

## Longitudinal Associations between Respiratory Infections and Asthma in Young Children

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## Abstract

We examined temporal dependencies between repeated assessments of respiratory infections and asthma in children in the Leicester Respiratory Cohort, Leicestershire, UK. Asthma (doctor diagnosis, health care visits, wheeze frequency) and respiratory infections (cold duration and frequency, cough with colds, ear infections) in the previous 12 months were assessed repeatedly at ages 1, 4, and 6 years for children born between April 1996 and April 1997. We determined associations between contemporaneous and lagged measures of asthma and respiratory infections using structural equation modelling. In 1995 children, asthma was positively associated with contemporaneous infections. Asthma at age 6 was positively associated with asthma at age 4 (regression coefficient = 0.87; 95% CI 0.76, 0.97), but not with asthma at age 1 (-0.01; -0.14, 0.11). We found no evidence for direct protective effect of infections at age 1 on asthma either at age 4 (-0.20; -0.51, 0.10) or 6 (0.24; -0.04, 0.52). Adjusting for potential confounders did not qualitatively change those relationships. Our findings suggest that asthma at age 6 is directly influenced by asthma history and only indirectly if, at all, by earlier infection episodes. We found little support for a protective effect of preschool infections on asthma at early school age.

**Keywords:** Asthma in children – respiratory infections – longitudinal structural equation modelling

**Abbreviations:** structural equation modelling (SEM), root mean square error of approximation (RMSEA), comparative fit index (CFI), full information maximum likelihood estimation (FIML), respiratory syncytial virus (RSV)

## Introduction

Respiratory tract infections and asthma are major components of acute and chronic morbidity in childhood, and they place a considerable burden on children, their families, and society.[1, 2] Asthma has multiple causes that include genetic predisposition, environmental exposures, and interactions between these factors and a child's developmental stage.[3] Although many studies have revealed a strong association between microbial (including viral) infections and asthma in children from infancy to school age,[4, 5] the direction of causation is unclear and may be bidirectional.[3] Several pathways from microbial infections to atopic conditions have been proposed: microbial infections can have protective effects (the hygiene hypothesis),[6, 7] or be provocative[8-12] of subsequent asthma. A reverse causation mechanism has also been proposed in which atopic conditions increase the susceptibility to infections.[3, 13-15] The association between infections and childhood asthma also may not be causal, but due to shared genetic components that induce susceptibility to both.[16]

Research on asthma is hampered by the complexity of asthma presentation; asthma is particularly difficult to assess before 6 years of age.[17] The interpretation of results from observational studies is not straightforward because at an early age asthma symptoms are often not easily distinguished from those of respiratory tract infections (RTI),[18, 19] which are the most frequent trigger of wheezing in early childhood.[20] In addition, many studies include large proportions of children at high risk of atopy,[11, 12] which casts doubt on the generalisability of the conclusions. Associations between asthma and respiratory infections are likely to vary with age,[5, 9] but few studies have systematically compared associations at different ages in the same cohort.[21, 22]

In this study, we quantified the relationships between assessments of respiratory tract infections and asthma repeated from infancy to school age in a large, population-based cohort to assess the plausibility of four hypotheses about contemporaneous and lagged associations: 1) asthma and

infections are highly correlated at each age, 2) both asthma and infections longitudinally track throughout childhood, 3) infections in preschool children modify later risk of developing asthma by 3a) reducing the risk (protective effect) or 3b) increasing the risk (provocative effect), and 4) wheezing infants are more susceptible to respiratory infections later in childhood.

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## Methods

### *Study population*

We used data from a population-based cohort of children of Leicestershire, UK. For all children we obtained routine data including demographic and perinatal data from the Leicestershire Health Authority Child Health Database (Table 1). Questionnaires with detailed questions on upper and lower respiratory symptoms, treatments and health care use, family history of respiratory and atopic diseases, socioeconomic and demographic factors, and environmental exposures were mailed to parents. Relevant exposures and outcome measures for this study are summarized in Table 2. We included 1995 children born between 21 April 1996 and 30 April 1997 for whom surveys were returned in each of the three years 1998 (mean age 1.5, range 1.0-1.99, SD 0.3), 2001 (mean age 4.8, range 3.9-5.6, SD 0.3), and 2003 (mean age 6.5, range 6.0-7.5, SD 0.3). We refer to these as the 1 year, 4 year, and 6 year age groups. Response rates were 78%, 60%, and 49% for the respective groups in 1998, 2001 and 2003. The Leicester cohorts are described in detail elsewhere.[23] The study protocol was approved by the Leicestershire Health Authority Research Ethics Committee.

