

ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Clinical correlates of rhinovirus infection in preschool asthma

Tuomas Jartti¹ | Unna Liimatainen¹ | Paraskevi Xepapadaki² | Tero Vahlberg³ |
 Claus Bachert⁴ | Susetta Finotto⁵ | Marek L Kowalski⁶ | Anna Sobanska⁶ |
 Heikki Lukkarinen¹ | Maria Pasioti² | Tytti Vuorinen⁷ | Nan Zhang⁴ |
 Theodor Zimmermann⁸ | Nikolaos G Papadopoulos^{2,9}

¹Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku, Finland

²Allergy Department, 2nd Pediatric Clinic, National and Kapodistrian University of Athens, Athens, Greece

³Department of Biostatistics, University of Turku, Turku, Finland

⁴Upper Airway Research Laboratory, Ghent University Hospital, Ghent, Belgium

⁵Department of Molecular Pneumology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Universitätsklinikum Erlangen, Erlangen, Germany

⁶Department of Immunology, Rheumatology and Allergy, Central University Hospital, Lodz, Poland

⁷Department of Clinical Microbiology, Turku University Hospital and Institute of Biomedicine, University of Turku, Turku, Finland

⁸Department of Pediatrics and Adolescent Medicine, Department of Allergy and Pneumology, Children's Hospital, Friedrich-Alexander-Universität Erlangen-Nürnberg, Universitätsklinikum Erlangen, Erlangen, Germany

⁹Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, UK

Correspondence

Unna Liimatainen, Vinku Study Group,
 Department of Pediatrics and Adolescent
 Medicine, Turku University Hospital, 20520
 Turku, Finland.
 Email: unna.l.liimatainen@utu.fi

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Abstract

Background: Investigation of preschool asthma is important since not all children outgrow their illness during this age. Data are scarce on the role of rhinovirus (RV) infections in this patient group.

Objectives: To investigate the role of RV infections in preschool asthma: (i) susceptibility factors, (ii) clinical course, and (iii) medium-term outcome.

Methods: A total of 130 asthmatic children aged 4–6 years from the multinational PreDicta cohort were prospectively followed for a 12-month period. Allergy tests and a standard health questionnaire were carried out at study entry. Respiratory virus presence in nasopharyngeal washes was studied at illness visits and at 3 scheduled visits.

Results: At study entry, mean age of the children was 5.3 years. Of 571 visits, 54% were positive for any virus and 39% for RV. Patient characteristics were only assessed with RV infection due to low number of other viruses. The use of supplementary vitamin D was inversely associated with RV infection ($P < .05$). RV infection was associated with more severe course of acute illness in terms of more severe nighttime coughing, more sleep disturbances, and more days with runny nose (all $P < .05$). RV infection was also associated with more severe disease course during the 12-month

Abbreviations: ICAM-1, intercellular adhesion molecule 1; IL, interleukin; I κ B α , inhibitor of NF- κ B; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; PCR, polymerase chain reaction; RV, rhinovirus.

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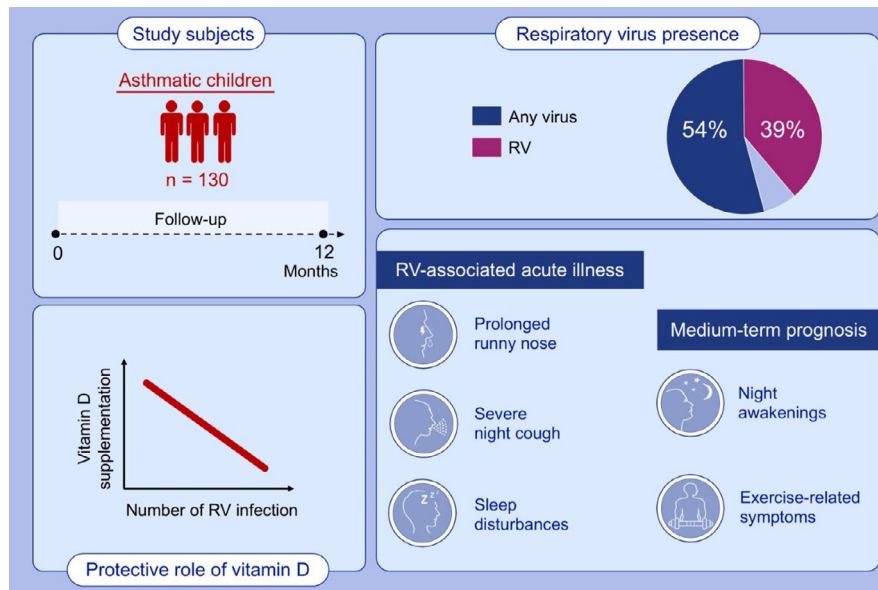
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follow-up in terms of more nights with awakenings and more days of exercise-related symptoms (both $P < .05$).

