Recurrent Rhinovirus Detections in Children Following a Rhinovirus-Induced Wheezing Exacerbation: A Retrospective Study

Sofie Halmø Hürdum[1,2], Guicheng Zhang[1,3], Siew-Kim Khoo[1,4], Joelene Bizzintino[1,4], Kimberley Marie Franks[1,4], Katie Lindsay[5], Anthony David Keil[5], Desmond William Cox[1,6], Jack Goldblatt[1], Yury Alexandrovich Bochkov[7], James Gern[7], Charlotte Suppli Ulrik[2], Peter Neils Le Souëf[1] and Ingrid Alisa Laing[1,4,*]

[1]*School of Paediatrics and Child Health, The University of Western Australia, GPO Box D184, Perth Western Australia, 6840, Australia*

[2]*Department of Pulmonary Medicine, Hvidovre Hospital, University of Copenhagen, Kettegård Allé 30, 2650 Hvidovre, Denmark*

[3]*School of Public Health, Curtin University, GPO Box U1987, Perth, Western Australia, 6845, Australia*

[4]*Telethon Kids Institute, The University of Western Australia, PO Box 855, West Perth, Western Australia, 6872, Australia*

[5]*Department of Microbiology, Pathwest Laboratory Medicine WA, Princess Margaret Hospital for Children, GPO Box D184, Perth, Western Australia, 6840, Australia*

[6]*Our Lady's Children's Hospital, Crumlin, Dublin 12 Ireland*

[7]*Department of Pediatrics, University of Wisconsin Clinical Science Center 600 Highland Avenue Madison, Wisconsin, USA*

Abstract: *Introduction:* It is unclear if children with a rhinovirus (RV)-induced wheezing exacerbation are more susceptible to viruses longitudinally, and whether a parental history of asthma and/or allergy impacts their susceptibility.

The objective of this study was to determine if RV, RV-A and RV-C related wheezing exacerbations in children were associated with prior or subsequent viral detections and investigate the role of parental history of asthma and allergy.

Materials and methods: Children presenting to hospital with acute wheeze were prospectively recruited and tested for respiratory viruses. Data on viruses detected in other respiratory samples (May 1997 to December 2012) were collected from hospital microbiology records and additional RV testing was performed on stored hospital respiratory samples (September 2009 to December 2012). A positive parental history was defined as either parent with self-reported asthma and/or allergy.

Results: At recruitment, RV was detected in 69.2% of samples from children with an acute wheezing episode (n=373, 0–16 years of age), with RV-C the most common virus (65.5%). Children with a history of parental asthma and/or allergy and RV at recruitment had a 14-fold increased incidence rate ratio (IRR) of subsequent RV detection (IRR 14.0, 95% CI 1.9–104.1; p=0.01) compared with children without RV at recruitment. Children without this parental history had a reduced incident rate ratio for samples assessed during this time (IRR 0.5, 95% CI 0.3–0.9; p=0.03).

Conclusion: Children with a parental history of asthma and/or allergy may become more susceptible to recurrent symptomatic RV infections.

Keywords: Allergy, asthma, attack, childhood, respiratory virus, wheeze.

1. INTRODUCTION

Acute wheezing triggered by respiratory viral infections is a highly prevalent cause of hospital admission for children in developed countries [1-3]. Rhinovirus (RV) has been recognized as the main pathogen associated with these acute episodes [2-8], and possibly increases the risk of wheezing later on in life in both non-hospitalized [9] and hospitalized children [10].

Studies suggest that genetic predisposition to asthma and/or allergic disease may play a role in wheezing with RV and the respiratory outcome in children [11-13]. Infants with maternal disposition to atopic asthma were more likely to be infected with RV than respiratory syncytial virus (RSV) and suffered from more severe RV infection compared with infants without this disposition [11]. In a prospective birth cohort study including children predisposed to asthma

*Address correspondence to this author at the Telethon Kids Institute and School of Paediatrics and Child Health, The University of Western Australia, PO Box 855, West Perth, Western Australia, 6872, Australia; Tel: +61 8 9489 7777; Fax: + 61 8 9489 7700; E-mail: Ingrid.laing@telethonkids.org.au

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and/or allergy, RV was the most prevalent virus in recurrent moderate to severe wheezing episodes in infancy [12] and a similar study revealed that early-life RV wheezing illnesses were a strong predictor of wheezing and asthma at school age [13].

