented in Figure E2)

	Difference in fetal length growth				Difference in infant length growth			
	Intercept (SD length)	P- value	Slope (SD length/ week)	P- value	Intercept (SD length)	P- value	Slope (SD length/ month)	P- value
Never wheezing	Reference		Reference		Reference		Reference	
Early wheezing	0.009 (-0.163, 0.179)	0.922	-0.002 (-0.008, 0.003)	0.397	-0.062 (-0.157, 0.034)	0.204	0.002 (-0.007, 0.010)	0.714
Late wheezing	0.092 (-0.314, 0.498)	0.656	-0.005 (-0.018, 0.009)	0.507	-0.003 (-0.234, 0.229)	0.983	-0.005 (-0.026, 0.016)	0.618
Persistent wheezing	0.165 (-0.073, 0.402)	0.174	-0.007 (-0.014, 0.001)	0.084	-0.062 (-0.197, 0.073)	0.367	-0.002 (-0.013, 0.010)	0.791
No asthma	Reference		Reference		Reference		Reference	
Asthma	0.218 (-0.081, 0.517)	0.154	-0.008 (-0.018, 0.002)	0.112	-0.096 (-0.265, 0.073)	0.264	0.004 (-0.010, 0.019)	0.547
	Difference in fetal weight growth				Difference in infant weight growth			
	Intercept (SD weight)	P- value	Slope (SD weight/ week)	P- value	Intercept (SD weight)	P- value	Slope (SD weight/ month)	P- value
Never wheezing	Reference		Reference		Reference		Reference	
Early wheezing	-0.008 (-0.157, 0.142)	0.921	-0.001 (-0.006, 0.003)	0.619	-0.011 (-0.103, 0.081)	0.812	0.007 (-0.001, 0.015)	0.080
Late wheezing	0.089 (-0.275, 0.452)	0.631	-0.001 (-0.012, 0.010)	0.844	0.058 (-0.155, 0.272)	0.593	-0.007 (-0.025, 0.011)	0.440
Persistent wheezing	0.041 (-0.173, 0.255)	0.708	-0.002 (-0.009, 0.004)	0.508	0.138 (0.008, 0.268)	0.037	-0.007 (-0.018, 0.004)	0.211

No asthma	Reference		Reference		Reference		Reference	
Asthma	0.105 (-0.161, 0.371)	0.439	-0.004 (-0.012, 0.004)	0.379	-0.037 (-0.199, 0.125)	0.654	0.003 (-0.010, 0.017)	0.626

Values are intercepts and slopes from repeated measurement regression models, which were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for child's sex, gestational age at birth, ethnicity, breastfeeding, and pet keeping. Rint analyses were additionally adjusted for time period of the measurement.

Table S4 Associations of fetal and infant growth characteristics per SD from conditional growth models with respiratory outcomes
(presented in Figure 3)

	Rint	Wheezing patterns			Asthma
		Early wheezing	Late wheezing	Persistent wheezing	
	Difference (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
	n=3,954	n=1,373	n=225	n=608	n=5,066
2nd trimester					
Femur length n=5,933	-0.08 (-0.17, 0.02)	0.96 (0.89, 1.04)	0.95 (0.81, 1.12)	0.98 (0.89, 1.08)	0.98 (0.88, 1.09)
Weight n=5,901	-0.13 (-0.23, -0.04)**	0.96 (0.88, 1.03)	1.01 (0.85, 1.19)	0.94 (0.84, 1.04)	0.99 (0.89, 1.10)
3rd trimester					
Femur length n=6,094	-0.11 (-0.21, -0.01)*	0.99 (0.92, 1.07)	0.88 (0.75, 1.04)	0.92 (0.84, 1.02)	0.97 (0.87, 1.08)
Weight n=6,074	-0.12 (-0.22, -0.02)*	0.96 (0.89, 1.04)	0.94 (0.80, 1.11)	0.95 (0.86, 1.05)	0.95 (0.85, 1.06)
Birth					
Length n=4,106	-0.00 (-0.11, 0.11)	0.95 (0.87, 1.02)	1.08 (0.92, 1.27)	1.01 (0.91, 1.11)	0.97 (0.86, 1.10)
Weight n=6,259	-0.11 (-0.21, -0.01)*	0.96 (0.89, 1.03)	1.12 (0.95, 1.31)	1.01 (0.91, 1.12)	1.02 (0.92, 1.14)