Conclusions: Vitamin D supplementation may have an anti-rhinovirus effect. Both short- and medium-term outcomes suggest RV infection to be an important clinical marker of instable preschool asthma.

KEYWORDS

asthma, child, preschool, rhinovirus, vitamin D



GRAPHICAL ABSTRACT

This study investigated the role of rhinovirus (RV) infections in preschool asthma in 130 asthmatic children from PreDicta cohort over 1-year follow-up. During all visits, RV was the most commonly detected virus. Vitamin D supplementation was associated with decreased number of rhinovirus infections in preschool children with asthma. Detection of RV was associated with more severe course of acute illness (more severe nighttime coughing, more sleep disturbances, and more days with runny nose) and more compromised medium-term prognosis (more nights with awakenings and more days of exercise-related symptoms).

1 | INTRODUCTION

Asthma is the most common chronic disease among children in industrialized countries. In Europe, the prevalence of childhood asthma has increased during last decades; in 2017, the prevalence at the age of 4 years varied from 2% to 13% depending on country.¹ Thus, asthma creates a major burden on public health, with the mean cost per patient per year estimated to be 1700 € in Europe considering all asthmatics.²

Respiratory viral infections are the almost exclusive trigger of wheezing episodes in early life, a condition affecting approximately 30% of children.³ Rhinovirus (RV) is the most commonly identified culprit, and interestingly, RV-induced wheezing has been shown to be a strong predictor of recurrent wheezing and a major risk factor for school-age asthma (odds ratios up to 20-45 depending on concomitant asthma risk allele and/or aeroallergy).⁴⁻⁶ Other main

risk factors of asthma include atopic dermatitis, family history of asthma, aeroallergen sensitization, exposure to smoke, and male gender.⁷ Among children with asthma, RV infections are also the main trigger of acute exacerbations.^{7,8} However, data are scarce on the role of RV infections in preschool age children with asthma.^{9,10} The investigation of preschool asthma is important since many children outgrow their illness during this age while in others the disease persists.

RV is a potentially valuable clinical marker of asthma persistence/morbidity in preschool age children with recurrent wheezing/asthma. Therefore, our goal was to examine factors associated with susceptibility to virus infections as well as clinical aspects of such infections in asthmatic preschoolers, with a special focus on RV. We had three specific aims: first, to identify characteristics of children with preschool asthma associated with (susceptibility to) symptomatic or asymptomatic RV infections; second, to assess whether RV

infections are associated with more severe clinical course of acute respiratory illness in comparison to other viruses or where no virus was identified, and third, to evaluate whether susceptibility to RV infections is associated with a more compromised 12-month asthma course. We hypothesized that there are likely to be factors that increase susceptibility for RV infections and, further, that RV infections may be associated with more severe short- and medium-term clinical course of preschool asthma.

2 | METHODS

2.1 | Subjects

This study was part of the PreDicta cohort (postinfectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases). Recruitment was carried out in five study centers in Europe: in Athens, Greece, Turku, Finland, Lodz, Poland, Ghent, Belgium, Erlangen, and Germany. Characteristics of the subjects, inclusion and exclusion criteria, and study design have been described in detail before.¹¹ In brief, main inclusion criteria consisted of a written informed consent from the child's parents/guardians, age 4-6 years, gestational age of 36 weeks or above and a doctor-diagnosed asthma within the last 2 years, mild to moderate persistence's severity according to GINA.¹² Main exclusion criteria were severe or brittle asthma, children receiving immunotherapy, other chronic nonatopic disease. The study was commenced only after obtaining written informed consent from the parents or a guardian. The study protocol was approved by the Ethics Committee of all participating hospitals.

