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ORIGINAL ARTICLE

Rhinovirus-induced wheeze was associated with asthma development in predisposed children

Idun Holmdahl[1,2] | Sofia Lüning[1,2] | Sabina Wärnberg Gerdin[1,2] | Anna Asarnoj[1,2] | Angela Hoyer[1,2] | Anastasia Filiou[1,2] | Anders Sjölander[3] | Anna James[1,4] | Magnus P. Borres[3,5] | Gunilla Hedlin[1] | Marianne van Hage[6,7] | Cilla Söderhäll[1,2] | Jon R. Konradsen[1,2]

[1]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

[2]Astrid Lindgren's Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

[3]Thermo Fisher Scientific, Uppsala, Sweden

[4]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

[5]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

[6]Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

[7]Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence
Idun Holmdahl, Department of Women's and Children's Health, Karolinska Institutet, KPE Barnallergiforskningen, QB:84, Karolinska vägen 37, SE-171 76 Solna, Sweden.
Email: idun.holmdahl@ki.se

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Abstract

Aim: This study explored whether early-life factors, such as rhinovirus-induced wheeze and allergic sensitisation, were related to asthma at 11 years of age.

Methods: We focused on 107 children aged 6–48 months, who attended the paediatric emergency department at Astrid Lindgren's Children's Hospital in Stockholm, Sweden, with acute wheeze in 2008–2012. They also attended follow-up visits at 11 years of age and were compared with 46 age-matched healthy controls. Odds ratios (OR) with 95% confidence intervals (CI) were calculated with logistic regression.

Results: We found that 62.6% of the acute wheeze cases had asthma at 11 years of age. Rhinoviruses at inclusion were the only common airway viruses associated with an increased asthma risk (OR 2.4, 95% CI 1.02–5.6). Other increased risks were parental heredity for asthma and/or allergies (adjusted OR 3.4, 95% CI 1.1–9.9) and allergic sensitisation at 2 years of age (adjusted OR 3.0, 95% CI 1.02–8.7). The highest prevalence of asthma was when children had both rhinovirus-induced wheeze at inclusion and allergic sensitisation at 7 years of age.

Conclusion: Our findings highlight the importance of hereditary factors and allergic sensitisation on the development of asthma and suggest that rhinoviruses are associated with asthma development in predisposed children.

Abbreviations: EDN, eosinophil-derived neurotoxin; FeNO, fractional exhaled nitric oxide; FEV1/FVC, forced expiratory volume in 1 s/forced vital capacity; GEWAC, Gene Expression in Wheezing and Asthmatic Children; IgE, Immunoglobulin E; RSV, respiratory syncytial virus.

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KEYWORDS
allergic sensitisation, asthma, heredity, preschool wheeze, rhinoviruses

1 | INTRODUCTION

Asthma is a complex and widely heterogeneous disease that affects millions of people worldwide. Acute wheeze in preschool children may progress to asthma later in life.[1] Factors that have been associated with this development are allergic sensitisation, asthma in first-grade relatives and severe and frequent wheezing episodes.[2,3] Allergic asthma, driven by type 2 inflammation, is the most common form during childhood, followed by non-allergic asthma, where the symptoms may be triggered by viral infections and/or exercise.[4,5]

Rhinoviruses are the most frequent trigger of wheeze among children over 1 year of age and have been associated with an increased risk of developing asthma at school-age.[6–8] It has been suggested that the combined effect of rhinovirus-induced wheeze and allergic sensitisation play a greater role than either of these factors on their own.[9] One theory is that the antiviral response is impaired among children with wheeze triggered by rhinoviruses. This response may be further reduced by the presence of allergic sensitisation, resulting in airway epithelial damage and the inception of asthma.[10] The heterogeneous nature of asthma makes it difficult to identify predictive biomarkers, particularly in children. In a clinical setting, blood eosinophil counts and fractional exhaled nitric oxide (FeNO) are used to diagnose and monitor asthma. Serum eosinophil-derived neurotoxin (EDN) has shown promising results as a novel biomarker of eosinophilic inflammation in childhood asthma.[11]

The Gene Expression in Wheezing and Asthmatic Children (GEWAC) study comprises a high-risk asthma cohort. Most of the children with wheeze were admitted to the Swedish hospital at inclusion and had a high prevalence of asthma at 7 years of age.[7] A rhinovirus-induced wheeze at inclusion, as well as the severity of preschool wheeze, were both shown to be associated with asthma development at 7 years of age.[7]

The aim of this study was to identify early-life factors associated with asthma at 11 years of age. We wanted to explore the significance of rhinovirus-induced wheeze at inclusion and allergic sensitisation at different time-points during childhood. The study also longitudinally investigated potential markers of asthma, such as lung function measurements, FeNO, blood eosinophil counts and EDN.