3 months

Length n=3,202	-0.09 (-0.22, 0.05)	1.02 (0.94, 1.10)	1.06 (0.88, 1.29)	1.01 (0.90, 1.14)	0.96 (0.84, 1.11)
Weight n=3,789	0.03 (-0.08, 0.15)	1.07 (0.99, 1.16)	1.00 (0.85, 1.17)	1.17 (1.04, 1.31)**	1.03 (0.91, 1.17)

6 months

Length n=4,217	-0.16 (-0.28, -0.04)**	0.99 (0.91, 1.08)	0.96 (0.79, 1.15)	0.95 (0.85, 1.07)	1.04 (0.91, 1.19)
Weight n=4,736	-0.06 (-0.17, 0.05)	1.08 (0.99, 1.18)	1.04 (0.86, 1.25)	1.06 (0.94, 1.20)	1.04 (0.92, 1.18)

Age 1

Length n=4,358	-0.08 (-0.19, 0.04)	1.01 (0.93, 1.09)	1.04 (0.85, 1.27)	0.97 (0.86, 1.10)	1.00 (0.88, 1.14)
Weight n=4,362	-0.05 (-0.17, 0.06)	1.08 (0.99, 1.17)	0.97 (0.81, 1.17)	1.00 (0.89, 1.12)	1.00 (0.88, 1.15)

Values are Rint's z-score change or odds ratios for wheezing patterns, or asthma (95% confidence interval) from conditional analysis in which associations are independent of the associations at other time points, and reflect the risk per standard deviation of residual increase in length or weight. *p < 0.05 and **p < 0.01. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, gestational age, ethnicity, breastfeeding, and pet keeping. Rint analyses were additionally adjusted for time period of the measurement.

Table S5 Associations of fetal and infant growth characteristics per SD with overall wheezing

	Cross-sectional	Conditional
	Odds Ratio (95% CI)	Odds Ratio (95% CI)
	n=4,847	n=4,847
2nd trimester		
Femur length n=5,933	1.00 (1.00, 1.00)	0.98 (0.94, 1.01)
Weight n=5,901	1.00 (1.00, 1.00)	0.96 (0.92, 0.99)*
3rd trimester		
Femur length n=6,094	1.00 (1.00, 1.00)	0.99 (0.95, 1.02)
Weight n=6,074	1.00 (1.00, 1.00)	0.98 (0.94, 1.02)
Birth		
Length n=4,106	0.99 (0.94, 1.03)	0.98 (0.95, 1.05)
Weight n=6,259	0.97 (0.93, 1.00)	0.99 (0.95, 1.03)
3 months		
Length n=3,202	0.99 (0.93, 1.05)	1.00 (0.95, 1.05)

Weight n=3,789	1.16 (1.10, 1.22)**	1.07 (1.02, 1.13)**
6 months		
Length n=4,217	0.97 (0.92, 1.02)	0.98 (0.94, 1.02)
Weight n=4,736	1.11 (1.06, 1.17)**	1.03 (0.98, 1.07)
Age 1		
Length n=4,358	0.96 (0.91, 1.01)	0.98 (0.93, 1.02)
Weight n=4,362	1.09 (1.04, 1.15)**	1.02 (0.98, 1.06)

Values are odds ratios (OR) (95% confidence interval) from generalized estimating equation models. *p < 0.05 and **p < 0.01 and reflect the risk per standard deviation score increase in length or weight. Cross sectional models were dependent of growth at other time points, conditional models were independent of growth at other time points. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, ethnicity, breastfeeding, and pet keeping and postnatal additionally for birth weight and gestational age at birth.