2.2 | Study protocol

At study entry, a guardian filled out a standardized questionnaire on symptoms of asthma, rhinitis, eczema, infections, immunizations, environmental factors, and family history in the preceding 12-month period. In order to be included at baseline, children had to be asymptomatic for at least 4-6 weeks. Baseline blood tests and nasopharyngeal swab samples were obtained (see below). Follow-up visits were arranged at 6-month intervals for 2 years, where guardians were asked to fill out a questionnaire on symptoms of asthma/rhinitis activity (Figure S1). Respiratory symptoms and medications were also recorded in a daily symptom diary.

In addition to the scheduled visits, guardians were instructed to contact the study center if the child developed signs of a respiratory tract infection, exacerbation of asthma, a symptom score according to the diary cards of 4 or over, or a decrease in forced expiratory volume in one second >15% or in peak expiratory flow >30%. An additional visit to the clinic was then arranged, for pathogen identification.

At each visit, nasopharyngeal sample was collected with the use of a nasal applicator swab, which has a tip with flocked soft nylon

fiber (ESwab 482CE; Copan Italia). Immediately after collection, the specimen was transported to the laboratory and stored at -80°C for later analysis.

2.3 | Definitions

Childhood asthma refers to repeated attacks of airway obstruction and intermittent symptoms of increased airway responsiveness to triggering factors, such as exercise, allergen exposure, and viral infection.^{12,13} "Wheezing" refers to expiratory breathing difficulty as a result of airway obstruction with a high-pitched bilateral sound during expiration. The presence of allergic sensitization was defined by having one or more positive values for allergen-specific IgE (>0.35 kU/L, Phadiatop Combi; Phadia, Uppsala, Sweden). Skin prick tests were performed optionally for additional atopy determination using 7 common allergens: normal saline, histamine (10 mg/mL), dermatophagoides mix, cockroach, cat, alternaria, and grass mix in all centers. In addition, *birch and Ambrosia* were used in Ghent, Lodz, Erlangen and Turku and *Parietaria* and olive in Athens. Virus infection refers to a positive virus detection using PCR, irrespective of respiratory symptoms.

2.4 | Outcomes

Aim 1: The first study question investigated whether certain host characteristics at baseline were associated with the presence of RV vs. non-RV. RV infection was defined as a positive RT-PCR test of nasopharyngeal swab sample whether symptomatic or not during the 24-month follow-up period. RV group may include other viruses. Non-RV group includes other virus and virus-negative findings.

Aim 2: The second study question investigated whether the detection of RV vs. non-RV in an acute respiratory illness was associated with more severe disease course. The symptom data were based on the diary records.

Aim 3: The third study question investigated whether susceptibility RV vs. non-RV infection as defined in aim 1 was associated with more severe medium-term (12-month) prognosis, based on all clinical visits and diary records.

2.5 | Laboratory methods

Virus transport mediums (ESwab 482CE; Copan Diagnostics, Murrieta, CA, USA) were transported from study centers on dry ice and analyzed for respiratory viruses at the Department of Virology, University of Turku. Enter-, rhino- (species A, B, and C), and respiratory syncytial viruses were detected using an *in-house* RT-PCR test.¹⁴ The first one hundred Finnish samples were also tested for adenovirus, human metapneumovirus, coronavirus 229E/NL63, parainfluenza viruses 1, 2, or 3, influenza viruses A or B, respiratory syncytial virus A or B, rhinovirus A/B, and coronavirus OC43/HKU1

by a commercial multiplex PCR test (Seeplex RV12 ACE Detection kit). Thereafter, the test was replaced by a new test version of the same manufacturer and all remaining samples were assessed by Anyplex RV16 Detection kit (Seegene, Soul), which identifies adenovirus, influenza A and B, parainfluenza viruses types 1-4, rhinovirus A, B, and C, respiratory syncytial virus A and B, human bocavirus 1-4, metapneumovirus, coronavirus 229E, NL63 and OC43, and enterovirus.

2.6 | Statistics

The association between categorical baseline characteristics and susceptibility to rhinovirus infections was analyzed using chi-squared test or Fisher's exact test and continuous baseline characteristics using Mann-Whitney *U* test. Analyses were extended to group baseline characteristics as number of sensitizations, allergic diseases ever (atopic eczema, allergic rhinitis diagnosis, adverse reaction to food, bee, wasp or latex, and/or skin prick test positive for aeroallergens at study entry); parental allergic illnesses ever (eczema, allergic rhinitis, asthma and/or adverse reaction to food); moderate-to-severe asthma (asthma severity at least moderate persistent, asthma treatment during last 3 months, peak expiratory flow <80%), exposure to any air impurity factor (animal exposure on a weekly basis, parental smoking, trucks through street at least frequently though the day, mold spots, and/or window condensation at home), and exposures to animal and other air impurity factors separately. The associations between RV infection and symptoms of asthma were examined with Mann-Whitney *U* test. The associations between continuous parameters describing activity of asthma and RV were analyzed using Mann-Whitney *U* test, because the continuous variables were not normally distributed. A two-sided $P < .05$ was considered statistically significant, and no adjustment was made for multiplicity. We used SAS/STAT(r) version 9.4 of the SAS System for Windows (SAS Institute Inc, Cary, NC, USA).