Key notes

- This Swedish study comprised 107 adolescents, who had attended the emergency department for acute wheeze when they were 6–48 months of age.
- We found that 62.6% had asthma at 11 years of age and rhinoviruses were the only common airway viruses associated with increased asthma risk at that age.
- Other increased risks were parental heredity for asthma and/or allergies and allergic sensitisation at 2 years of age.

2 | MATERIALS AND METHODS

2.1 | Study population

Children aged 6–48 months with acute wheeze were included in the GEWAC study when they attended the emergency department at Astrid Lindgren's Children's Hospital in Stockholm, Sweden, between 2008 and 2012. A total of 156 were enrolled. The exclusion criteria were prematurity, any chronic disease or simultaneous complications such as sepsis, diabetes or pneumonia, at the time of inclusion (Table S1). During the same period, 102 age-matched healthy controls without acute wheeze were recruited from the surgical day care ward at the hospital, as previously described.[12] The exclusion criteria for the controls were prematurity, a history of asthma and known aeroallergen sensitisation (Table S1). The cases, who had acute episodes of preschool wheeze, were re-examined after 3 months and were followed prospectively until 11 years of age. Due to the COVID-19 pandemic, the 11-year follow-up was extended to include 107 cases and 46 healthy controls at 10–14 years of age (Figure 1). All children attending the 11-year follow-up were included in this study. The majority were seen in person, but the legal guardian of 10 cases and nine healthy controls were interviewed by telephone.

2.2 | Sample collection

The study protocol included standardised questionnaires. These covered demographics, breastfeeding, exposure to tobacco smoke, parental asthma and/or allergies, previous infections and wheeze, current atopic and asthma symptoms and use of asthma medication. Nasopharyngeal swabs were used to detect viruses and were taken during the emergency visit and analysed as previously described.[12] The viruses that were analysed included rhinoviruses, the respiratory syncytial virus (RSV) and enteroviruses. We also included other common airway viruses: adenoviruses, bocavirus, parainfluenza viruses type 1, 2 and 3, metapneumovirus, coronaviruses (229E, HKU1, NL63), influenza A, influenza A/H1N1 and influenza B. The Asthma Control Tests for children aged 4–11 years and adolescents over 12 years of age and the Asthma Quality of Life Questionnaire were completed at the 11-year follow-up. Blood samples were collected and a complete blood count was performed at the Karolinska University

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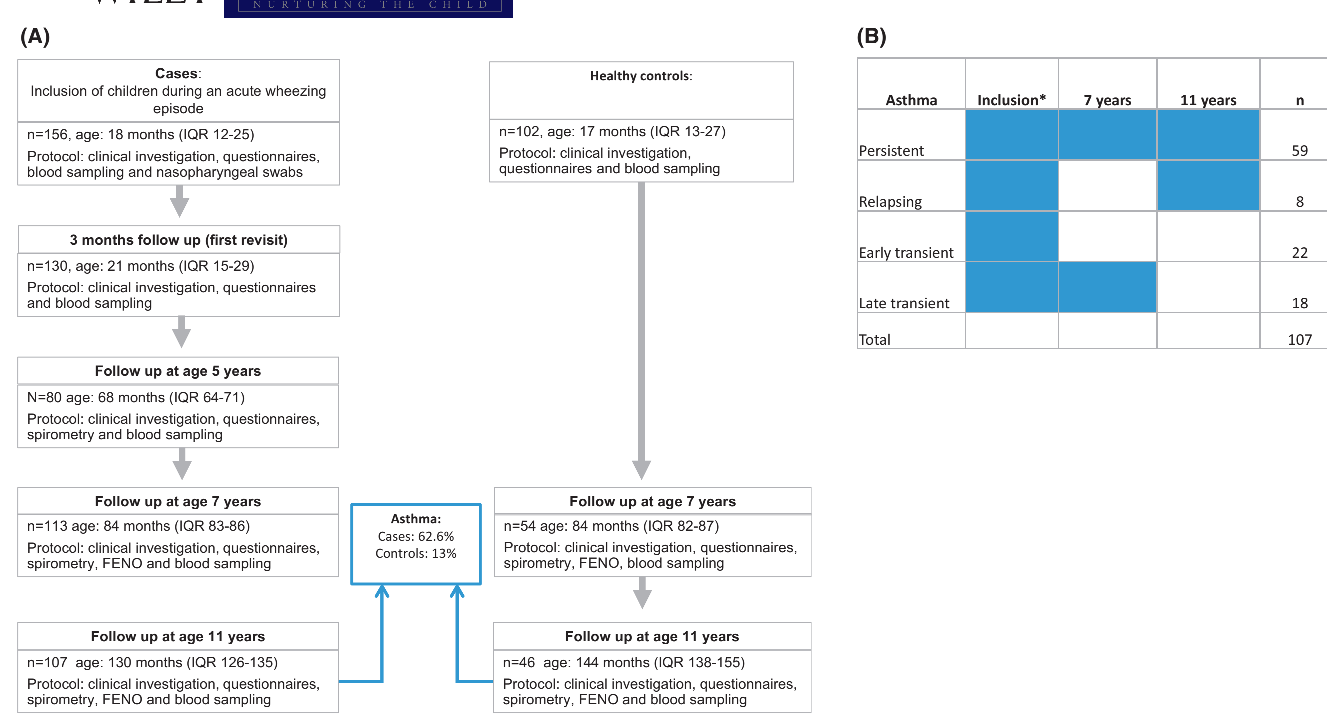

