JCM Journals.ASM.org

CASE REPORT

Chronic Rhinovirus Infection in an Adult with Cystic Fibrosis

William G. Flight,[a,b] Rowland J. Bright-Thomas,[a,b] Peter Tilston,[c] Kenneth J. Mutton,[b,c] Malcolm Guiver,[c] A. Kevin Webb,[a,b] Andrew M. Jones[a,b]

Manchester Adult Cystic Fibrosis Centre, University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom[a]; Institute of Inflammation & Repair, University of Manchester, Manchester, United Kingdom[b]; Department of Virology, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom[c]

Rhinovirus is a common cause of exacerbations of cystic fibrosis (CF) and is usually considered a self-limiting infection. We report a case of chronic infection with rhinovirus A type 33 in a 43-year-old male with CF which has persisted for over 2 years.

CASE REPORT

A 43-year-old man with cystic fibrosis (CF) was diagnosed with chronic rhinovirus A infection through a combination of PCR assays and genetic sequencing. His CF genotype was F508del/G542X, and he had moderate to severe CF-related lung disease (forced expiratory volume in 1 s, 42% [predicted at baseline]). The patient was known to have allergic bronchopulmonary aspergillosis (ABPA) and chronic endobronchial infection with *Pseudomonas aeruginosa*. He had received high-dose inhaled corticosteroids (fluticasone at 1,000 μg per day) and oral itraconazole (100 mg) twice daily for the previous 4 years.

In January 2011, the patient enrolled in an observational study investigating the role of respiratory viruses in adults with CF (1). At recruitment to the study, he was clinically stable and had no symptoms suggestive of an acute respiratory tract infection. In-house PCR assays for a total of nine respiratory viruses were performed on sputum and nose and throat swab specimens following total nucleic acid extraction using a QIAamp Virus Biorobot MDx instrument (Qiagen, Hilden, Germany). The rhinovirus PCR assay targeted the 5′ noncoding region in line with the method reported by Scheltinga et al. (2). Details of the primers and probes are given in Table 1. The sputum sample was PCR positive for rhinovirus, with a cycle threshold value of 33 cycles. Both upper-airway swabs were negative for viral pathogens.

During the following 12 months, the patient provided respiratory samples for virological analysis on a total of eight occasions. At seven of the study visits, rhinovirus was identified in at least one respiratory tract sample (see Table 2). On the eighth occasion, nose and throat swabs were negative for rhinovirus but the patient was unable to expectorate sputum. The patient's clinical course during this period was complicated by the diagnosis of adrenal insufficiency in August 2011. Itraconazole treatment was stopped, and the dose of inhaled fluticasone was reduced to 400 μg daily. Replacement oral hydrocortisone was commenced and has continued to the present day.

In March 2013, more than 2 years after its initial identification, the patient provided a further sputum sample which was again PCR positive for rhinovirus. Overall, only three of the patient's rhinovirus-positive episodes were associated with symptoms of an upper respiratory tract infection and just one met predefined criteria for a pulmonary exacerbation of CF lung disease (3).

Genetic sequencing of the rhinovirus 5′ untranslated region was performed in five of the patient's specimens using an Applied Biosystems 3130xl Genetic Analyzer and following the method reported by Lee et al. (4). Details of the primers used

TABLE 1 Primers and probes used in the rhinovirus PCR and sequencing assays

Assay, primer, or probe	Sequence
Rhinovirus in-house PCR	
Forward primer 1	GACARGGTGTGAAGAGCC
Forward primer 2	GACATGGTGTGAAGACYC
Reverse primer	CAAAGTAGTYGGTCCCATCC
Probe	VIC-TCCTCCGGCCCCTGAAT GYGGCTAA-TAMRA[a]
Rhinovirus sequencing	
Forward primer P1 (all steps)	CAAGCACTTCTGTYWCCCC
Reverse primer P3 (first-round PCR assay)	ACGGACACCCAAAGTAG
Reverse primer P2-1 (seminested PCR and sequencing reaction)	TTAGCCACATTCAGGGGC
Reverse primer P2-2 (seminested PCR and sequencing reaction)	TTAGCCACATTCAGGAGCC
Reverse primer P2-3 (seminested PCR and sequencing reaction)	TTAGCCGCATTCAGGGG

[a] TAMRA, 6-carboxytetramethylrhodamine.

