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## Short communication

## The role of rhinovirus infections in young children with cystic fibrosis

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## ABSTRACT

Rhinovirus (RV) is an important virus in children with chronic respiratory conditions such as asthma; however, little is known about its role in CF. Our aim was to examine the prevalence and clinical impact of different RV species in young children with CF.

We collected clinical data and nasal swabs on patients at home and in the hospital setting. Parents filled out symptom diaries and collected nasal swabs when their children were symptomatic and asymptomatic. A novel RV typing PCR assay was used to determine the RV species present.

We collected 55 nasal swab samples from ten preschool CF patients over a six month period. The quality of parent collected samples at home was sufficient for PCR analysis. RV was the most common virus detected in young children with CF. There was no difference in the frequency of RV species between symptomatic and asymptomatic subjects. However, parental home-sampling is an acceptable and feasible approach to monitoring young children with CF.

## 1. Background

Cystic Fibrosis (CF) is the most common inherited genetic disease found in Caucasians [1]. CF lung disease is characterised by recurrent respiratory infections leading to progressive pulmonary injury and respiratory failure. Children with CF are born with structurally normal lungs; studies have shown that CF lung disease begins early in childhood [2–4]. The factors that drive its progression are multifactorial, involving both genetic and environmental factors, including the repeated exposure to respiratory infections over time [5].

The role pulmonary exacerbations due to bacterial infections play in the development of CF lung disease is well documented [6,7]. However, viruses are the major cause of acute respiratory illnesses in infants and young children, and there is increasing evidence that they play a role in the development of pulmonary exacerbations and CF lung disease in children with CF [8,9]. Additionally, rhinovirus (RV) has emerged as a significant pathogen in the development of asthma and recurrent wheeze in young children [10].

RV is prevalent and associated with pulmonary exacerbations in both adults and children with CF [9,12–14]. While the role of RV species is well studied in children with asthma [15,16], there is limited evidence on their clinical impact in children with CF [12,17]. Previous studies have used nasal swabs (NS) collected at home by parents.

However, the optimal sampling collection methodology in a community setting has not been fully validated in a young CF population [18].

## 2. Objectives

We conducted a pilot study to examine the prevalence and clinical impact of different RV species in young children with CF and to assess the feasibility of parental home-sampling in this population.

## 3. Study design

The study was conducted over a six month period (October 2015–April 2016) at Children's Health Ireland, Crumlin (CHI) in Dublin, Ireland. Children with CF under the age of six attending CHI were invited to participate. Ethical approval was obtained from the hospital ethics committee and informed consent was obtained from parents of participants.

Parents were taught how to take NS and provided with kits to collect samples and return by post. Flocked NS (eSwab, Copan™) containing a transport medium were used for this study. Parents were instructed to collect a nasal swab within 48 h of their child developing acute respiratory symptoms. If the sample was taken on a weekend day, then we requested that the parent would keep the specimen refrigerated until

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Monday and then send it in the post to us. They recorded the child's symptoms in a symptom diary for the duration of the study, including details on respiratory symptoms, absence from playschool, and treatment prescribed. We collated clinical information using the symptom diaries and medical records. NS were also collected at any scheduled or unscheduled hospital encounters by nursing staff. In addition, paired throat swabs as well as NS were collected by nursing staff if the parents reported symptoms at these hospital attendances.

Samples were initially stored at -80C at CHI and were then sent to the National Virus Reference Laboratory for analysis. A lab developed (LDT) real-time RT PCR targeting the 5'untranslated region of Enteroviruses (EV) and RV was used to detect the presence of RV in all samples [27,28]. Positive RV specimens were further characterised using a novel RV typing assay [19]. Nucleic acid was extracted using a Roche Magna Pure 96 (Roche, Switzerland). A semi-nested PCR was used to amplify a variable segment of the 5' untranslated region and sanger sequencing was performed. RIVM enterovirus online genotyping tool was used for characterisation [11]. The Luminex Respiratory Pathogen Panel RPP was used to test for the presence of other viral respiratory pathogens (Luminex Corp, USA).

Statistical analysis was performed with chi-squared testing to compare the presence of respiratory symptoms with RV detection using SPSS 24, IBM.

#### 4. Results

Ten participants were recruited to the study (50 % male). The median age was 33 months (range 3–54 months). Half (50 %) of the cohort was homozygous for the  $\Delta$ F508 genotype. Over the six month period, 55 NS were collected, 39 (70.9 %) of which were taken from symptomatic subjects and 16 (29.1 %) from asymptomatic at the time of sampling. Rhinorrhoea, cough, and wheeze were the most common respiratory symptoms reported in the symptom diaries. The median number of samples collected per subject over the study period was  $6.0 \pm 1.54$  (range 3–8). Of the 55 NS collected, 55/55 (100 %) were adequate for viral PCR analysis.

Rhinorrhoea was the most common symptom recorded in the symptom diaries and the symptom was logged by parents in 85.7 % of encounters. Cough was the next most frequently recorded symptom, reported in 67.9 % of encounters. Other common symptoms reported were wheeze (25 %) and fever (14.2 %).