FIGURE 1 (A) Study protocol of the GEWAC cohort. Cases that attended to the follow-up at 11 years of age were included in the analyses. (B) The course of asthma from inclusion, 7 and 11 years in the GEWAC cohort, based on cases who attended the 11-year follow-up. Blue denotes asthma/wheeze at inclusion and asthma diagnosis at 7 and 11 years. *Asthma at inclusion is defined as asthma-like symptoms (wheeze) at the time of inclusion in the study.

Hospital laboratory. Serum EDN levels were measured at Thermo Fisher Scientific, Uppsala, Sweden, using a microarray-based semi-quantitative research assay, as previously described.[13] Allergen-specific immunoglobulin E (IgE) antibodies were measured at the first revisit, at a median age of 2 years, and at the follow-up visits at 7 and 11 years. The allergens analysed included common food allergens measured with Fx5 (milk, egg white, wheat, codfish, peanut and soya bean) (ImmunoCAP System, Thermo Fisher Scientific). We also measured common airborne allergens measured with Phadiatop (house dust mites, cat, horse, dog, timothy, birch, and mugwort and *Cladosporium herbarum*) (Thermo Fisher Scientific). Allergic sensitisation was defined as an allergen-specific IgE level of ≥0.35 kU_A/L. Fractional exhaled nitric oxide (FeNO) was measured using the non-invasive NIOX VERO apparatus (Circassia AB, Uppsala, Sweden). Spirometry and airway reversibility were assessed using the Medikro Pro spirometer (Medikro Oy, Kuopio, Finland). Reversibility was evaluated 15 min after inhalation, with 400 μg of salbutamol using a spacer. Legal guardians were advised not to give their children their asthma medication for up to 24 h before the follow-up visit, namely salbutamol, corticosteroids or leukotriene receptor antagonists. Early-life factors were defined as factors collected at the inclusion visit and at the revisit 3 months later. The definition of asthma at 11 years of age was a doctor's diagnosis, plus at least one of the following three criteria. The first criterion was lower respiratory symptoms, including cough, shortness of breath and waking at night, because of wheeze, for 5 days or longer in the preceding 12 months. The second was using asthma medication in the preceding 12 months. The third was airway reversibility of more than 12% after using bronchodilator with salbutamol (Table S2), as previously described.[7]

2.3 | Statistical analyses

SPSS statistics, version 27.0 (IBM Corp, New York, USA) was used for all the analyses. The Chi-squared test was used to compare proportional differences between two groups and Fisher's exact test was used on small sample sizes. An unpaired *t*-test was used to analyse continuous variables with normal distribution and the nonparametric Mann–Whitney *U*-test was used for skewed continuous variables. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using logistic regression for the associations between asthma at 11 years of age and early-life risk factors and aetiology of viral wheeze. The variables shown to significantly affect the risk of asthma at 11 years of age (Table 1) were included in the multivariate, adjusted logistic regression analysis. Differences in forced expiratory volume in 1 s/forced vital capacity (FEV1/FVC) were examined using the Wilcoxon signed rank test for evaluating paired differences between two time-points. p values of <0.05 were considered statistically significant.

2.4 | Ethical approval

The study received ethical approval from the Regional Ethics Committee at Karolinska Institutet, Stockholm (Dnr 2008/378-31/4, Dnr 2014/399-31/3 and Dnr 2017/2527-32). The study was

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TABLE 1 Early-life factors in asthmatic and non-asthmatic cases.