3 | RESULTS

3.1 | Study cohort

The enrollment of the study subjects and data sets for each study aim are shown in Figure 1. Originally, 167 children were recruited into the case group (diagnosed asthma) and 75 children into the control group in 5 study centers. Over 1-year period, one patient dropped out after the baseline visit, 70 patients were not sampled for viruses from follow-up visits, while 41 patients had data from only 1 follow-up visit. A total of 131 patients, one of which in control group, had virus data from at least 2 follow-up visits. Finally, we have analyzed 130 patients for whom virus data from at least 2 follow-up visits were available (Turku = 42, Lodz $n = 39$, Athens $n = 26$, Ghent $n = 12$, Erlangen $n = 11$).

3.2 | Patient characteristics

Baseline characteristics of 130 study children who had virus data available from at least two scheduled visits are shown in Table 1, and Table S1. At study entry, 62% of them were boys, 55% had aeroallergen sensitization, 38% had active atopic eczema, and 38% had vitamin D supplementation. The severity of asthma was mainly mild persistent (59%) or intermittent (25%), and 83% of subjects had peak expiratory flow in normal range. Inhaled corticosteroid was the most common asthma control medication (83%), 34% of parents smoked and 42% had indoor pets. Of all 571 visits (scheduled and illness visits), viruses were detected in 307 (54%). RV was the most common finding (39%), whereas each other virus was found in <5% of visits. RV was detected significantly more often at illness compared to scheduled visits (58% vs 34%, $P < .0001$). Virus coinfections were found in 6.7% (38/571) of all visits (Table S2). No difference in their prevalence was found between RV-positive (16/222, 7.2%) vs. RV-negative groups (22/349, 6.3%) ($P = .67$).

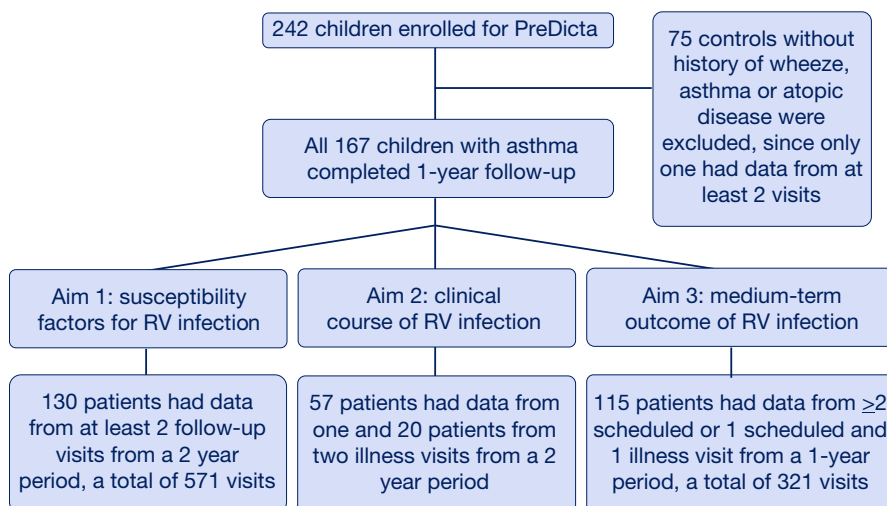


FIGURE 1 Study flowchart

3.3 | Susceptibility to rhinovirus infections

Patient characteristics at baseline were only compared with RV ($n = 109$) vs. non-RV ($n = 21$) infection due to low number of other virus findings. The use of vitamin D supplementation was inversely associated with RV infection at any visit (scheduled and illness visits) (proportion of children with supplementation, RV+ vs RV- group, 76% vs 89%, $P = .049$, Table S3). The association persisted after adjusting to study center, $P = .0496$). Use of vitamin D-fortified food items (milk, cereal, or yoghurt) was not associated with RV detection ($P > .1$, Table S2). We further analyzed the association between vitamin D supplementation at study entry and symptomatic rhinovirus illnesses but no association was found: positive RV findings in children without vit-D vs with vit-D, 69.6% (32/46) vs 70.0% (14/20), $P > .9$. No other associations were found (Table S3) even if baseline characteristics were grouped as number of sensitizations, atopic disease ever, parental allergic illnesses ever moderate-to-severe asthma, or any pollutant exposure (all $P > .1$, data not shown).