Table S6 Associations of fetal and infant growth characteristics per SD with respiratory outcomes

	Rint	Wheezing patterns			Asthma
		Early wheezing	Late wheezing	Persistent wheezing	
	Difference (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
	n=3,954	n=1,373	n=225	n=608	n=5,066
2nd trimester					
Femur length n=5,933	-0.08 (-0.18, 0.02)	0.98 (0.91, 1.06)	0.96 (0.81, 1.13)	0.99 (0.89, 1.09)	0.99 (0.89, 1.10)
Weight n=5,901	-0.13 (-0.23, -0.03)*	0.98 (0.91, 1.06)	1.00 (0.85, 1.19)	0.97 (0.87, 1.07)	1.01 (0.91, 1.13)
3rd trimester					
Femur length n=6,094	-0.13 (-0.23, -0.04)**	0.98 (0.90, 1.05)	0.87 (0.74, 1.03)	0.92 (0.83, 1.02)	0.96 (0.86, 1.07)
Weight n=6,074	-0.16 (-0.25, -0.06)**	0.96 (0.89, 1.04)	0.95 (0.81, 1.12)	0.93 (0.84, 1.03)	0.95 (0.85, 1.05)
Birth					
Length n=4,106	-0.05 (-0.16, 0.05)	0.93 (0.86, 1.00)	1.09 (0.91, 1.29)	0.98 (0.89, 1.09)	0.96 (0.85, 1.07)
Weight n=6,259	-0.18 (0.28, -0.08)**	0.93 (0.87, 1.01)	1.07 (0.91, 1.26)	0.96 (0.87, 1.07)	0.98 (0.87, 1.09)
3 months					
Length n=3,202	-0.23 (-0.41, -0.05)*	1.04 (0.93, 1.17)	1.03 (0.78, 1.35)	0.95 (0.80, 1.13)	0.86 (0.69, 1.06)

Weight n=3,789	-0.02 (-0.13, 0.17)	1.16 (1.04, 1.28)**	0.95 (0.76, 1.18)	1.22 (1.06, 1.41)**	1.02 (0.85, 1.21)
6 months					
Length n=4,217	-0.32 (-0.47, -1.18)**	1.00 (0.91, 1.10)	0.94 (0.75, 1.16)	0.93 (0.86, 1.00)	1.06 (0.90, 1.25)
Weight n=4,736	-0.08 (-0.22, 0.05)	1.16 (1.06, 1.27)**	1.04 (0.86, 1.26)	1.20 (1.06, 1.36)**	1.09 (0.94, 1.27)
Age 1					
Length n=4,358	-0.23 (-0.36, -0.09)**	1.01 (0.92, 1.11)	1.00 (0.81, 1.23)	0.92 (0.81, 1.04)	0.98 (0.84, 1.15)
Weight n=4,362	-0.11 (-0.25, 0.02)	1.18 (1.08, 1.30)**	0.99 (0.81, 1.21)	1.11 (0.98, 1.26)	1.03 (0.88, 1.19)

Values are Rint's z-score change or odds ratios (OR) (95% confidence interval) from linear or logistic regression models without taking account for the same growth measure at other ages. *p < 0.05 and **p < 0.01 and reflect the risk per standard deviation score increase in length or weight. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, ethnicity, breastfeeding, and pet keeping and postnatal additionally for birth weight and gestational age at birth. Rint analyses were additionally adjusted for time period of the measurement.