Variables	Total number included	Asthmatic cases ($n = 67$)	Non-asthmatic cases ($n = 40$)	p value
Early-life factors				
Age in months at inclusion, median (IQR)	107 (67/40)	18 (11–27)	15 (12–22.7)	0.41
Age in months at the first revisit, median (IQR)	100 (61/39)	22 (14–30.5)	19 (15–26)	0.51
Male sex, *n* (%)	107 (67/40)	45 (67.2)	28 (70.0)	0.76
Caucasian mother and/or father, *n* (%)	105 (67/38)	60 (89.6)	36 (94.7)	0.36
Parental asthma/allergy, *n* (%)	104 (66/38)	56 (84.8)	26 (68.4)	**0.048**
First time wheeze at inclusion, *n* (%)	100 (61/39)	11 (18.0)	9 (23.1)	0.54
Hospitalised at inclusion, *n* (%)	100 (61/39)	48 (78.7)	34 (87.2)	0.28
Doctor's diagnosis of asthma at the revisit, *n* (%)	100 (61/39)	32 (52.5)	22 (56.4)	0.70
History of atopic dermatitis at inclusion, *n* (%)	105 (67/38)	20 (29.9)	5 (13.2)	0.054
A rhinovirus-induced wheeze at inclusion, *n* (%)	105 (67/38)	33 (49.3)	11 (28.9)	**0.043**
Respiratory syncytial virus-induced wheeze at inclusion, *n* (%)	104 (67/37)	16 (23.9)	5 (13.5)	0.21
Food allergen positive at the first revisit, *n* (%)	98 (61/37)	17 (27.9)	6 (16.2)	0.19
Aeroallergen positive at the first revisit, *n* (%)	98 (61/37)	11 (18.0)	0 (0)	**0.006**
EDN at inclusion (AU), median (IQR)	56 (33/23)	2771.2 (1646.5–4233.7)	1878.4 (1485.2–3222.2)	0.28
EDN at revisit (AU), median (IQR)	79 (50/29)	4377.2 (2725.5–6953.9)	3256.4 (2225.4–6902.3)	0.30
Blood eosinophil count at the revisit 10^9/L, median (IQR)	96 (60/36)	0.4 (0.2–0.57)	0.3 (0.1–0.47)	0.12

Note: Bold text indicates $p < 0.05$.

Abbreviation: EDN, Eosinophil-Derived Neurotoxin, presented in arbitrary units (AU).

performed in accordance with the Helsinki Declaration. Written, informed consent was obtained from the parents or legal guardians.

3 | RESULTS

3.1 | Baseline characteristics

The 11-year follow-up of the current study comprised 107 cases who had experienced preschool wheeze and 46 healthy controls (Table S3). At 11 years of age, 62.6% of the cases and 13.0% of the non-wheeze controls had asthma (Figure 1). In addition, cases had higher blood eosinophil counts and lower FEV1/FVC ratios than the controls (Table S3). Dropout analysis revealed that the cases who attended the 11-year follow-up had a higher prevalence of parental asthma and/or allergy and a more frequent history of atopic dermatitis than those who did not attend (Table S4). Figure 1B shows the course of asthma from inclusion to the 11-year follow-up. We found that 55.0% had persistent asthma and 7.5% were categorised as relapsing.

3.2 | Early-life factors associated with asthma at 11 years of age

We compared early-life factors between asthmatic and non-asthmatic cases at 11 years (Table 1). Asthmatic cases at this age were more likely to have parents with asthma and/or allergies. These cases were also more likely to be sensitised to aeroallergens and have had rhinovirus-induced wheeze at inclusion. No difference was seen regarding wheeze induced by respiratory syncytial virus (RSV) at inclusion between asthmatic and non-asthmatic cases at 11 years of age.

TABLE 2 Viral aetiology of wheeze at inclusion and asthma risk at 11 years of age.

Viral aetiology of wheeze at inclusion	Crude odds ratios OR (95% CI)	Adjusted odds ratios[a] OR (95% CI)
Rhinoviruses	2.4 (1.02–5.6)	2.9 (1.2–7.2)
Respiratory syncytial virus	2.0 (0.7–6.0)	2.9 (0.9–9.3)
Enteroviruses	2.3 (0.6–8.8)	2.1 (0.5–8.7)
Other[b]	0.9 (0.3–2.4)	0.8 (0.3–2.3)

[a]Adjusted for all viruses included in the crude analysis.

[b]Metapneumovirus, adenoviruses, bocavirus, coronaviruses (229E, HKU1, NL63), parainfluenza viruses type 1, 2 and 3, influenza A, influenza A/H1N1 and influenza B.

A viral aetiology was identified in 72/107 (68.6%) of cases at inclusion and 25.0% of the cases had more than one virus. We also found that 41.9% of the cases had a rhinovirus-induced wheeze at inclusion and this was associated with an increased risk of asthma at 11 years of age, when adjusting for wheezing with RSV, enteroviruses and other common viruses. Wheezing with RSV affected 20.2% of the cases, 13.3% had enterovirus-induced wheeze and 20.0% had wheezing caused by other common viruses. None of these were associated with asthma at 11 years of age (Table 2).