In children and adults diagnosed with asthma, acute exacerbations are often associated with RV infections [7, 14-16]. Sensitization to common aeroallergens may be a risk factor for RV-related asthma exacerbations leading to paediatric hospital admissions [16] and allergic asthma has been associated with impaired components of the RV antiviral response, suggesting that children with allergic illness may have an increased susceptibility to RV [17].

There are three species of RV; A (RV-A), B (RV-B) and C (RV-C) [6, 18]. We previously reported that RV-C was the most common virus detected and associated with more severe wheezing exacerbations in children presenting to hospital compared with RV-A, RV-B and other viruses [2]. Furthermore, RV-C was associated with an increased risk of subsequent respiratory hospital admission particularly in atopic subjects [19]. However, there is a paucity of data on the recurrence of RV and RV species in children presenting to hospital with acute wheeze and the role of family predisposition to asthma and allergy. Although RV-C is often detected in children presenting to hospital with acute wheeze, it is unknown whether these children have an inherent susceptibility to this RV species

The aim of this study was to determine the rate of viruses detected longitudinally in children who had presented to hospital with a RV-induced wheezing exacerbation.

2. MATERIALS AND METHODS

2.1. Study Subjects

Children were enrolled from August 2002 to October 2012 in the Perth Childhood Acute Asthma Study (PCAAS) and the Mechanisms of Acute Viral Respiratory Infections in Children (MAVRIC) study as previously described [2, 20]. Children aged 0–18 years were prospectively recruited when they presented with an acute wheezing episode to the Emergency Department (ED), Princess Margaret Hospital (PMH), Perth, Australia. Children were excluded if they had any other underlying chronic disorder, apart from asthma, or were born prematurely (< 36 weeks). All children (n=373) had data available on parental history of asthma and/or allergy and RV detection at recruitment.

The PMH Human Ethics Committee approved the study and written informed consent was obtained from at least one parent/guardian for each subject.

2.2. Data and Sample Collection

2.2.1. Recruitment

At recruitment, an ED physician determined each subject's diagnosis independent of the study including: acute asthma exacerbation, viral-induced wheeze, exercise-induced wheeze, wheeze with no specific trigger and other respiratory diagnoses. Exacerbation severity was scored according to a modified NIH score of clinical parameters as mild (0–2), moderate (3–6), or severe (7–10) [2, 21, 22]. A nasal specimen (nasopharyngeal aspirate (NPA), flocked swab or nasal wash) was obtained for testing of common respiratory viruses.

History of parental asthma and/or allergy was defined as a positive response to the question “Does anyone in the family have asthma or allergy?” and an answer of “mother” or “father” to the question “What is their relationship to your child?” in a parent/guardian administered questionnaire.

2.2.2. Respiratory Samples Assessed Longitudinally

Data and samples other than recruitment were collected retrospectively as part of an audit from the PMH Department of Microbiology. Most of these samples were likely collected at other hospital presentations with a clinical indication for collection of a respiratory sample. Children were recorded as either positive or negative for each of the common respiratory viruses depending on the detection results of these samples. All samples collected and tested within seven days intervals were recorded as one sample. Data on respiratory samples (NPA, nose or throat swabs) were ascertained from May 1997 when the PMH Department of Microbiology records commenced through to December 2012. Where available, samples stored between September 2009 and December 2012 (3.2 years) by the PMH Department of Microbiology underwent further RV testing and typing in our research laboratory.

2.2.3. Respiratory Virus Detection at Recruitment and Longitudinally

Respiratory samples were routinely tested by the PMH Department of Microbiology for the presence of RSV, adenovirus, influenza virus A and B, parainfluenza virus 1, 2 and 3, as well as human

metapneumovirus using immunofluorescence, virus culture, or polymerase chain reaction (PCR). RV was tested on an ad hoc basis using semi-nested reverse transcription (RT)-PCR by PathWest Laboratory Medicine, Perth, WA, Australia [23].

