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No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease

P. Norja [a,1], I. Ubillos [b], K. Templeton [b], P. Simmonds [a,*]

[a] Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, United Kingdom
[b] Specialist Virology Centre, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, United Kingdom

Abstract

Background: WU virus (WUV) and KI polyomavirus (KIPyV) are newly discovered related human polyomaviruses detected in respiratory samples. To investigate their potential role in respiratory disease, we determined their frequencies of detection, clinical presentations and epidemiological characteristics among samples referred for diagnostic respiratory virus testing.
Methods: Anonymised samples and accompanying study subject information were obtained from the Edinburgh respiratory specimen archive. Samples were screened by nested PCR using two sets of primers conserved between WUV and KIPyV, as well for other respiratory viruses (respiratory syncytial virus [RSV], adenoviruses [AdV], influenza A/B and parainfluenza viruses 1–3, human bocavirus, B19).
Results and Conclusions: WUV and KIPyV were detected in 10 and 14 samples, respectively from 983 specimens (from 9 to 10 different individuals from 612 study subjects). Infections occurred in two types of study subject; those who were young (<2 years) with lower respiratory tract infections ($n = 8$), and almost invariably co-infected with other respiratory viruses (RSV, AdV), and a second, generally older group either without respiratory disease ($n = 6$) or with mild upper respiratory tract infections ($n = 5$) but who were generally clinically severely immunosuppressed from leukaemia or transplant therapy. Findings from either group do not support an aetiological link between infection with WUV or KIPyV and respiratory disease.
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1. Introduction

Virus discovery programmes based on molecular cloning have been increasingly successful in the identification and genetic characterisation of a wide range of novel viruses infecting humans and animals. Examples of viruses infecting humans discovered by such techniques include the parvoviruses, human bocavirus (HBoV) and PARV4 (Allander et al., 2005; Jones et al., 2005), anelloviruses (Nishizawa et al., 1997; Jones et al., 2005), hepatitis G virus or GB virus C (Linnen et al., 1996; Leary et al., 1996), following on from earlier discoveries of hepatitis C virus (HCV) and human herpesvirus 8 (HHV-8) (Choo et al., 1989; Chang et al., 1994). While in many cases their discovery could be directly linked to a specific disease syndromes (non-A, non-B hepatitis for HCV; Kaposi's sarcoma for HHV-8), others have much more tenuous links with human disease. Indeed, subsequent investigations of their epidemiologies, transmission routes and disease associations have often proven to be major challenges. There is an ongoing effort, for example, to determine precisely whether HBoV is a significant cause of childhood respiratory disease, why it is so often co-detected with other respiratory pathogens, and whether it exclusively a respiratory virus (reviewed in (MacIntosh, 2006; Mackay, 2007). Disease associations of PARV4 are even less well defined (Simmonds et al., 2007).

Recently, two new human polyomaviruses, WU virus (WUV) and KI polyomavirus (KIPyV) were cloned out of respiratory tract specimens (Allander et al., 2007; Gaynor et al., 2007). They are related members of a new group (or possibly sub-family) within the *Polyomaviridae*, small DNA viruses with circular, covalently closed double-stranded DNA genomes infecting a wide range of mammalian and avian species. Polyomaviruses show narrow host specificities, and frequently establish persistent, lifelong infections characterised by highly restricted or latent infection. Infections with the two known human polyomaviruses, BK virus (BKV) and JC virus (JCV) are acquired through the respiratory routes at a young age, achieving seroprevalences of 75% or greater by adulthood (Stolt et al., 2003). Both spread systemically and establish sites of persistent infection in the kidneys and the central nervous system in the case of JCV. Disease associations of BKV and JCV are rare and are almost invariably associated with immunodeficiency. Lack of immune control of virus replication and the consequent greater likelihood of acquiring mutations in transcription control regions (Ault and Stoner, 1993; Chatterjee et al., 2000) triggers unregulated replication leading to progressive multifocal leukoencephalopathy by JCV, and renal disease by BKV (Coleman et al., 1978; de Silva et al., 1995).