RV was the most frequent virus detected in both symptomatic and asymptomatic subjects, being identified at least once in 80 % of subjects. While RV was present in 21/55 (38.2 %) nasal samples, it was detected in 14/39 (35.9 %) samples taken when the child was symptomatic (Table 1). The next most commonly identified viruses were: coronavirus in 5/39 (12.8 %); RSV in 3/39 (7.7 %); and parainfluenza in 2/39 (5.1 %). Other viruses were detected in 4/39 (10.3 %), and no virus was detected in 11/39 (28.2 %) cases. Dual infections were identified in 4/39 (10.3 %) cases.

RV typing was successful on 11/14 (78.6 %) RV positive samples on symptomatic subjects (Table 1). RV-A was the commonest RV species detected, being present in 5 (35.8 %) of the samples, RV-C was found in

**Table 1**  
Microbiology results of symptomatic versus asymptomatic subjects.

	Symptomatic (n = 39)	Asymptomatic (n = 16)
RV positive	14 (35.9)	7 (43.8)
RV-A	5/14 (35.8)	0/7 (0.0)
RV-B	2/14 (14.3)	1/7 (14.3)
RV-C	4/14 (28.6)	4/7 (57.1)
RV species (un-typed)	3/14 (21.4)	2/7 (28.6)
Other virus	14 (35.8)	0 (0)
No virus	11 (28.2)	9 (56.3)
Bacterial culture positive	10/17 (58.8)	4/16 (25.0)

4 (28.6 %), RV-B in two (14.3 %), and three (21.4 %) samples had an RV that could not be typed.

In asymptomatic subjects, RV was identified in the 7/16 (43.8 %) of samples collected; RV-C was identified in 4 (57.1 %), RV-B in 1 (14.3 %), RV-A in none, and 2 (28.6 %) samples had an RV that could not be typed (Table 1). No virus was detected in 9 (56.2 %) subjects

Re-infection with a different RV species was seen in 40 % of subjects. The median time to reinfection in subjects with another RV species was  $40 \pm 39.7$  (range 28–138) days. The same RV species was seen in one subject four weeks after their initial swab was collected and symptoms had resolved.

Paired throat swabs for bacterial culture were collected on 17/39 (43.6 %) symptomatic subjects. Only 10/17 (58.8 %) samples had a significant bacterial growth on culturing. *Staphylococcus aureus* was the most common bacterium identified, representing 5/10 (50 %) samples. *Haemophilus influenzae* 3/10 (30 %) and *Streptococcus pneumoniae* 2/10 (20 %) were the other bacteria detected on cultures. In subjects who were symptomatic, 9/17 (52.9 %) were treated with a two week course of antibiotics.

In asymptomatic children, 4/16 (25 %) samples had a significant bacterial growth on culturing. *Staphylococcus aureus* was the most common bacterium detected, seen in 3/4 (75 %) of samples and *Haemophilus influenzae* was detected in one (25 %) sample.

In all subjects, the median number of symptomatic days was  $3.0 \pm 3.73$ , range 1–15. There was no difference in the median number of days of symptoms for virus positive patients ( $3.0 \pm 3.4$ , range 2–12 days) versus virus negative patients ( $3.5 \pm 4.53$ , range 1–15 days). RV positive subjects were not more likely to have symptoms when compared with RV negative subjects (chi-square = 1.01,  $p = 0.31$ ). Although RV-A was detected in 35.8 % of samples from symptomatic subjects, and in 0% of samples from asymptomatic subjects, numbers were not sufficient for further statistical analysis on whether there was a difference between RV species in relation to symptoms.

#### 5. Discussion

This pilot study demonstrated the feasibility of parents collecting viable samples repeatedly from children with CF in their home environment. The high microbiological yield from home sampling and the use of up-to-date molecular RV typing methods are significant strengths of the study. Our study demonstrated that 100 % of the samples collected were of good quality which is comparable to other similar studies [17]. However, other studies have reported issues with the transport and viability of samples on arrival by mail [18,26] that we did not experience.

This study suggests that although RV is the most prevalent virus in young children with CF, it is not always associated with symptomatic infections. Previous studies also found a high prevalence of RV infections in asymptomatic individuals with CF [9,20,21]. Others have suggested that RV more frequently and persistently infects children with CF when compared with healthy controls [21]. This may be because of diminished antiviral defences in CF patients [22,23].

RV-A was the most common species identified in symptomatic subjects. However, little data exists on the prevalence of RV species in children with CF [12,17,24]. Previous studies indicate that RV-C is associated with more severe and frequent asthma and respiratory exacerbations in CF [15–17,25] while another identified RV-A as the major RV species in CF exacerbations [12]. The prevalence rates of different RV species require further investigation in larger cohorts using methods to identify pathogenicity of different viruses.

In conclusion, while the small number of subjects studied is a limitation of the study, our reported prevalence rates are similar to larger studies of similar cohorts [9,20,21]. Significantly, this is the first study in Ireland to report data on the prevalence of different RV species in an Irish CF cohort using molecular RV typing methods and parent-led sampling, demonstrating the feasibility of these methods for studies in

the future.

### CRedit authorship contribution statement

**D.W. O Loughlin:** Investigation, Writing - original draft, Writing - review & editing. **S. Coughlan:** Formal analysis, Writing - original draft, Conceptualization. **C.F. De Gascun:** Supervision, Writing - review & editing. **P. McNally:** Formal analysis, Conceptualization. **D.W. Cox:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Conceptualization.

### Declaration of Competing Interest

None.

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