Received 20 June 2013 Returned for modification 14 July 2013
Accepted 15 August 2013
Published ahead of print 21 August 2013
Address correspondence to William G. Flight, williamflight@hotmail.com.

doi:10.1128/JCM.01604-13

TABLE 2 Serial rhinovirus PCR results and symptomatology from an adult with cystic fibrosis[a]

Date of specimen collection	Symptom(s) of URTI?	Pulmonary exacerbation?	Rhinovirus PCR result (cycle threshold value [specimen no.])			Sample sequenced?
			Nose swab	Throat swab	Sputum	
21 January 2011	No	No	Neg	Neg	Pos (33)	No
4 March 2011	Yes	Yes	Pos (41 [M11914546])	Pos (38 [M11914547])		Yes
3 June 2011	No	No	Neg	Neg	Pos (34)	No
8 July 2011	No	Yes	Neg	Neg		No
31 August 2011	Yes	No	Neg	Neg	Pos (33)	No
2 November 2011	No	No	Neg	Neg	Pos (31 [M11960169])	Yes
30 November 2011	Yes	No	Neg	Neg	Pos (34)	No
4 January 2012	No	No	Pos (39)	Neg	Pos (35 [M12890805])	Yes
21 March 2013	No	No			Pos (35 [M13907462])	Yes

[a] Specimen identity numbers beginning with "M" identify the samples shown in Fig. 1. All samples from the patient were PCR negative for adenovirus, influenza A and B virus, metapneumovirus, parainfluenza 1 to 3, and respiratory syncytial virus. URTI, upper respiratory tract infection; Neg, negative; Pos, positive.

are shown in Table 1. Sequencing was conducted in two batches, with four of the patient's samples analyzed in batch one and the other in batch two (sample number M13907462). The remaining rhinovirus-positive specimens collected from the patient were unfortunately not available at the time the sequencing was performed. A number of steps were taken to minimize cross-contamination in the laboratory during the sequencing analysis. Full aseptic precautions were taken throughout, and separate laboratories were used for nucleic acid extraction, PCR reagent preparation, and steps involving the handling of amplified DNA.

The five sequenced samples from the patient represented four discrete episodes over a period of 24 months. Each of the sequences was consistent with the presence of *Human rhinovirus A*, and phylogenetic analysis suggested that they all belonged to the same strain. Three samples (M11914547, M11960169, and M12890805) were identical at the nucleotide level, with a maximum divergence of 1% across all five specimens. There was 98% similarity between the sequences of the isolates from the patient and the sequence of rhinovirus A serotype 33 reported by Lu et al. (NCBI GenBank accession number EU096020.1) (5). Figure 1 shows a phylogenetic tree to demonstrate the genetic relatedness of the patient's samples to the reference rhinovirus strains reported by Lee et al. (4) and to samples from other patients in the Manchester cohort (sequences beginning M but without the symbol ◆ in Fig. 1). The tree was constructed in MEGA 5.1 using the neighbor-joining method with bootstrap values of 500. The findings of this analysis are consistent with the presence of chronic lower respiratory infection with rhinovirus A type 33.

We have demonstrated for the first time that rhinovirus A has the potential to cause chronic infection of the respiratory tract in a patient with CF. This observation is of importance to the understanding of virus-induced exacerbations of CF lung disease and raises further questions as to the true meaning of rhinovirus positivity in the respiratory tract.

Rhinovirus belongs to the family *Picornaviridae*, and among members of the general population, it is the major cause of the common cold. Rhinovirus is also the principal viral pathogen affecting patients with CF and is associated with significant morbidity in this population (6–8). Adults with CF experience an average of at least 1.6 cases of rhinovirus infection per year (1). At present, the typical duration of rhinovirus PCR positivity in patients with CF is not known but some information can be inferred from other populations. Data from a cohort of children at risk of asthma who had acute rhinovirus infection suggests that in the vast majority of cases, PCR positivity lasts less than 2 weeks (9). Other investigators have found that rhinovirus infections may persist for up to 6 weeks in a proportion of children with asthma (10).

Chronic rhinovirus infection, however, appears rare. All such cases documented in the literature relate to patients with a significant degree of immunosuppression. Three individuals who had undergone lung transplantation, for instance, have been reported as having persistence of rhinovirus in the respiratory tract for over 12 months (11, 12). Patients with hypogammaglobulinaemia also appear to be prone to prolonged rhinovirus shedding, with PCR positivity continuing for up to 55 days in a small group of such patients compared with 15 days in healthy individuals (13).

Chronic rhinovirus infection has not been reported in CF before, although we have previously described a case of prolonged influenza A/H1N1 infection in another adult CF patient receiving maintenance oral corticosteroids (14). The patient reported here was taking high-dose inhaled corticosteroids and itraconazole, a combination which is known to enhance serum levels of corticosteroids and lead to adrenal suppression (15). Chronic rhinovirus infection persisted after itraconazole administration was stopped and the dose of inhaled fluticasone was reduced. It is possible that an iatrogenic excess of corticosteroids led to a degree of immunosuppression and contributed to the failure of our patient to clear the initial rhinovirus infection.