In our laboratory, RV was examined in respiratory samples collected at recruitment and retrieved from the PMH Department of microbiology, using a two-step PCR amplification of the RV 5' non-coding region as previously described [2, 6, 24]. If positive, PCR products were DNA sequenced and RV species were determined by alignment with the RV sequence of 101 classical serotypes and the 53 recently identified types using phylogenetic tree reconstruction with Clustal X Software. The species assignment of these new types was previously confirmed by analysing the 420-bp VP4-VP2 sequences of the coding region of representative samples of each type [20, 25]. Further genetic analyses of all RV positive samples were done to make sure that each illness represented a new infection.

2.3. Statistical Analyses

Respiratory samples assessed before and after recruitment as well as children with and without a parental history of asthma and/or allergy, were analysed separately.

Within each group, children with RV, RV-A and RV-C were compared with those that had another virus or no virus detected at recruitment. Separate analyses were not performed on children with RV-B at recruitment due to the small numbers of children with this species. Co-infections were recorded separately; hence children with both RV and another virus at recruitment or at other hospital attendances were analysed as separate viral detections.

We used STATA version 13 and a Poisson regression model to determine the incidence rate ratio (IRR). The IRR compared the mean number of respiratory samples assessed and viruses detected (RV-A, RV-B, RV-C and other viruses (RSV, adenovirus, influenza virus A and B, parainfluenza 1, 2 and 3, and human metapneumovirus) during a study period (before and after recruitment) between recruitment samples with and without RV, RV-A or RV-C.

Models were adjusted for age, gender and observation period, to adjust for different length of follow up for each child according to recruitment time.

3. RESULTS

3.1. Study Population

Children with a wheezing exacerbation at recruitment (n=373) had a mean age of 4.5 years (standard deviation [SD] = 3.3, range 0.1–15.6) and were mostly male (60.1%) (Table **1**). The majority of children with acute wheeze had a moderate severity score (65.0%) and a parental history of asthma and/or allergy (61.9%). Of children with a genetic predisposition to asthma and/or allergy, most children had only one parent with asthma and/or allergy (78.4%), which was primarily maternal (53.0%) (Table **2**).

Table 1: Study Population Demographics

Study subjects, n	373 †
Gender male, n (%)	224 (60.1)
Age at recruitment in years, mean ± SD (range)	4.5 ± 3.3(0.1-15.6)
Season recruited, n (%)	
Spring	87 (23.3)
Summer	26 (7.0)
Fall	79 (21.2)
Winter	181 (48.5)

† If not otherwise stated.

Table 2: Clinical Data on Study Population at Recruitment

Diagnosis at recruitment, n (%)	309 (100)
Acute asthma exacerbation	177 (57.3)
Viral induced wheeze	75 (24.3)
Wheeze, exercise-induced or no specific trigger	7 (2.3)
Other respiratory diagnosis	50 (16.2)
Acute asthma severity score, n (%)	300 (100)
Mild (0–2)	35 (11.7)
Moderate (3–6)	195 (65.0)
Severe (7–10)	70 (23.3)
Parental history of asthma and/or allergy, n (%)	231 (61.9)
Uniparental	181 (78.4)
Mother	96 (53.0)
Father	85 (47.0)
Biparental	50 (21.6)

3.2. Respiratory Viruses Detected at Recruitment

A respiratory virus was detected in 79.9% of children recruited with a wheezing exacerbation (n=298

virus detected/373 children tested). The majority of cases had RV (69.2%, n=258/373) (Figure **1**), either as a single detection (61.1%, n=228/373) or co-detected with another respiratory virus (8.0%, n=30/373). A different respiratory virus was detected in 10.7% of children (n=40/373) of which RSV was the most common (7.8%, n=29/373). Of the 258 RV-positive respiratory specimens, all were successfully typed. The most common species was RV-C (65.5%, n=169/258), with RV-A and RV-B detected in 32.9% (n=85/258) and 2.3% (n=6/258), respectively. Dual detection of RV-A and RV-C occurred in two samples (recorded separately).