### *Data on asthma and respiratory infections*

From each survey, we derived the following information relating to asthma during the previous 12 months: diagnosis of asthma, asthma-related healthcare visit general practitioner or accident/emergency department, episodes of wheeze (current wheeze), number of wheeze attacks (0, 1 to 3, 4 to 12, more than 12), and use of antiasthma medication including short- and long-acting  $\beta_2$ -agonists and inhaled corticosteroids. We also noted information on infections during the previous 12 months that included frequency and duration of colds, cough with colds, and ear infections (see Web Appendix 1 for detail on the questions).

All variables with more than two response categories were recoded as binary variables. We used thresholds that identified approximately the 10% to 20% most severe cases for wheeze and

infections at each survey (Table 2). We defined frequent wheeze as  $\geq 4$  attacks of wheeze, frequent colds as  $\geq 7$  episodes, colds of long duration as colds lasting  $\geq 2$  weeks, and frequent ear infections as  $\geq 2$  ear infections in the last 12 months.

### *Statistical analyses*

We used longitudinal structural equation modelling (SEM) to assess associations between repeated assessments of asthma and respiratory infections at ages 1, 4, and 6 years. Longitudinal SEM is a multivariate method that allows modelling of the temporal relationships between unobserved, latent variable constructs derived from observed variables.[24] In our model, diagrammed in Web Figure 1, we defined “asthma” and “infections” as latent constructs corresponding to the observed indicator variables listed above. The circled variables in the diagram represent the unobserved, latent constructs that are measured on a continuous scale. Variables depicted in squares represent observed indicator variables. Directed arrows are hypothesised causal paths. Effect sizes are modelled as either probit regressions of indicators on latent variables (measurement model), or linear regressions between the latent constructs (structural model). Given the multiplicity of hypotheses, we allowed for all possible paths between latent variables for which cause precedes effect. Bidirectional paths represent bivariate associations and account either for the covariance of contemporaneous latent constructs or for correlations between repeated measurements of indicator variables over time. Further detail on SEM estimation based on dichotomous data is provided in Web Appendix 2.

We used diagonally weighted least squares (DWLS) to estimate the model parameters with their corresponding 95% confidence intervals. The full weight matrix was used to compute robust standard errors.[25] P-values of path coefficients were obtained by the Wald test and were further corrected for multiple statistical testing by controlling for the false discovery rate at the 5% level using the Benjamini–Hochberg procedure.[26]

Interpreting effect sizes in longitudinal SEM is meaningful only if the factors corresponding to the same construct (“infections” or “asthma”) have the same meaning over time, i.e. they are

time invariant. This can be assessed by establishing the invariance of factor loadings (metric invariance) and factor variances over time (factor variance invariance; Web Appendix 3 and Web Appendix 4). For model comparison, we considered as acceptable goodness of fit a comparative fit index (CFI) value  $\geq 0.95$  and a root mean square error of approximation (RMSEA)  $\leq 0.06$ . [27] We also regarded a decrease in CFI (delta CFI) of 0.01 and an increase of RSMEA (delta RSMEA) of 0.015, which have been found to be more sensitive for detecting lack of invariance in studies with large sample sizes, [28] as criteria indicative of unacceptable decrease in (or poorer) model fit.

The baseline SEM model was adjusted for variables that were assessed only at age 1 year, which might have confounded the associations assessed in our model: sex, ethnicity, maternal asthma, maternal smoking during pregnancy, breastfeeding, presence of older siblings, family education, birth season, and economic deprivation (Table 1). We assessed the influence of varying response rates at the three survey times on our SEM estimates by doing a sensitivity analysis based on all 1-year-old children who responded at the first survey and keeping those children in the analysis independently of their participation to the subsequent questionnaires. We used a full information maximum likelihood estimation (FIML) to account for the missing information on outcomes in the SEM procedure (Web Appendix 2). [29] Data were prepared and analysed using Stata 13.1 (Stata Corporation, Austin, Texas). SEM models were implemented with the *lavaan* [25] package (version 0.5-20) in R (version 3.1.1).