This case report raises a number of important questions for future research. First, it is currently unclear how long the average CF patient harbors rhinovirus after an acute infection. Potential risk factors for prolonged carriage of respiratory viruses, which might include the use of systemic corticosteroid therapy or coinfection with bacterial pathogens such as *Pseudomonas aeruginosa* (16), need to be identified. Another uncertainty is whether chronic viral infection actively contributes to the cycle of airway inflammation characteristic of CF or whether it is simply a marker of disease severity. Finally, it is not known whether CF patients with chronic respiratory virus

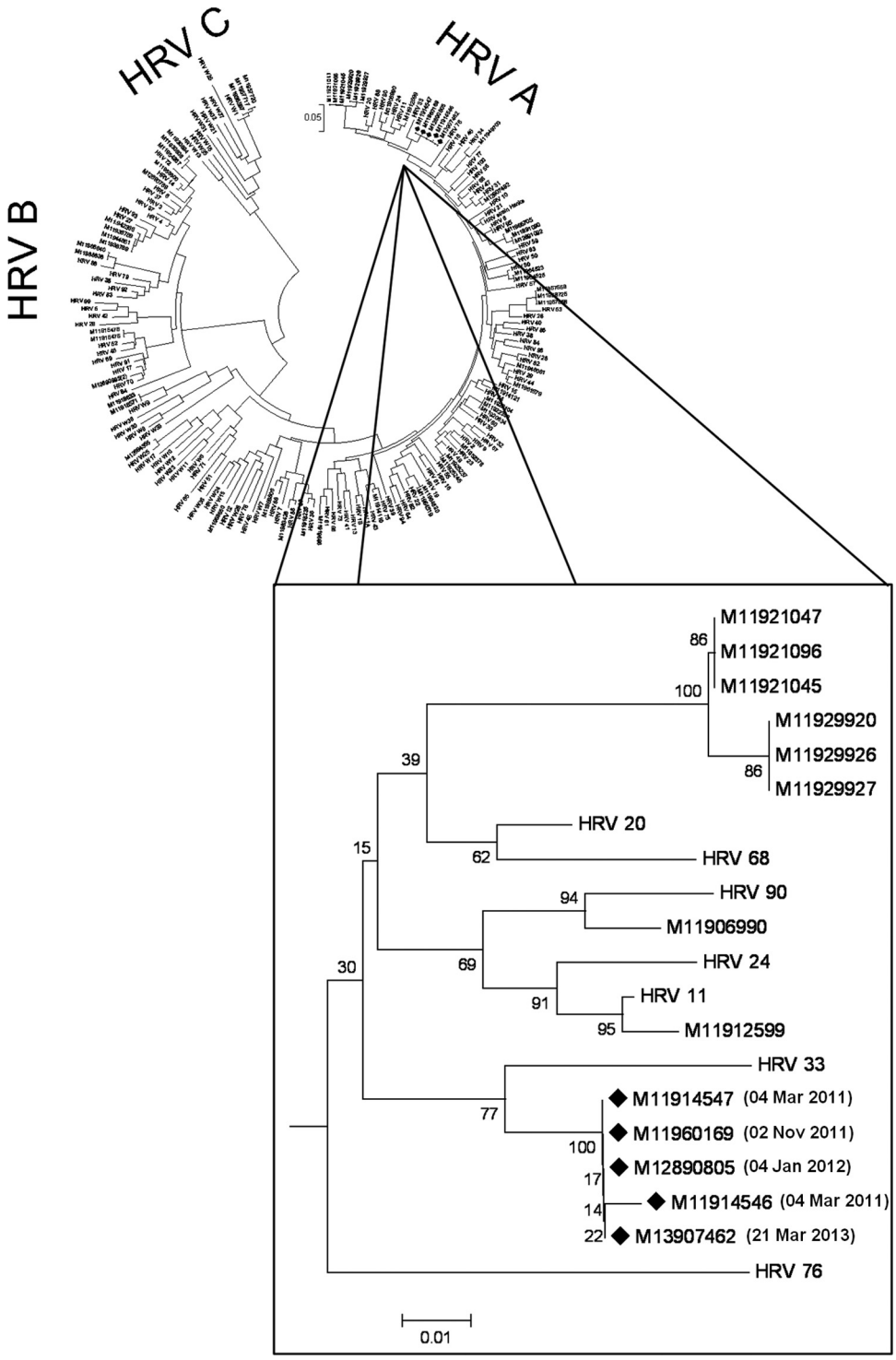

FIG 1 Phylogenetic tree to illustrate the persistence for more than 2 years of a strain of rhinovirus A in an adult with CF. Sequences marked with a diamond symbol (◆) indicate samples from the patient described in this report and had a genomic length of 287 bases. Other sequences marked "M" relate to different patients from our CF clinic. The two clusters of three identical sequences indicated at the top of the figure (from M11921047 to M11929927) relate to separate patients who were positive for the same strain of rhinovirus in sputum and nose and throat swabs collected at the same visit. HRV, human rhinovirus.

infection are contagious and represent an infection control risk. Until such issues are addressed, this case report serves to highlight that CF lung disease may be complicated by chronic respiratory infection with viruses as well as bacterial and fungal pathogens.