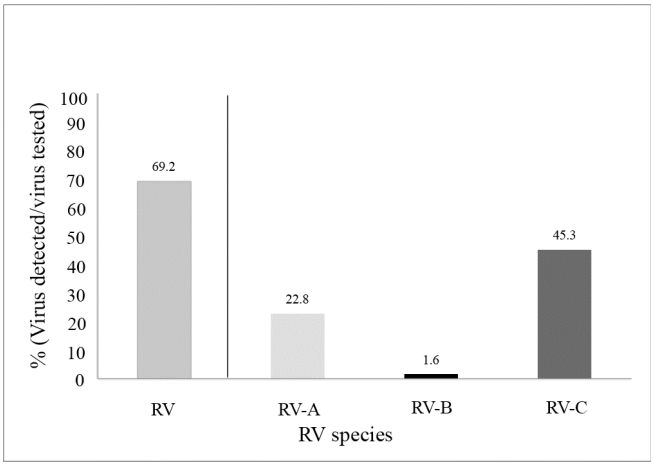

Figure 1: Percentage of RV species detected at recruitment n=298 viruses detected (373 children tested); two subjects had RV-A and C co-infection.

3.3. Respiratory Samples Assessed Longitudinally

Data from respiratory virus assessments completed by the PMH Department of Microbiology between May 1997 and December 2012 were used to determine the rate of respiratory viruses in these 373 children. The mean study duration of data collected before and after these children were recruited was 4.1 (SD 2.7) and 4.0 years (SD 3.0), respectively, and the mean age at the end of the study period was 8.4 years (SD 5.4) (Table **3**). Subjects had a higher mean number of respiratory samples assessed before than after recruitment (0.7 [SD 1.5] vs. 0.3 [SD 0.8], respectively). There was a correlation between age at recruitment and the mean number of respiratory samples assessed

Table 3: Follow-up Data

Age at end of follow up time in years, mean ± SD (range)	8.4 ± 5.4 (0.4–23.9)
Follow-up time before recruitment in years, mean ± SD (range)	4.1 ± 2.7 (0.1–3.0)
Follow-up time after recruitment in years, mean ± SD (range)	4.0 ± 3.0 (0.2–0.3)

before (β-coefficient = 0.6, 95% confidence interval (95% CI) 0.4–0.9; p<0.001) and after recruitment (*β*-coefficient = 0.5, 95% CI 0.3–0.6; p<0.001). For samples potentially available from the PMH Department of Microbiology over the previous 3.2 years (September 2009-December 2012), 64.1% samples were located (n=109 retrieved/170 potentially available respiratory samples) and most were collected after recruitment (59.6%, n=65/109). The proportion of samples tested for RV was therefore lower before recruitment (28.0%, n=77 RV tested/275 respiratory samples assessed) than after recruitment (59.3%, n=73/123).

3.4. Respiratory Viruses Detected Longitudinally

RV was the most common virus detected in virus-positive samples both before (55.0%, n=60 RV detected/109 virus positive samples) and after (71.0%, n=49/69) recruitment (Figure **2**). Of the RV-positive samples, 58.3% (n=35 typed/60 HRV positive)

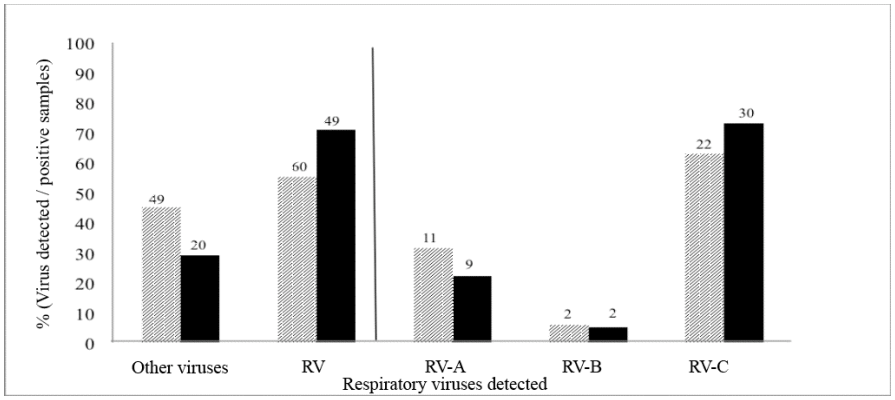

Figure 2: Percentage of viruses detected longitudinally. Numbers above columns refer to number of viruses detected; shaded columns: viruses detected before recruitment from n (respiratory samples) = 275; solid columns: viruses detected after recruitment from n (respiratory samples) = 123; other viruses: respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza 1,2 and 3, human metapneumovirus; RV (Rhinovirus).