Table S7 Birth characteristics and respiratory outcomes at the age of 6 years

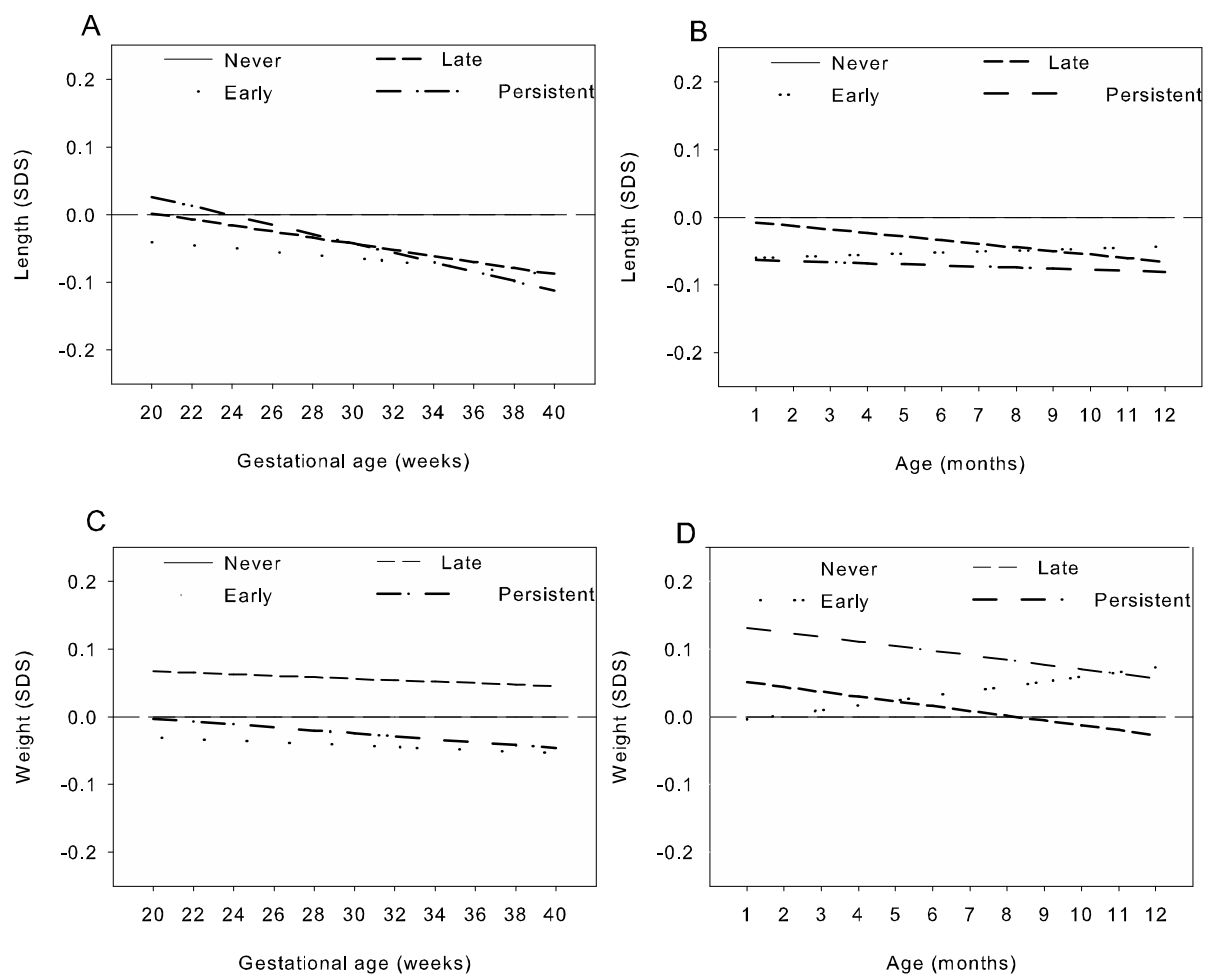
	Overall wheezing	Wheezing patterns			Asthma
		Early wheezing	Late wheezing	Persistent wheezing	
	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)	Odds Ratio (95% CI)
	n=4,847	n=1,373	n=225	n=608	n=5,066
Gestational age					
Preterm birth (<37 weeks) n=293	1.55 (1.32, 1.92)**	1.95 (1.40, 2.71)**	0.83 (0.30, 2.24)	1.53 (0.96, 2.43)	2.14 (1.47, 3.14)**
Term birth (37- 42 weeks) n=5,445	Ref	Reference	Reference	Reference	Reference
Post term birth (>42 weeks) n=300	0.96 (0.81, 1.12)	0.90 (0.66, 1.25)	0.91 (0.44,1.89)	0.90 (0.59, 1.38)	0.69 (0.39, 1.20)
<i>Trend (per week)</i>	<i>0.94 (0.92, 0.96)**</i>	<i>0.91 (0.87, 0.95)**</i>	<i>1.01 (0.91, 1.13)</i>	<i>0.93 (0.88, 0.98)**</i>	<i>0.90 (0.85, 0.95)**</i>
Absolute birth weight					
Low birth weight (<2500 grams) n=268	1.48 (1.22, 1.80)**	1.61 (1.13, 2.30)**	1.10 (0.48, 2.49)	1.50 (0.93, 2.44)	2.04 (1.36, 3.07)**
Normal birth weight (2500-4500 grams) n=5,634	Ref	Reference	Reference	Reference	Reference
High birth weight (>4500 grams) n=136	0.82 (0.65, 1.03)	0.78 (0.49, 1.26)	0.85 (0.31, 2.35)	0.80 (0.42, 1.53)	0.73 (0.33, 1.59)
<i>Trend (weight per 500 grams)</i>	<i>0.90 (0.97, 0.94)**</i>	<i>0.87 (0.81, 0.93)**</i>	<i>1.06 (0.90, 1.25)</i>	<i>0.91 (0.83, 1.00)*</i>	<i>0.87 (0.79, 0.96)**</i>