3.4 | Clinical characteristics of virus infections

Clinical characteristics of acute events were studied by investigating the connection between the virus findings during the course of an acute respiratory illness and symptom severity. Of 130 asthmatic children, 57 children had virus information available from illness visit 1 and 20 children from illness visit 2 (Table S4). RV infection was associated with increased cough during the night ($P = .029$) and sleep disturbances ($P = .015$), but less ophthalmic symptoms ($P = .041$) at illness visit 1 and with increased runny nose ($P = .008$) at illness visit 2 (Figure 2, Online Table E3).

3.5 | Virus findings and asthma activity

The connection between RV infections at any time-point (scheduled or illness visits) and asthma activity was analyzed within 1-year follow-up (Table S5). It was found that RV infection was associated with increased activity of asthma in terms of more nights with awakenings ($P = .026$) and more days with exercise-related symptoms ($P = .041$, Figure 3). Moreover, RV infection at baseline was associated with increased days of rhinitis within the 12-month follow-up (50% vs 25% had rhinitis >4 days per week within 6 months, $P = .024$).

4 | DISCUSSION

This study had three important findings in children with preschool asthma: First, of many personal (age, sex, atopic characteristics,

TABLE 1 Baseline characteristics

Characteristics	N = 130
Age, mean (SD)	5.3 (0.7%)
Male	80 (62%)
Allergic diseases ever	
Atopic eczema	74 (57%)
Symptoms within past 12 months	50 (38%)
Allergic rhinitis diagnosis	91 (70%)
Symptoms within past 12 months	109 (84%)
Adverse reaction to food	48 (37%)
Adverse reaction to bee or wasp	20 (15%)
Adverse reaction to latex	1 (1%)
Asthma severity	
Intermittent	32 (25%)
Mild persistent	77 (59%)
Moderate persistent	21 (16%)
Severe persistent	0 (0%)
Asthma treatment during last 3 months	
Inhaled corticosteroid	108 (83%)
Leukotriene receptor antagonist	55 (42%)
Long-acting beta2-agonist	20 (15%)
Status at study entry	
Skin prick test positive for aeroallergens	72 (55%)
Peak expiratory flow	
$>80\%$	101/121 (83%)
60%-80%	16/121 (13%)
$<60\%$	4/121 (3%)
Parental smoking	44 (34%)
Parental allergic illnesses ever	
Eczema	47 (36%)
Allergic rhinitis	81 (62%)
Asthma	51 (39%)
Adverse reaction to food	34 (26%)
Animal exposure on a weekly basis	
Indoor pets	54 (42%)
Cat	24/77 (31%)
Dog	30/76 (39%)
Farm animal	4/76 (5%)
Other	17/76 (22%)
Breastfed	110 (85%)
Vitamin D supplementation	45/119 (38%)
In milk	62/112 (55%)
In cereal	40/103 (39%)
In yogurt	48/112 (43%)

Note: Data are shown as number of patients (%) unless otherwise expressed.

See more patient characteristics in Table S1.