The associations between early-life factors and asthma in the cases at 11 years of age were analysed using univariate regression models. These showed that having a rhinovirus-induced wheeze at inclusion

and allergic sensitisation at 2 years of age were associated with asthma at 11 years of age (Table 3). Multivariate regression showed that allergic sensitisation at 2 years and parental asthma and/or allergies were associated with an increased risk of asthma at 11 years (Table 3).

3.3 | Allergic sensitisation

Aeroallergen sensitisation was more common in asthmatic cases than in non-asthmatic cases at all measured time-points. All 11 cases with aeroallergen sensitisation at 2 years of age developed asthma by 11 years of age. Food sensitisation at 11 years of age was more frequent in asthmatic cases than in non-asthmatic cases (Tables 1 and 4).

TABLE 3 Early-life factors associated with asthma development at 11 years of age.

Early-life factors	Crude odds ratios, OR (95% CI)	Adjusted odds ratios,[a] OR (95% CI)
Parental asthma and/or allergy	2.6 (0.99–6.7)	3.4 (1.1–9.9)
Sensitisation at 2 years of age	2.9 (1.05–8.1)	3.0 (1.02–8.7)
Rhinovirus-induced wheeze	2.4 (1.02–5.6)	1.9 (0.8–4.8)

[a]Adjusted for all variables in the crude analysis.

3.4 | Rhinovirus-induced wheeze plus sensitisation

Asthma at 11 years of age was more common in cases with a rhinovirus-induced wheeze plus allergic sensitisation at 7 years than cases with a rhinovirus-induced wheeze without allergic sensitisation (92.9% vs. 57.1%, $p = 0.03$) (Figure 2). Our comparison of cases without a rhinovirus-induced wheeze, with and without allergic sensitisation, revealed no statistically significant differences in asthma prevalence at 11 years.

3.5 | Clinical characteristics and markers of asthma

Asthmatic cases at 11 years of age had lower Asthma Control Test and Asthma Quality of Life Questionnaire scores than non-asthmatic cases (Table 4). Furthermore, asthmatic cases had lower FEV1/FVC ratios at both 7 and 11 years of age than non-asthmatic cases (Figure 3A and Table 4). The FEV1/FVC ratios declined in cases, independent of asthma development, but this did not happen in the controls (Figure 3A).

Asthmatic cases had higher FeNO levels at the 11-year follow-up and higher blood eosinophil counts at both 7 and 11 years of age than non-asthmatic cases (Figure 3B,C and Table 4). In addition, asthmatic cases exhibited higher EDN levels at the 7-year follow-up, but not at inclusion or when they revisited 3 months later (Figure 3D, Tables 1 and 4).

TABLE 4 Clinical characteristics at 5, 7 and 11 years in asthmatic and non-asthmatic cases.

Variables	Total number included	Asthmatic cases, $n = 67$	Non-asthmatic cases, $n = 40$	p value
Follow-ups at 5 and 7 years				
Age in months at the 7-year follow-up, median (IQR)	92 (58/34)	84 (83–85)	84 (83–86)	0.78
FEV1/FVC at 5 years of age, median (IQR)	80 (51/29)	92.8 (84.6–99.1)	96.4 (90.6–100)	0.11
FEV1/FVC at 7 years of age, median (IQR)	91 (57/34)	87.3 (80.6–91.9)	90.3 (83.4–96.9)	**0.035**
Food allergen positive at 7-year follow-up, *n* (%)	83 (53/30)	13 (24.5)	3 (10.0)	0.11
Aeroallergen positive at 7-year follow-up, *n* (%)	83 (53/30)	22 (41.5)	3 (10.0)	**0.003**
EDN at 7-year follow-up (AU), median (IQR)	83 (54/29)	4660.5 (3201.4–7465.9)	3090.1 (2072.6–4524.7)	**0.004**
Blood eosinophil count at 7-year follow-up 10^9/L, median (IQR)	78 (49/29)	0.4 (0.25–0.7)	0.2 (0.15–0.45)	**0.014**
FeNO at 7-year follow-up, median (IQR)	33 (22/11)	10.5 (6–14.5)	8 (7–12)	0.61
Follow-up at 11 years				
Age in months at 11-year follow-up, median (IQR)	107 (67/40)	130 (126–135)	129 (126–135)	0.74
Asthma Control Test in %, median (IQR)	107 (67/40)	89 (78–93)	100 (96–100)	**<0.001**
Asthma Quality of Life Questionnaire, median (IQR)	95 (62/33)	6.6 (5.9–7)	7 (6.9–7)	**<0.001**
FEV1/FVC at 11-year follow-up, median (IQR)	97 (63/34)	83.1 (77.7–88.9)	87.0 (81.3–90.6)	**0.018**
Food allergen positive at 11-year follow-up, *n* (%)	83 (54/29)	23 (42.6)	5 (17.2)	**0.02**
Aeroallergen positive at 11-year follow-up, *n* (%)	82 (54/28)	29 (53.7)	7 (25.0)	**0.013**
Blood eosinophil count at 11-year follow-up 10^9/L, median (IQR)	76 (52/24)	0.3 (0.2–0.5)	0.2 (0.05–0.2)	**0.001**
FeNO at 11-year follow-up, median (IQR)	93 (59/34)	8 (5–18)	6 (5–8.3)	**0.037**