## Results

### *Study population*

We analysed the complete-case data of the 1995 children who were 1 year old in 1998 whose parents responded to all three surveys in 1998, 2001, and 2003 (Table 1). Slightly more than half were male and 15% were of South Asian ethnic origin, while the others were white.

### *Prevalence of asthma/wheeze and respiratory infections*

Prevalence of an asthma diagnosis doubled from 10% at age 1 to about 20% at ages 4 and 6 years (Table 2). The use of inhaled corticosteroids increased as well, but bronchodilator use remained constant. Current wheeze decreased from 31% at age 1 to about 13% at age 6, while asthma-related visits decreased from 11% to 4% over the same period. Frequent colds, ear infections, and long lasting colds also decreased in prevalence. The prevalence of cough with colds remained high at 65-70% throughout early childhood (Table 2).

### ***Validation of SEM construct, model fit***

The variables wheeze with colds and current wheeze were highly correlated (correlation coefficient  $\rho=0.95$ ), so only current wheeze was kept for further SEM model building. Measurement invariance of the two constructs “infections” and “asthma” was confirmed with difference in CFI of concurrent models below 0.01 (Web Table 1, Web Appendix 1), suggesting that factor loadings and variances and residual variances of the regressions of indicators on the latent variables can be considered invariant across time (Web Table 2, Web Table 3). This means the two constructs have the same interpretation with respect to the questionnaire items regardless of age at response.[30] The baseline model (Web Figure 2) showed an acceptable model fit with CFI of 0.995 and RMSEA of 0.028 (90% confidence interval 0.026, 0.030).[27] We show results for this model. All indicator variables had significant loadings on their respective latent variable (Web Table 3).

### ***Relationships between asthma and susceptibility to infections***

At each survey, the latent constructs “infections” and “asthma” were positively correlated, with covariances of 0.27 (95% confidence interval 0.20, 0.34) at age 1, 0.17 (0.11, 0.23) at age 4, to 0.08 (0.04, 0.11) at age 6 (Web Figure 2, latent construct variances decreased similarly and are reported in Web Table 2). Adjusting the SEM model for confounding variables minimally changed these values (Table 3 and Web Table 4).



We found strong evidence for the constructs “asthma” and “infections” tracking as children grew older (Web Figure 2, Table 3): factor scores at age 4 were strongly and positively related to those at age 1, with path coefficients of 0.68 (0.56, 0.81) and 0.36 (0.11, 0.61) for “asthma” and “infections”, respectively. Dependencies of factor scores at age 6 on previous corresponding values at age 4 were even stronger, with path coefficients of 0.87 (0.76, 0.97) for “asthma” and 0.88 (0.45, 1.32) for “infections”. There was no evidence of a direct effect from age 1 to 6 years for both constructs.

We found little evidence of cross-lagged relationships between “asthma” and “infections” between ages 1 and 4, 4 and 6, or 1 and 6 years. In particular, higher “infections” scores at 1 year were not associated with higher “asthma” scores at age 4 (-0.20 [-0.51, 0.10]) or at age 6 (0.24 [-0.04, 0.52]) (Web Figure 2, Table 3). We found no evidence that wheezing infants were more susceptible to respiratory infections at ages 4 (0.07 [-0.03, 0.17]) or 6 (0.00 [-0.15, 0.16]).

Adjusting the model for potential confounders changed the path between “infections” at ages 1 and 4 to a nonsignificant association (0.08, 95% confidence interval -0.79, 0.96;  $P=0.850$ ), but led to a stronger dependency of “asthma” at 4 years on “asthma” at 1 year (2.22 [0.83, 3.62],  $P=0.002$ ) (Table 3). Little evidence of cross-lagged associations between “asthma” and “infections” was also found after adjusting for potential confounders. More “infections” at age 1 was negatively related to “asthma” at age 4 (-4.26 [-8.0, -0.49],  $P=0.027$ ), but the protective effect failed to reach significance after accounting for multiple statistical testing (Table 3).

