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Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis

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Abstract

Background: Human rhinoviruses (HRVs) are some of the earliest identified and most commonly detected viruses associated with acute respiratory tract infections (ARTIs) and yet the molecular epidemiology and genomic variation of individual serotypes remains undefined.
Objectives: To molecularly characterise a novel HRV and determine its prevalence and clinical impact on a predominantly paediatric population.
Study design: Nucleotide sequencing was employed to determine the complete HRV-QPM coding sequence. Two novel real-time RT-PCR diagnostic assays were designed and employed to retrospectively screen a well-defined population of 1244 specimen extracts to identify the prevalence of HRV-QPM during 2003.
Results: Phylogenetic studies of complete coding sequences defined HRV-QPM as a novel member the genus *Rhinovirus* residing within the previously described HRV-A2 sub-lineage. Investigation of the relatively short VP1 sequence suggest that the virus is resistant to Pleconaril, setting it apart from the HRV A species. Sixteen additional HRV-QPM strains were detected (1.4% of specimens) often as the sole micro-organism present among infants with suspected bronchiolitis. HRV-QPM was also detected in Europe during 2006, and a closely related virus circulated in the United States during 2004.
Conclusions: We present the molecular characterisation and preliminary clinical impact of a newly identified HRV along with sequences representing additional new HRVs.

Keywords: Rhinovirus; ARTI; LRTI; Newly identified virus; Respiratory infection; Phylogeny; Characterisation; RT-PCR; Epidemiology

1. Introduction

Acute respiratory tract infections (ARTIs) are a leading cause of human morbidity worldwide and are most frequently viral in origin. Many newly identified viruses have recently been associated with ARTI, including human metapneumovirus (HMPV; van den Hoogen et al., 2001), human coronaviruses (HCoV) NL63 (van der Hoek et al., 2004) and HKU1 (Woo et al., 2005), human bocavirus (HBoV; Allander et al., 2005) and the KI and WU polyomaviruses (Allander et al., 2007; Gaynor et al., 2007).

Human rhinoviruses (HRVs) are the most common infectious agents associated with ARTIs, comprising the largest genus of human pathogens, *Rhinovirus*, in the family *Picornaviridae*. Despite a significant and growing body of evidence

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to implicate HRVs in more serious clinical outcomes (Gern and Busse, 1999; Hayden, 2004), the presence and role of more than 100 genetically and serologically distinct HRVs in illness is often unexplored. Additionally, the viruses themselves are not considered to be individual entities with distinct circulation patterns as other respiratory viruses are. Studies have produced unusual HRV-like sequences or made cursory reports of untypeable or variant HRV strains in the United Kingdom (Mori and Clewley, 1994), Switzerland (Deffernez et al., 2004), Finland (Savolainen et al., 2002), Belgium (Loens et al., 2006), Australia (Arden et al., 2006) and the United States (Lamson et al., 2006). Many of these strains may represent novel HRV serotypes.

During an investigation of picornavirus PCR products we identified a cluster of more than 30 nucleotide sequences demonstrating <20% identity with any sequence on GenBank (Arden et al., 2006). We proposed that these belonged to a novel genetic sub-lineage, HRV-A2. We further investigated one of these putative viruses and herein present the complete polyprotein coding sequence of a novel HRV, HRV-QPM, which was first detected in an infant with bronchiolitis.

2. Materials and methods

2.1. Specimen population

Clinical specimens ($n = 1244$) were predominately nasopharyngeal aspirates (NPA; 92%) collected from patients (52.9% male) aged between 1 day and 80 years (mean age 9.2 years, median age 1.3 years) who had presented to Queensland hospitals or general practitioners with symptoms of ARTI during 2003. NPAs and purified nucleic acid extracts were stored at −70 °C. Extracts were tested by RT-PCR (OneStep, QIAGEN®) for HMPV, HRVs and enteroviruses (HEVs), all four non-SARS HCoVs, HBoV, respiratory syncytial virus (HRSV), influenza A and B viruses, parainfluenzaviruses and human adenoviruses (Arden et al., 2006).

2.2. Reverse-transcription and PCR

Real-time RT-PCR employed 0.2–0.4 pmol of oligonucleotide. Amplicons were purified when necessary and both strands sequenced (Big Dye v3.1, Applied Biosystems). Real-time RT-PCR was performed on the Rotor-Gene™ 3000 (Corbett Research).

First strand cDNA synthesis (Transcriptor RT, Roche) using $(T)_{17}$ATA or gene specific primers was followed by second strand synthesis and amplification (Platinum® *Pfx*, Invitrogen). Oligonucleotide sequences are available upon request.

2.3. Cell culture

Human cell lines (HeLa-Ohio, A549, MRC-5 and WI-38) were inoculated with patient respiratory secretions stored at −70 °C. Inoculated cells were rolled at 33 °C, watched daily for cytopathic effect and HRV-QPM RNA levels were monitored by real-time RT-PCR.

2.4. Phylogenetic diversity

Nucleotide or amino acid sequences were aligned with the program MUSCLE (Edgar, 2004) and, where necessary, backtranslated with the program Protogene (Moretti et al., 2006). Maximum likelihood trees were constructed using a $GTR + I + \Gamma_4$ model of nucleotide substitution in PAUP* (Swofford, 2003), with outgroups as indicated. Trees were visualized with TreeEdit (http://evolve.zoo.ox.ac.uk/software.html). The nucleotide distance matrix for neighbour-joining trees was generated using the Kimura two-parameter estimation in MEGA 3.1 (Kumar et al., 2004). Nodal confidence values indicate the results of bootstrap resampling ($n = 1000$).

2.5. Determination of the complete HRV-QPM coding sequence

Initial HRV-QPM sequences were used to design new PCR primers permitting determination of the continuous coding sequence from overlapping fragments, bracketed by partial untranslated regions (UTRs). Additional primers from HRV (Loens et al., 2006) and HEV (Caro et al., 2001; Oberste et al., 2004; Simmonds and Welch, 2006) studies supplemented the process. To confirm that our primary sequence belonged to a distinct virus and was not the result of mixed template in the patient's specimen, a second round of RT-PCR and sequencing of overlapping fragments was performed using newly designed HRV-QPM-specific primers targeting RNA from the original specimen extract. Discrepant sequence results were confirmed by a third round of RT-PCR and sequencing.

3. Results

3.1. Detection of HRV-QPM in Brisbane, Australia

To determine whether HRV-QPM was a distinct pathogen endemically circulating in Brisbane, 1244 specimen extracts spanning all of 2003 were examined (Fig. 1). HRV-QPM 1B RNA was detected in 1.4% ($n = 17$) of extracts by real-time RT-PCR and verified using a confirmatory assay targeting the 1D region. HRV-QPM prevalence peaked in winter but was also detected in spring and summer. No other micro-organisms were detected in 64.7% ($n = 11$) of positives (Table 1). There were six co-detections with: HCoV-NL63, HCoV-NL63 and HBoV, HBoV, HMPV or HCoV-229E.

Overall respiratory picornaviruses were the most frequently detected micro-organisms at 41.0% of microbial detections ($n = 346$) followed by HBoV (12.0%,