Note: Bold text indicates $p < 0.05$.

Abbreviations: EDN, eosinophil-derived neurotoxin, presented in arbitrary units (AU); FeNO, Fractional Exhaled Nitric Oxide; FEV1/FVC, The ratio of Forced Expiratory Volume in 1 s/Forced Vital Capacity.

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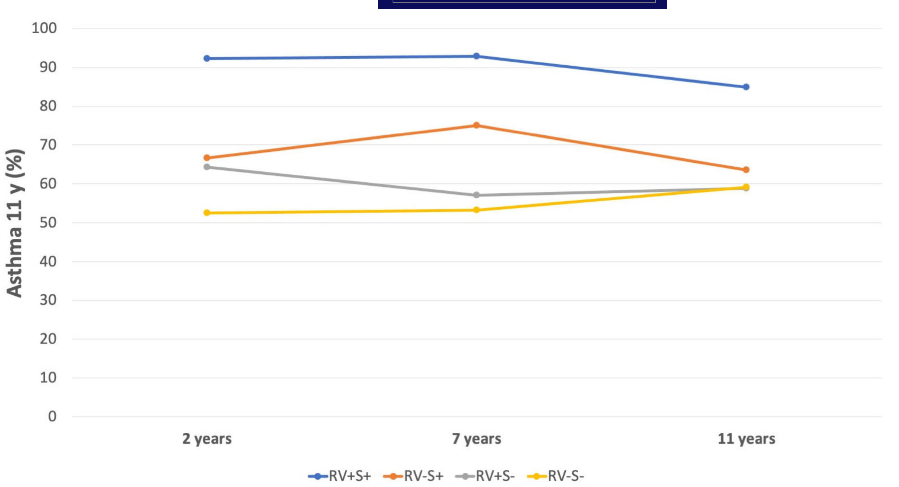

FIGURE 2 Prevalence of asthma at 11 years of age based on rhinovirus ($RV^{+/-}$)-induced wheeze and allergic sensitisation ($S^{+/-}$) at 2, 7 and 11 years. RV^+S^+ at 2 years ($n=13$), 7 years ($n=14$) and 11 years ($n=20$) of age. RV^-S^+ at 2 years ($n=15$), 7 years ($n=16$) and 11 years ($n=22$) of age. RV^+S^- at 2 years ($n=28$), 7 years ($n=21$) and 11 years ($n=17$) of age. RV^-S^- at 2 years ($n=40$), 7 years ($n=30$) and 11 years ($n=22$) of age.