assessed before and 83.7% (n=41/49) assessed after recruitment, were typed. RV-C was detected in the majority of typed samples both before (62.9%, n=22 RV-C/35 typed) and after recruitment (73.2%, n=30/41). RV-A was the next most common species, identified in 31.4% (n=11 RV-A/35 typed) of samples before and in 22.0% (n=9/41) of samples after recruitment.

3.5. Incidence Rate Ratio of Respiratory Sample Assessment and Viral Detection Longitudinally

3.5.1. Recurrent Sampling and Viral Detection before Recruitment

There was a trend towards an increased IRR for prior RV detection in children with a parental history of asthma and/or allergy who had RV at recruitment compared with children with no RV detected at recruitment (IRR 2.3, 95% CI 0.9–5.8; p=0.09). For all other analyses of children with and without parental asthma and/or allergy, there were no differences in the mean number of respiratory samples assessed or viruses detected before recruitment between children with and without RV, RV-A or RV-C at recruitment.

3.5.2. Recurrent Sampling and Viral Detection after Recruitment

Children with no parental history of asthma and/or allergy that had RV detected at recruitment had a lower rate of respiratory samples assessed (IRR 0.5, 95% CI 0.3–0.9; p=0.03) and a trend towards a lower incidence of other viruses detected (IRR 0.1, 95% CI 0.01–1.3; p=0.09) after recruitment than children without RV at recruitment (Figure **3**).

In the group of children with a parental history of asthma and/or allergy, subjects with RV at recruitment had a higher incidence of respiratory samples assessed (IRR 3.0, 95% CI 1.6–5.7; p=0.001) and a 14-fold increase in IRR for recurrent RV (IRR 14.0, 95% CI 1.9–104.1; p=0.01) relative to children without RV at recruitment. In addition, there was a trend towards a six-fold increase in IRR for detection of other viruses (IRR 6.6, 95% CI 0.9–50.3; p=0.07).

Furthermore, children in this high-risk group that had a RV-C wheezing exacerbation at recruitment had a trend towards more recurrent RV-C detections (IRR 3.2, 95% CI 0.9–11.4; p=0.07) following recruitment than children without RV-C at recruitment.

There were no other associations between RV, RV-A or RV-C detected at recruitment and samples assessed or viruses detected after recruitment.

4. DISCUSSION

This study revealed that children with a genetic predisposition to asthma and/or allergy that presented