Adjusting the path model for time-invariant confounding variables did not change the relationships among and between the latent constructs to any large extent (Table 3). Results of these analysis (Web Table 4; Web Appendix 3) showed that male sex and the presence of older siblings were both associated with higher scores for “infections” at age 1, while higher scores of “asthma” at age 1 were associated with male sex, presence of older siblings, maternal asthma, and not having been breastfed. There was no evidence of associations between any of maternal smoking, family education level, ethnicity, socioeconomic deprivation level, or birth season with

latent constructs “infections” or “asthma” at age 1 year. Breastfeeding was associated with a significant reduction of the “asthma” score (-0.17 [-0.28, -0.06],  $P=0.004$ ), but not of the “infections” score (-0.03 [-0.09, 0.02],  $P=0.210$ ) at age 1 year (Web Table 4).

The sensitivity analysis based on all children who participated to the first survey and were 1 year old at the time ( $n=3983$ ) led to a very similar picture of the SEM diagram, except that 1) most coefficients were larger in the complete-case SEM than in the FIML SEM approach, and 2) the path from infections at 4y to asthma at 6y became significant in the FIML SEM approach but not in the complete-case SEM (Web Table 5; Web Appendix 5). Yet, the coefficient of this path was small compared to the other significant path coefficients in both SEM models. The model fit of the FIML SEM was poorer than that of the model that included the children who answered the three questionnaires.

## Discussion

This study’s results support hypothesis 1: the risks of respiratory infections and asthma were positively related to each other at each age investigated. In addition, infections in infancy led to more infections in subsequent years, and asthma symptoms in infancy similarly led to more asthma symptoms the following years, which supports hypothesis 2. In the unadjusted model, there was little evidence of lagged effects from infections to asthma or from asthma to infections as children grew older. After adjusting for potential confounding factors assessed at baseline, we found a protective effect of early respiratory infections on asthma at early school age which, however, did not reach significance after adjusting for multiple testing, rejecting hypothesis 3.

Early wheezers did not appear to be more susceptible to respiratory infections when they reached school age, which rejects hypothesis 4. Male sex, maternal asthma, and older siblings were associated with high scores for both asthma and infections at age 1, while breastfeeding significantly reduced asthma but not infection scores at age 1 year (Web Table 4).

This study is one of the first to examine the associations between respiratory infections and asthma symptoms from infancy to early school age using a multivariate approach. Its findings

corroborate results from previous, mostly cross-sectional studies in which respiratory infections and asthma were strongly associated,[8, 10] and various viral and bacterial agents were shown to have elicited similar asthmatic symptoms in young children.[5, 31] It also confirmed that the trajectories of wheeze and infection phenotypes remain tightly associated to each other during childhood.[18] The tracking of both asthma symptoms and respiratory infections from infancy to school age became stronger with age showing stronger relationships from age 4 to 6 years than from age 1 to 4 years. This may indicate that symptom patterns are more variable in preschool years. We know in particular that the predominant phenotype in preschool children, virus-associated wheeze, tends to remit, while schoolchildren more often present with persistent wheeze associated with atopy.[32-34]

We found no evidence of a direct association between upper and lower respiratory tract infections in early childhood and increased asthma at age 6-7 years. This observation does not support the commonly held hypothesis that respiratory tract infections in early life can initiate a chronic disease trajectory leading to recurrent wheeze later in childhood, and thus may be responsible in part for asthma development.[35] A prospective cohort study reported that nearly 50% of children who experienced severe RSV bronchiolitis at 12 months of age or less had a subsequent asthma diagnosis at age 6,[36] but the study did not include a control group of infants without severe RSV infections. It is therefore difficult to assess the causal relationships between severe RSV bronchiolitis and higher risk of asthma later in life. The numbers of children considered in most previous studies[35] have been rather low, at best consisting of few hundred participants. In addition, the association between respiratory infection and later asthma sometimes disappeared after adjusting for the frequency of respiratory episodes,[4] suggesting nonspecific association between the viral trigger with later asthma development. A study in a large population-based sample of twins (n>8000) conducted in Denmark concluded that severe RSV infections that lead to hospitalization do not cause asthma, but may serve as indicator of genetic predisposition to asthma.[16] That study also noted that models in which asthma