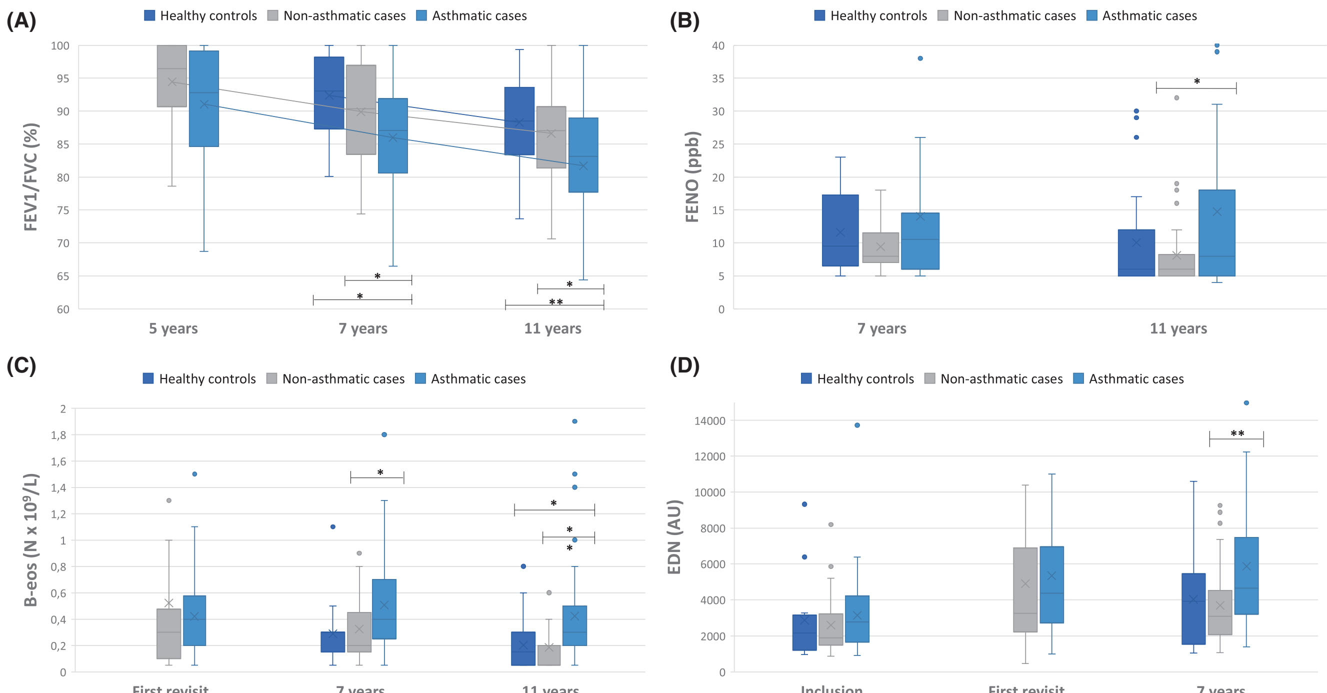

FIGURE 3 Measurement of fractional exhaled nitric oxide (FeNO), blood eosinophils, eosinophil-derived neurotoxin (EDN) and lung function (FEV1/FVC) at different time-points in asthmatic and non-asthmatic cases at 11 years of age as well as in controls. (A) FEV1/FVC measured at 5, 7 and 11 years. (B) FeNO at 7 and 11 years in cases and healthy controls. (C) Blood eosinophils measured at the first revisit at 2 years, then 7 and 11 years. (D) EDN measured at inclusion, the first revisit at 2 years and at 7 years. *$p<0.05$; **$p<0.01$. †The differences in FEV1/FVC between two time-points, presented as a trend line in figure (A). FEV1/FVC in controls between 7 and 11 years ($p=0.088$). FEV1/FVC in non-asthmatic cases between 5 and 11 years ($p<0.001$) and 7 and 11 years ($p=0.011$). Asthmatic cases between 5 and 11 years ($p<0.001$) and between 7 and 11 years ($p=0.001$).

4 | DISCUSSION

This study focused on 107 children, who were originally recruited from a Swedish emergency department during an episode of acute wheeze. They were evaluated for an asthma diagnosis at 11 years of age. Asthma at that age was associated with a rhinovirus-induced wheeze at inclusion and heredity and allergic sensitisation at all measured time-points. Sensitised children with a rhinovirus-induced wheeze had a higher prevalence of asthma at 11 years than non-sensitised children with a rhinovirus-induced wheeze.

Both a rhinovirus-induced wheeze at inclusion and allergic sensitisation at 2 years of age were associated with asthma at 11 years of age in the univariate analysis. In the multivariate analysis, parental asthma and/or allergies and allergic sensitisation at 2 years were the strongest early-life factors associated with asthma at 11 years of age. Asthma is a highly heritable disease.[14] Several genome-wide association studies have identified more than 100 genes associated with asthma.[15] The combination of specific genetic variants at the 17q21, a childhood asthma risk locus and a rhinovirus-induced wheeze have been shown to increase the risk of asthma development.[16] This

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suggests that a rhinovirus infection may be an associated factor in children predisposed to developing asthma.[17]

In this study, a rhinovirus-induced wheeze at inclusion and allergic sensitisation were more common in 11-year-old asthmatic cases. Rhinovirus-induced wheeze[18] and allergic sensitisation[9] in early life have both been shown to be associated with asthma development among wheezing children. The combination of both have contributed to an even higher risk of asthma.[9,19,20] We found that cases with rhinovirus-induced wheeze at inclusion had a higher prevalence of asthma at 11 years of age if they did, rather than did not, have allergic sensitisation at 7 years of age. This suggests a possible interaction between a rhinovirus-induced wheeze and allergic sensitisation. The mechanisms underlying the increased risk of asthma due to the combination of these conditions are not completely understood and different theories have been proposed. For example, rhinovirus infections may trigger an inflammatory response, leading to epithelial damage and mucus production, and repeated rhinovirus infections may eventually remodel the airways.[21] The damaged airway epithelial barrier would facilitate enhanced absorption of aeroallergens and thereby increase allergic inflammation.[22] Moreover, increased expression of IgE receptors on plasmacytoid dendritic cells has been associated with reduced rhinovirus-induced interferon secretion. This led to further epithelial damage caused by the rhinoviruses.[23] A rhinovirus-induced wheeze could also be a sign of an already impaired antiviral response among children predisposed to allergic disease.[6]