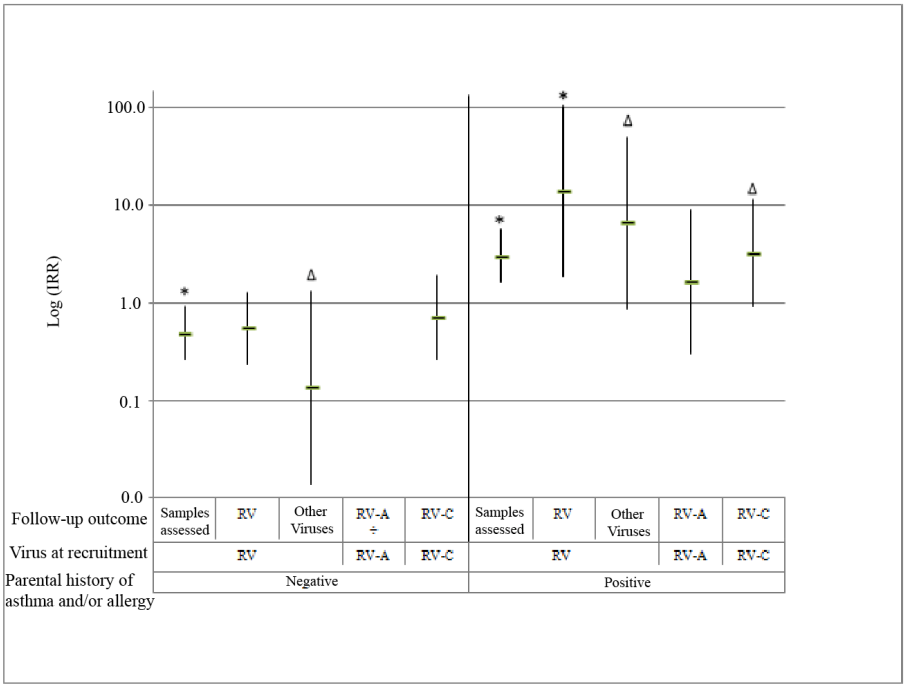

Figure 3: Incidence rate ratio for samples assessed and virus detection longitudinally, after recruitment. n study subjects = 373; Δ p < 0.1; * p < 0.05; ÷ result not presented in figure due to value = 0; this result is non-significant); other viruses: respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza 1, 2 and 3, human metapneumovirus; RV (Rhinovirus).

to hospital with RV-related wheezing were more likely to have subsequent viral testing and detection of RV than children without this predisposition.

To our knowledge, this is the first study to report the relationship between RV and RV species in children attending hospital with acute wheeze and repeated childhood RV detection and furthermore, to suggest that genetic predisposition may play a critical role in a child's propensity to develop severe recurrent viral infections.

We previously showed that RV-C is associated with the majority and most severe acute wheezing episodes in children presenting to hospital [2]. RV-C was also associated with subsequent respiratory hospital admissions [19] and this study found limited evidence of increased recurrent detection of RV-C in children who had attended hospital-based care. These findings suggest that children with a genetic predisposition to asthma and/or allergic disease and an RV-C related wheezing exacerbation could be more susceptible to further RV-C and that this RV species may induce more severe respiratory illnesses. It is most likely that an illness that necessitated sample collection and testing for respiratory viruses was more severe, thus viral detection is likely to have occurred as a result of a symptomatic infection. However, we cannot preclude that episodes of RV illness occurred at other times in hospital or the community but were of insufficient severity to prompt sample collection and thereby be recorded in this study.

The PMH Department of Microbiology does not routinely investigate respiratory samples for RV or RV strain. We retrieved most samples stored by the PMH Department of Microbiology over the previous three years from 2012. However lack of availability of further samples and missing results may contribute to the limited evidence of RV species-specific recurrence after recruitment and may also have masked association between RV or RV species detected at recruitment and an increased rate of viral detection before recruitment. The possibility that children at high risk for asthma and/or allergy are more susceptible to RV overall, can therefore not be precluded. Our findings suggest that increased routine testing for RV in children at high risk of asthma and/or allergic disease presenting to hospital with respiratory illnesses may repeatedly detect RV and thus identify children at high risk of developing recurrent wheezing exacerbations.

The association between RV detection and further respiratory illnesses in children genetically at high risk for developing allergic respiratory diseases has previously been reported [12, 13, 26-28]. Community-based cohort studies of children with parental history of atopy have demonstrated that early life aeroallergen sensitization predisposes children to subsequent RV-related wheezing illnesses [26] and wheezing with RV during infancy was associated with the subsequent development of asthma at age 5 in sensitized children [13]. Similar studies revealed that RV-induced wheezing episode in the first year of life was a significant risk factor for wheezing at 3 years of age, [27] and an increasing frequency of RV-related wheezing illnesses during the first 3 years of life was associated with development of asthma at age 6 [28], suggesting RV recurrence may impact asthma development.

Samples for the present study were collected from children in a hospital setting, suggesting more severe respiratory illnesses compared with the previously described cohorts. Furthermore, separate analyses were performed on children with and without a genetic predisposition to asthma and/or allergic disease, identifying a difference within the high-risk group but not the low risk group for several parameters investigated. To our knowledge, previous studies have not been able to distinguish the role of atopy on wheeze and RV infections since their recruitment was limited to children only at high-risk of atopy and asthma. The results of this study indicate that a parental history of asthma and/or allergy may be a critical factor in severe recurrent RV-induced wheezing illnesses in children. A limitation of the present study was the lack of clinical information associated with the assessment of respiratory samples; hence it remains unknown whether RV detections in samples collected at other times were associated with wheezing. RV can be detected in children without respiratory symptoms, implying that presence of RV does not always reflect symptomatic infection. However, samples in this study were collected in a hospital setting most likely as a result of more severe infection as they would prompt more comprehensive testing.