"causes" RSV hospitalization fit the data better than models in which RSV hospitalization "causes" asthma. Therefore, higher susceptibility and inflammatory response to respiratory tract infections may be related to the fact that asthmatic patients have an altered epithelial immune response to viral and bacterial agents and a higher likelihood of developing lower respiratory tract infections in relation to these.[3, 37]

Some studies have suggested that asthma may predispose children to respiratory infections,[38] or colonisation by microbes.[3, 13-15] This early wheezing-later infections relationship may be related to diminished antiviral activity and a defective immune function against microbes of asthmatic patients.[3] However, previous studies have used high-risk children with virus-induced wheeze during early childhood, which increases the chance of confounding and limits the generalizability of their conclusions. We did not find that early wheezing is a direct indicator of higher susceptibility to respiratory infections at school age.

A major strength of our study is its large, population-based sample, which included the full spectrum of disease severity. This afforded the use of a model that simultaneously assessed a large number of relationships between repeated assessments of infections and asthma over time. In the first years of life, the distinction between respiratory infections and asthma symptoms or between upper and lower respiratory infections is not always clear-cut and diagnoses differ according to clinician's experience and preferences. In contrast to most previous studies, we thus used a data-driven approach, by which "infections" at a specific age is modelled as a continuous latent (unobserved) variable composed of multiple observed indicators of respiratory infections, each contributing to a different degree, which is determined by fitting the model to the data. This in turn allowed testing several hypotheses in a single multivariate model, which is an approach we have argued should receive greater attention in respiratory research.[39] This also helped reduce confounding of, for example, the estimated effect of infections on later asthma without considering earlier asthma, which can easily arise when assessing these relationships separately. Our study was based on a large sample size and in a population that included the full spectrum of

disease severity. Infections and asthma were defined as latent constructs based on multiple variables. This avoided subjective decisions commonly involved in defining these outcomes.

Although participation rates were lower in the second and third surveys, our sensitivity analysis including all children responding at age one regardless of later participation suggests that selection bias did not materially influence the direction of our findings or the strength of the associations. Therefore, we believe that the results obtained from the subgroup of children who participated in all surveys are robust and can be extrapolated to the entire study population.

The limitations of our study include the fact that all variables in our model were reported by parents and did not include objective measurements. Laboratory measurements were available for only a subset of the cohort participants at one time-point, so could not have been used for this longitudinal study. Also, there is no single laboratory parameter that tells us whether a child has had asthma or respiratory infections in the past year. We also had limited information on severity of infections and our latent variable “infection” does only partly reflect severity. RSV bronchiolitis for instance is a severe infection, but being a disease of infancy, was only asked at age one year. So we could not use this information to construct a variable reflecting vulnerability to infections with the same meaning in different ages. Our latent variable “infection” thus uses data that are available in all age groups and indicate frequent and long-lasting infections rather than single severe episodes. However, all four variables used (frequent colds, long duration of colds, coughing with every cold, repeated ear infections; Web Table 3) were significantly associated with the likelihood of having a bronchiolitis or a pneumonia in our study population (data not shown), so that our latent variable infection is to some degree also a proxy for severe infections. In addition, our questionnaires mostly targeted respiratory infections and did not assess other infections such as those of the urinary and gastro-intestinal tracts.[40, 41] In Although the latent constructs took into account different aspects of disease that, for asthma, included physician diagnosis, health care use, asthma medication, and frequency of symptoms, our approach was not entirely free of subjective decisions because we had to select the variables

used to define the constructs. Our latent variables “infections” that capture the common variation in the observed indicators of respiratory infections explained only a small proportion of the variation in each of the indicator variables, underlining the multidimensional nature of “infections” variables. We suggest that future work should investigate different aspects of infections (different germs, different locations of infections, and differences in severity) and their interplay with asthma, to complement and expand on the findings obtained in this study.