The FEV1/FVC ratio was reduced in asthmatic cases, compared to non-asthmatic cases at 11 years of age, supporting asthma diagnoses at this age. A lower FEV1/FVC ratio was observed at 7 and 11 years of age and this declining trend was even present from the first spirometry measurements at 5 years of age. The decline in the FEV1/FVC ratio was seen in both asthmatic and non-asthmatic cases at 11 years of age, but not in the controls. It is widely accepted that the FEV1/FVC ratio decreases from childhood to early adolescence, due to a more rapid increase in FVC than FEV1. This decrease is temporarily reversed during adolescence and continues to decrease during adult life.[24] One explanation for the decrease in the cases, but not in controls, could be because the latter group were older at the 11-year follow-up (Table S3). Even though age could partly explain the difference in decreases in the FEV1/FVC ratio over time, we cannot entirely rule out the possibility that a history of wheeze may have affected lung function. Recurrent wheeze in early life,[25] and early allergic sensitisation, have previously been associated with diminished lung function in adolescence.[26]

EDN and eosinophil cationic protein are markers for eosinophil cell activation and degranulation.[13,27] We found that, at 7 years of age, the EDN levels were elevated in cases that developed asthma at 11 years of age, in line with our previous results.[13] It has been suggested that EDN is a better marker of asthma control than an eosinophil count, because it reflects active inflammation better. EDN has previously been found to be increased among school-age children with atopic asthma and the levels correlated with airway hyperresponsiveness and asthma severity.[28] In adults, both EDN and eosinophil cationic protein have been associated with current asthma, but with different asthma characteristics. EDN has been associated with wheeze and asthma attacks, while eosinophil cationic protein has been associated with chronic bronchitis.[27]

Adolescents with asthma at 11 years of age had increased levels of FeNO and increased blood eosinophil counts at both 7 and 11 years of age. These further supported asthma diagnoses at 11 years of age. Both FeNO and blood eosinophil counts are well-known markers of type 2 inflammation and can be helpful tools when diagnosing and monitoring of asthma.[29,30]

4.1 | Strengths and limitations

The main strength of this study was the unique cohort of preschool children recruited from the same hospital during an episode of acute wheeze and the longitudinal follow-up to early adolescence. These subjects all underwent clinical examinations, blood sampling and spirometry measurements. The limitations of the study included possible recall bias, as the questionnaires asked about symptoms and medications over the last year. Furthermore, although about two-thirds of the cases and half of the healthy controls attended the 11-year follow-up, selection bias due to dropout cannot be ruled out. A dropout analysis of the cases showed that those who attended the 11-year follow-up had increased heredity for asthma and/or allergies. These may have influenced our results. Some of the subgroups were small and this limited the statistical power to detect differences.

5 | CONCLUSION

Our findings provide evidence of the importance of allergic sensitisation and hereditary factors on the development and persistence of asthma among preschool children with wheeze. Although a rhinovirus-induced wheeze was associated with asthma at 11 years of age, our findings suggest that rhinoviruses might be an associated factor in predisposed individuals, rather than an actual trigger for the inception of asthma.

AUTHOR CONTRIBUTIONS

Idun Holmdahl: Writing – original draft; conceptualization; investigation; methodology; visualization; formal analysis; writing – review and editing. **Sofia Lüning:** Writing – review and editing. **Sabina Wärnberg Gerdin:** Writing – review and editing; investigation. **Anna Asarnoj:** Conceptualization; visualization; methodology; writing – review and editing; supervision. **Angela Hoyer:** Writing – review and editing. **Anastasia Filiou:** Writing – review and editing; investigation. **Anders Sjölander:** Writing – review and editing. **Anna James:** Writing – review and editing. **Magnus P. Borres:** Conceptualization; visualization; methodology; writing – review and editing; supervision. **Gunilla Hedlin:** Writing – review and editing; conceptualization. **Marianne van Hage:** Conceptualization;

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visualization; methodology; writing – review and editing; supervision. **Cilla Söderhäll:** Conceptualization; visualization; methodology; writing – review and editing; supervision; funding acquisition. **Jon R. Konradsen:** Conceptualization; investigation; methodology; visualization; writing – review and editing; supervision; resources; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

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ORCID

Idun Holmdahl https://orcid.org/0000-0002-3869-5202
Anna Asarnoj https://orcid.org/0000-0002-0797-2369
Anna James https://orcid.org/0000-0003-2698-8419

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