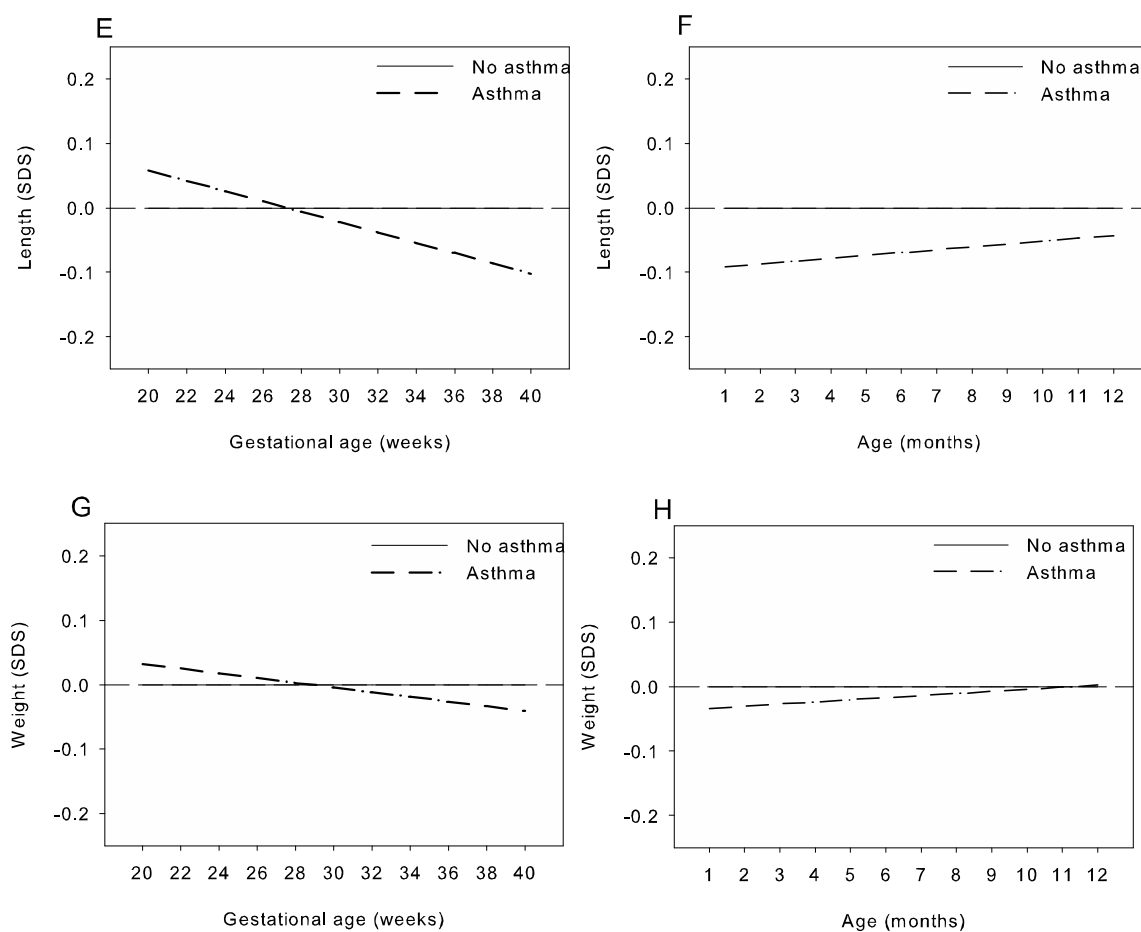
Birth weight for gestational age

Small size for gestational age n=314	1.06 (0.86, 1.29)	1.14 (0.82, 1.61)	0.65 (0.27, 1.54)	1.02 (0.64, 1.64)	0.92 (0.55, 1.52)
Appropriate size for gestational age n=5,631	Ref	Reference	Reference	Reference	Reference
Large size for gestational age n=314	0.85 (0.72, 1.01)	0.71 (0.51, 1.00)*	1.16 (0.63, 2.14)	0.84 (0.54, 1.32)	0.88 (0.53, 1.46)
<i>Trend (per SD)</i>	<i>0.96 (0.93, 1.00)*</i>	<i>0.93 (0.87, 1.01)</i>	<i>1.07 (0.91, 1.26)</i>	<i>0.96 (0.87, 1.07)</i>	<i>0.98 (0.87, 1.09)</i>

Values are odds ratios (OR) and, if continuously measured, reflect the risk of respiratory outcomes per 500 grams birth weight or week of gestational age or per SD of size for gestational age increase (95% confidence interval). We used never wheezing (n=2,641) and no asthma (n=4,675) as reference groups. *p < 0.05, **p < 0.01. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes, and for child's gender, ethnicity, breastfeeding habits, and pet keeping. Small size for gestational age was defined as the lowest 5% (SD=-1.74) and large size for gestational age was defined as the highest 5% (SD=1.59) of size for gestational age