In conclusion, susceptibility to respiratory tract infections and to asthma are strongly correlated and track throughout early childhood. Our study suggests that recurrent respiratory tract infections at preschool age do not increase the risk for asthma at school age except potentially through an indirect effect mediated by contemporaneous wheezing illness.

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Table 1. Baseline characteristics at age 1 year of the 1995 children born between April 1996 and April 1997 from the Leicester Respiratory Cohort, Leicestershire, UK

| Characteristics                                   | No.  | %  |
|---|------|----|
| Male sex  | 1049 | 53 |
| South Asian ethnicity                             | 308  | 15 |
| Low birth weight <sup>a</sup>                     | 130  | 7  |
| Preterm <sup>b</sup>                              | 121  | 6  |
| Attending nursery care                            | 574  | 29 |
| Older siblings                                    | 1082 | 54 |
| Mother smoking                                    | 234  | 12 |
| Father smoking                                    | 414  | 21 |
| Breastfed   | 1283 | 64 |
| History of parental wheeze or asthma <sup>c</sup> | 335  | 17 |
| Higher parental education <sup>c,d</sup>          | 932  | 47 |
| Higher deprivation categories <sup>e</sup>        | 475  | 24 |

<sup>a</sup>Birth weight <2500 g.

<sup>b</sup>Gestational age <37 weeks.

<sup>c</sup>Either mother or father.

<sup>d</sup>Age at the end of education was >16 years.

<sup>e</sup>Higher deprivation categories consist of deprived and more deprived categories based on the following ranges of Townsend Deprivation scores: more affluent [-6.222, -2.635], affluent [-2.615, -0.707], average [-0.705, 1.859], deprived [1.861, 5.147], more deprived [5.160, 11.072].

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Table 2. Prevalence of asthma/wheeze and respiratory infections for children born between April 1996 and April 1997 from the Leicester Respiratory Cohort, Leicestershire, UK

| Indicator variables                      | 1 year |    | 4 years |    | 6 years |    |
|--|--------|----|---------|----|---------|----|
|  | No.    | %  | No.     | %  | No.     | %  |
| <b>Asthma/wheeze indicator variables</b> |        |    |         |    |         |    |
| Asthma diagnosis                         | 194    | 10 | 402     | 20 | 430     | 22 |
| Current wheeze                           | 611    | 31 | 305     | 15 | 259     | 13 |
| Wheeze with colds                        | 591    | 30 | 323     | 16 | 288     | 14 |
| Frequent wheeze <sup>a</sup>             | 201    | 10 | 106     | 5  | 97      | 5  |
| Asthma-related healthcare visit          | 224    | 11 | 132     | 7  | 83      | 4  |
| Bronchodilator use                       | 290    | 15 | 314     | 16 | 280     | 14 |
| Inhaled corticosteroid use               | 100    | 5  | 201     | 10 | 197     | 10 |
| <b>Infection indicator variables</b>     |        |    |         |    |         |    |
| Frequent colds <sup>b</sup>              | 370    | 19 | 124     | 6  | 93      | 5  |
| Long duration of colds <sup>c</sup>      | 249    | 13 | 119     | 6  | 103     | 5  |
| Cough with colds                         | 1299   | 65 | 1403    | 70 | 1403    | 70 |
| Frequent ear infections <sup>d</sup>     | 359    | 18 | 220     | 11 | 190     | 10 |

<sup>a</sup>Frequent wheeze is defined as  $\geq 4$  wheeze attacks in last 12 months.

<sup>b</sup>Frequent colds are defined as  $\geq 7$  cold episodes in last 12 months.

<sup>c</sup>Long duration of colds is defined as colds lasting  $\geq 2$  weeks in the last 12 months.

<sup>d</sup>Frequent ear infections are defined as  $\geq 2$  ear infections episodes in last 12 months.