REFERENCES

1. **Flight WG, Mutton K, Webb AK, Bright-Thomas RJ, Jones AM.** 2012. The incidence of respiratory virus infection in adults with cystic fibrosis. Pediatr. Pulmonol. 47(Suppl 35):315.
2. **Scheltinga SA, Templeton KE, Beersma MF, Claas EC.** 2005. Diagnosis

of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. J. Clin. Virol. **33:**306–311.
3. **Fuchs H, Borowitz D, Christiansen D, Morris E, Nash M, Ramsey B, Rosenstein B, Smith A, Wohl M.** 1994. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N. Engl. J. Med. **331:**637–642.
4. **Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, Jakiela B, Lemanske RF, Shult PA, Gern JE.** 2007. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. PLoS One **2:**e966. doi:10.1371/journal.pone.0000966.
5. **Lu X, Holloway B, Dare RK, Kuypers J, Yagi S, Williams JV, Hall CB, Erdman DD.** 2008. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. J. Clin. Microbiol. **46:**533–539.
6. **Collinson J, Nicholson K, Cancio E, Ashman J, Ireland D, Hammersley V, Kent J, O'Callaghan C.** 1996. Effects of upper respiratory tract infections in patients with cystic fibrosis. Thorax **51:**1115–1122.
7. **Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierrepoint M, Evans R, Doull I.** 2008. The role of respiratory viruses in cystic fibrosis. J. Cyst. Fibros. **7:**320–328.
8. **van Ewijk BE, van der Zalm MM, Wolfs TF, Fleer A, Kimpen JL, Wilbrink B, van der Ent CK.** 2008. Prevalence and impact of respiratory viral infections in young children with cystic fibrosis: prospective cohort study. Pediatrics **122:**1171–1176.
9. **Jartti T, Lee WM, Pappas T, Evans M, Lemanske RF, Gern JE.** 2008. Serial viral infections in infants with recurrent respiratory illnesses. Eur. Respir. J. **32:**314–320.
10. **Kling S, Donninger H, Williams Z, Vermeulen J, Weinberg E, Latiff K, Ghildyal R, Bardin P.** 2005. Persistence of rhinovirus RNA after asthma exacerbation in children. Clin. Exp. Allergy **35:**672–678.
11. **Kaiser L, Aubert JD, Pache JC, Deffernez C, Rochat T, Garbino J, Wunderli W, Meylan P, Yerly S, Perrin L, Letovanec I, Nicod L, Tapparel C, Soccal PM.** 2006. Chronic rhinoviral infection in lung transplant recipients. Am. J. Respir. Crit. Care Med. **174:**1392–1399.
12. **Tapparel C, Cordey S, Junier T, Farinelli L, Van Belle S, Soccal PM, Aubert JD, Zdobnov E, Kaiser L.** 2011. Rhinovirus genome variation during chronic upper and lower respiratory tract infections. PLoS One **6:**e21163. doi:10.1371/journal.pone.0021163.
13. **Peltola V, Waris M, Kainulainen L, Kero J, Ruuskanen O.** 2013. Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia. Clin. Microbiol. Infect. **19:**E322–E327.
14. **Flight WG, Bright-Thomas R, Mutton K, Webb K, Jones A.** 2011. Persistent oseltamivir-resistant pandemic influenza A/H1N1 infection in an adult with cystic fibrosis. BMJ Case Reports **2011:**bcr0220113874. doi: 10.1136/bcr.02.2011.3874.
15. **Gilchrist FJ, Cox KJ, Rowe R, Horsley A, Webb AK, Jones AM, Bright-Thomas RJ.** 2013. Itraconazole and inhaled fluticasone causing hypothalamic-pituitary-adrenal axis suppression in adults with cystic fibrosis. J. Cyst. Fibros. **12:**399–402.
16. **Chattoraj SS, Ganesan S, Faris A, Comstock A, Lee WM, Sajjan US.** 2011. Pseudomonas aeruginosa suppresses interferon response to rhinovirus infection in cystic fibrosis but not in normal bronchial epithelial cells. Infect. Immun. **79:**4131–4145.