In a study of 7–12-year-old children with asthma, increasing levels of serum allergen-specific Immunoglobulin E (IgE) antibody were associated with a substantial increase in the probability of RV-related wheeze, indicating that atopy plays a role in modifying the host anti-viral responses, [29] consistent with our findings. Our analyses used parental history of asthma and/or allergy as a surrogate because children with this family history have an increased likelihood of

developing these conditions [30] independent of their age at the time of study. Thus we avoided potential misclassification of some children as non-atopic since their recruitment at a young age may have preceded their development of skin prick test positivity.

Studies on the association between allergic respiratory illnesses and RV detection rates in children are limited. Nasal samples collected from asthmatic children during five consecutive weeks for at least one RV peak season did not indicate that children with atopic asthma were more prone to have RV detected than non-atopic asthmatic children, although atopic asthmatics did experience more symptomatic infections [31]. Since we focused on children presenting to hospital with a wheezing exacerbation rather than children with asthma in the community, the differences in these study designs possibly influenced their findings. Moreover, our study was strengthened by several factors: a longer study period after recruitment (4.0 years versus 3 years), sample collection not subject to the systematic bias caused by subjects lost to follow-up (in the study by Olenec. et al. most participants completed only one season during the 3 year study time), a greater number of participants (373 versus 42 children) [31] and minimising selection bias for those children with known asthma and/or allergy being more likely to seek care in a prospectively study design.

This study included data from 1997 when the PMH Department of Microbiology records commenced, thus, older children recruited at the beginning of the study may have had respiratory samples collected and tested for viruses before this date. Also, the follow-up time both before and after recruitment varied according to when a child was enrolled in the study. We adjusted for the variations in longitudinal analyses by including the duration of follow-up for each participant before and after recruitment in the statistical analyses.

Previous studies have shown that RV is an important pathogen in children at high-risk of asthma and/or allergy [11-13, 17, 26-28] and our findings suggest that such predispositions may underpin the relationship between RV and recurrent viral infections. There is evidence that asthmatics with an atopic tendency have altered immune responses to RV infection [17]. We postulate that this altered antiviral response predisposes children to repeated viral infections, particularly with RV, that results in symptoms severe enough to warrant medical attention. Whether RV infections induce or are a consequence of immunological changes requires further study. We have found that species-specific RV-C antibodies are low in children infected with RV regardless of asthmatic status [32], suggesting a less efficacious antiviral immune response to this species. However, further assessment of RV-specific antibody responses will help determine if there are differences in protection following RV infection between children with and without atopy.

CONCLUSION

Children predisposed to asthma and/or allergy with an RV wheezing exacerbation may be prone to recurrent RV detection whereas children without this predisposition do not. These findings may improve our ability to identify children at heightened risk of recurrent infections and illnesses.

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LIST OF ABBREVIATIONS

RV: Rhinovirus.

IgE: Immunoglobulin E.

IRR: Incidence rate ratio.

PMH: Princess Margaret Hospital for Children.

RSV: Respiratory syncytial virus.

SD: Standard deviation.

95% CI: 95% confidence interval.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTIONS

SHH recorded data and obtained samples from the PMH Department of Microbiology, and was responsible for data analysis and manuscript drafting. GZ supported the data analysis. SKK and KMF performed molecular detection and typing assays on the additional samples obtained from PMH Department of Microbiology. JB and DWC recruited study subjects, and were involved in sample and data collection. KL and ADK provided information on the techniques and system used by the PMH Department of Microbiology and supervised the audit. JEG and YAB developed the RV identification methods that were used for typing the additional samples obtained from the PMH Department of Microbiology. JG, CSU and PNLS initiated the study and revised the manuscript. IAL was involved in the study design, study initiation and management, and revision of the manuscript. All authors read and approved the final manuscript.

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