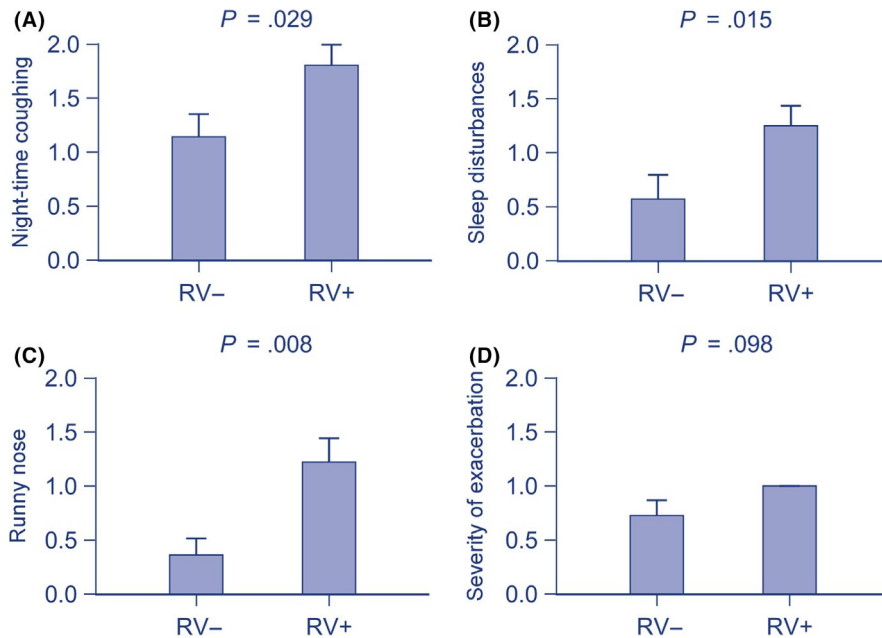


FIGURE 2 The association between rhinovirus detection and respiratory symptoms at illness visits. Significant and positive associations were found with (A) nighttime coughing, (B) sleep disturbances, (C) runny nose, and a tendency with (D) the severity of exacerbation. Data are shown as mean (standard error of mean) for better visualization and are analyzed using Mann-Whitney *U* test

asthma severity, physical activity, breast feeding, and vitamin D supplementation), family (atopic characteristics and education level), and environmental (pet ownership, farm animal exposure, and air pollutants) factors, only vitamin D supplementation was associated with decreased susceptibility to RV infections. Interestingly, atopic sensitization was not. Second, RV triggered acute respiratory illness was associated with more severe course of the illness; and third, susceptibility to RV infection was associated with increased asthma activity during the following 12-month period.

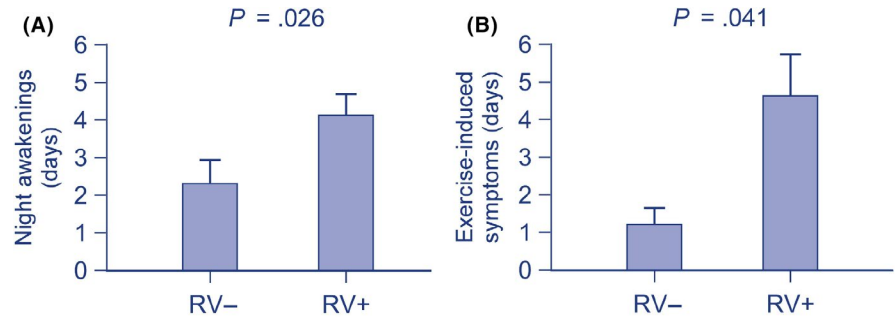
Our findings of potential protective role of vitamin D against respiratory infections are supported by many clinical studies in children and in vitro studies,¹⁵⁻¹⁸ although contradictory reports also exist in adults.^{19,20} Low levels of vitamin D or lack of supplementation have been associated with increased risk of respiratory infections as well as coinfections in children.^{15,16} Specific association to RV infections, however, has received less attention, and results have been inconclusive.^{21,22} High vitamin D levels may have partly explained the lack of meaningful correlations in some cases.²¹ Our findings support the protective role of vitamin D against RV infections which is particularly important in asthmatic children since RV is the major trigger of exacerbations²³ and vitamin D levels were inversely related to measures of asthma severity.²⁴ Collectively, available data strongly support antiviral effects of vitamin D3 supplementation (recommended 7.5-25 µg/day in many European countries but not necessarily during summer times) and its usage in young asthmatic patients. In addition, many food items are fortified with vitamin D. Our data support the view that vitamin D supplementation, and not vitamin D fortified food items, is the best source.