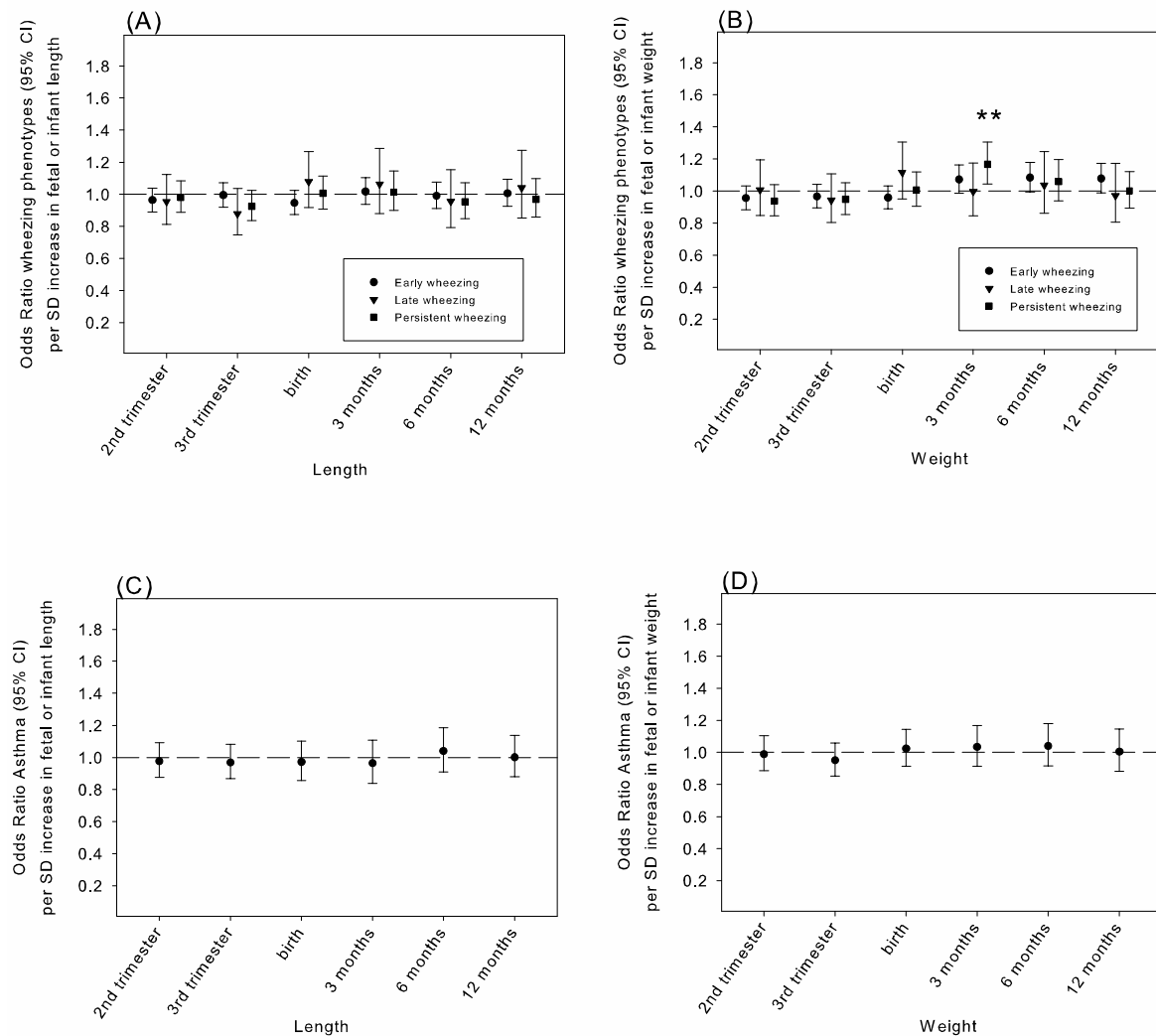
Figure S1 Differences in fetal and infant growth (length, weight) between wheezing and asthma groups at the age of 6 years





Lines are differences in length or weight growth in fetal life or infancy between the respiratory outcome groups wheezing pattern (never; early; late; persistent), or asthma (no; yes). Values were obtained from longitudinal repeated measurement models. * $p < 0.05$ and ** $p < 0.01$, given for the difference in growth over time. Specific estimates and p-values are given in table S3. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy, smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, gestational age, ethnicity, breastfeeding, and pet keeping.

Figure S2 Associations of independent fetal and infant growth characteristics per SD with wheezing and asthma at the age of 6 years



Values are odds ratios (OR) for wheezing patterns (A, B), or asthma (C, D) (95% confidence interval) from conditional analysis in which associations are independent of the associations at other time points, and reflect the risk per standard deviation of residual increase in length or weight. We used never wheezing and no asthma as the reference group (not shown). Specific estimates and p-values are given in online data supplement (Table S4). * $p < 0.05$ and ** $p < 0.01$. Models were adjusted for maternal age, body mass index, educational level, history of asthma or atopy,

smoking during pregnancy, parity, psychological distress, gestational hypertensive disorders and diabetes and for children's sex, gestational age, ethnicity, breastfeeding, and pet keeping.