Table 3. Path coefficients for pathways between asthma and infection latent variables for children born between April 1996 and April 1997 from the Leicester Respiratory Cohort, Leicestershire, UK

| Path directions   | Crude model (N=1995)  |             |                      | Adjusted model <sup>d</sup> (N=1807) |              |                     |
|---|-----------------------|-------------|----------------------|--------------------------------------|--------------|---------------------|
|   | Estimate <sup>a</sup> | 95% CI      | P-value <sup>b</sup> | Estimate                             | 95% CI       | P-value             |
| <b>Bidirectional paths between latent variable categories</b> |                       |             |                      |                                      |              |                     |
| Between “infections” (1Y) and “asthma”(1Y)                    | 0.27                  | 0.20, 0.34  | <0.001 <sup>c</sup>  | 0.29                                 | 0.21, 0.36   | <0.001 <sup>c</sup> |
| Between “infections” (4Y) and ASTHMA (4Y)                     | 0.17                  | 0.11, 0.23  | <0.001 <sup>c</sup>  | 0.165                                | 0.09, 0.22   | <0.001 <sup>c</sup> |
| Between “infections” (6Y) and ASTHMA (6Y)                     | 0.08                  | 0.04, 0.11  | <0.001 <sup>c</sup>  | 0.08                                 | 0.04, 0.12   | <0.001 <sup>c</sup> |
| <b>Unidirectional paths within latent variable categories</b> |                       |             |                      |                                      |              |                     |
| From “infections” (1Y) to “infections” (4Y)                   | 0.36                  | 0.11, 0.61  | 0.005 <sup>c</sup>   | -0.04                                | -0.89, 0.82  | 0.929               |
| From “infections” (1Y) to “infections” (6Y)                   | 0.08                  | -0.25, 0.41 | 0.630                | -1.06                                | -3.08, 0.97  | 0.306               |
| From “infections” (4Y) to “infections” (6Y)                   | 0.88                  | 0.45, 1.32  | <0.001 <sup>c</sup>  | 1.00                                 | 0.48, 1.52   | <0.001 <sup>c</sup> |
| From “asthma” (1Y) to “asthma” (4Y)                           | 0.68                  | 0.56, 0.81  | <0.001 <sup>c</sup>  | 2.17                                 | 0.88, 3.46   | 0.001 <sup>c</sup>  |
| From “asthma” (1Y) to “asthma” (6Y)                           | -0.01                 | -0.14, 0.11 | 0.824                | 0.48                                 | -0.24, 1.2   | 0.192               |
| From “asthma” (4Y) to “asthma” (6Y)                           | 0.87                  | 0.76, 0.97  | <0.001 <sup>c</sup>  | 0.75                                 | 0.59, 0.92   | <0.001 <sup>c</sup> |
| <b>Unidirectional paths across latent variable categories</b> |                       |             |                      |                                      |              |                     |
| From “infections” (1Y) to “asthma” (4Y)                       | -0.20                 | -0.51, 0.10 | 0.192                | -4.13                                | -7.64, -0.62 | 0.021               |
| From “infections” (1Y) to “asthma” (6Y)                       | 0.24                  | -0.04, 0.52 | 0.097                | -0.99                                | -2.77, 0.79  | 0.276               |
| From “infections” (4Y) to “asthma” (6Y)                       | -0.04                 | -0.32, 0.24 | 0.769                | 0.15                                 | -0.16, 0.47  | 0.330               |
| From “asthma” (1Y) to “infections” (4Y)                       | 0.07                  | -0.03, 0.17 | 0.174                | 0.23                                 | -0.1, 0.56   | 0.179               |
| From “asthma” (1Y) to “infections” (6Y)                       | 0.00                  | -0.15, 0.16 | 0.962                | 0.46                                 | -0.37, 1.29  | 0.274               |
| From “asthma” (4Y) to “infections” (6Y)                       | -0.01                 | -0.15, 0.14 | 0.918                | -0.07                                | -0.3, 0.17   | 0.581               |

Comparative fit index (CFI); root mean square error of approximation (RMSEA).

<sup>a</sup>Covariances for bidirectional paths and regression coefficients for unidirectional paths, respectively.

<sup>b</sup>P-value for testing the null hypothesis that the parameter equals zero in the population using the Wald statistical test.

<sup>c</sup>P <0.05 after correction for multiple testing. [26]

<sup>d</sup>Model adjusted at age 1 year for child sex, maternal asthma, maternal smoking, breastfeeding, presence of older siblings, level of family education, ethnicity, socioeconomic level and birth season (September-November, December-February, March-May, June-August).