Asthma-prone children appear to be more susceptible to RV infections than healthy children.⁷ Our findings in preschool children support these findings by showing that RV detection during acute illness was positively associated with measures of illness severity. However, the exact mechanisms are not precisely known but most

plausible explanation is that asthmatic children have weaker antiviral defense than healthy children. This is likely to be due to predisposition (genetically low interferon $\alpha/\beta/\gamma/\lambda$ levels and low interleukin [IL]-10 levels thereby weakened antiviral defense and anti-inflammatory control), T helper2 type risk factors of asthma (allergen exposure and high-affinity IgE receptor cross-linking have been shown to impair type I and III interferon production and increased production of T helper2 cytokines IL-4, IL-5, and IL-13 in airway secretions counteract T helper1 responses), and interactions between the underlying inflammatory pathways (RV infections and allergens enhance, for example, the airway epithelial cell production of IL-25 and IL-33, which promote T helper2 airway inflammation; T helper2 polarized immune responses may increase the expression of ICAM-1, and disrupted airway epithelium favors RV replication).⁷ Delayed maturation of antiviral responses may also contribute at this age range.²⁵ Thus, genetically weak antiviral mechanisms may be further attenuated by airway inflammation and, in our case, also by low vitamin D status. Moreover, antiviral mechanisms are likely to be, at least partly, RV-specific since genetic variants at the 17q21 locus and in CDHR3 allele may markedly increase the risk of severe RV infections although not investigated in our study.^{5,6}

Negative findings in many investigated personal, family, and environmental factors further support the compromised virus defense as the key element in the susceptibility to RV infections. More severe RV infections are known to be closely associated with atopic asthma,^{4,26} but surprisingly atopic characteristics (investigated separately and as a group) were not associated with susceptibility to RV infection even though a half of our children were sensitized to aeroallergens. Also, air pollutants, especially exposure to allergens, but also traffic pollutants such as NO₂, increase risk of virus-induced exacerbation of asthma.^{27,28} However, we did not find association between exposure to these pollutants (separately or as a group) and susceptibility to RV infections. The probable explanation for these

FIGURE 3 The association between rhinovirus detection and activity of asthma within 12 months. Significant and positive associations were found with (A) night awakenings and (B) exercise-induced symptoms. Data are shown as mean (standard error of mean) for better visualization and are analyzed using Mann-Whitney *U* test



null findings is that our children were using regular asthma controller medication. Parental smoking does not appear to have strong influence on the susceptibility to RV infections.^{26,29} As expected, breast feeding during infancy was not associated with virus susceptibility at preschool age.

Our findings also indicate that susceptibility to RV infections, whether symptomatic or asymptomatic, serves as an important clinical marker for more compromised prognosis of asthma. It is known previously that RV-induced wheezing is an important risk factor for subsequent wheezing and later asthma among young wheezing children.⁷ Already at the time of first severe wheezing episode, its presence can predict school-age atopic asthma (odds ratio 5.0 in normal population).²⁶ This risk value of RV detection in wheezing children has been extended to the first three years of life.⁴ It has been speculated that this risk may be limited to early wheezing only. Our results contrast these speculations and extend the prognostic value of RV infection in preschool to school-age asthma. RV detection among wheezing children appears to be particularly important since it also effectively predicts steroid-responders.^{30,31} It should be noted that increased RV expression in asthmatics may suppress secondary virus infections, thus further promoting the role of RV in asthmatic subjects.³²

The PreDicta cohort has many strengths including careful characterization of study subjects, serial virus detection as well as prospective, multi-center, and multinational design. *In-house* RT-PCR effectively covered all RV species.³³ There are also weaknesses. Sample size only allowed RV-specific analyses since the number of other virus detections was low. However, RV was our main focus. A longer than 12-month follow-up time may have revealed more risk factors. Days with exercise symptoms were determined subjectively by parents. Also, sample size for some risk factors was likely to be low, but analyses were repeated by uniting them into larger groups, for example, by uniting any atopic characteristics under one variable. Correction for multiple comparisons was not done due to explorative nature of the analysis. Due to large study setting and multidisciplinary approach, several predefined sub-analyses to assess causality and interrelations have not yet been completed.

In conclusion, vitamin D supplementation may have beneficial role in antiviral defense in preschool asthmatic children when used with recommended doses. However, an intervention study is warranted. Our findings also highlight the significance of RV infection as

a meaningful clinical marker for short- and medium-term outcomes in preschool asthma. This information adds to the potential of direct RV detection, as well as RV antibody detection,³⁴ to be used as an approach for personalized medication.³⁵

CONFLICTS OF INTEREST

The authors declare no conflict of interest in connection with this paper.

ORCID

Unna Liimatainen  <https://orcid.org/0000-0002-0214-3481>
 Paraskevi Xepapadaki  <https://orcid.org/0000-0001-9204-1923>
 Marek L Kowalski  <https://orcid.org/0000-0002-8442-2774>
 Nikolaos G Papadopoulos  <https://orcid.org/0000-0002-4448-3468>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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