WUV and KIPyV DNA sequences have been frequently detected in respiratory specimens (Gaynor et al., 2007; Bialasiewicz et al., 2007; Allander et al., 2007), and their aetiological role in childhood respiratory disease has been proposed (Gaynor et al., 2007; Bialasiewicz et al., 2007). Investigations to date have been based on large scale screening of respiratory samples referred for diagnostic testing from patients generally with moderate to severe respiratory disease. It is, however, difficult to assess the frequency of asymptomatic infection with these viruses, or indeed, whether WUV or KIPyV played an aetiological role in the respiratory diseases of patients from whom they were obtained. In the current study, we have screened an existing archive of respiratory specimens with accompanying epidemiological and clinical information to determine prevalences of infection with WUV and KIPyV. The archive also included a sub-group of samples from children and adults with no respiratory disease, thus providing a control group to assess more critically their aetiological role in respiratory disease.

2. Material and methods

2.1. Test specimens

The study was based on a total of 983 archived samples referred to the Specialist Virology Centre (SVC), Royal Infirmary of Edinburgh for respiratory virus testing. The samples were predominantly nasopharyngeal swabs or aspirates ($n = 825$, 84%), although other respiratory sample type were also included (47 brochoalveolar lavages, 21 tracheal swabs or aspirates, 23 sputa, 43 "other", 24 no specimen type recorded). All samples were examined routinely by nested PCR for respiratory syncytial virus (RSV), influenza A and B, parainfluenza types 1–3 (PIV1–PIV3) and human adenovirus (AdV) based on previously described assays (Templeton et al., 2004; Heim et al., 2003), modified for a nested format. Additionally, the majority of samples were screened for HBoV and parvovirus B19 as described in a previous study (Manning et al., 2006). All samples were first anonymised and deposited in the SVC respiratory sample archive prior to testing as previously described using a procedure approved by the Lothian Regional Ethics Committee to maintain confidentiality (Manning et al., 2006).

2.2. Detection of WU and KI polyomaviruses

Total nucleic acid was extracted from respiratory specimens as previously described (Manning et al., 2006). PCR was carried out using the same buffers, enzymes and cycling conditions as used for parvovirus PCR (Manning et al., 2006) with nested primers to enhance sensitivity and specificity of the amplification reaction. Because the original description of WU and KI viruses described primers capable of detecting WU and KI polyomaviruses individually, two new sets of primers hybridising to regions of sequence conservation between the virus groups were selected. Set A comprised (positions in the published complete genome sequence of KIPyV [NC_009238] shown in parentheses) sense outer (480) ATCRTAGCTGGAGGAGCAGAG, sense, inner (507) RTCAATTGCTGGWTCTGGAGCTGC, antisense, inner (782) TCCACTTGSACTTCCTGTTGGG and antisense, outer (815) CCYTGGGGATTGTATCCTGMGG primers, yielding amplicon lengths of 336 and 276 bps for first and second round amplification reactions. Set B comprised sense outer (2260) ACTRTTGGATGAAAATGGCATTGG, sense, inner (2299) RGGWAGATTGTACATWACTTGTGC, antisense, inner (2710) YATGCAAATGAATGTAACMAATGG and antisense, outer (2737) WTATATAGGCCTTACTGARTTTGC primers, yielding amplicon lengths of 478 and 412 bps for first and second round amplification reactions.

3. Results

3.1. Frequency of detection of WUV and KIPyV

A total of 983 respiratory samples from 612 different individuals (356 male, 251 female, 5 unknown) were examined. Routine screening by multiplexed nested PCR detected RSV (120 positive samples; 12.2%), influenza viruses A and B (4 [0.4%] and 46 [4.7%], respectively), parainfluenza viruses 1–3 (6 [0.6%], 27 [2.7%] and 2 [0.2%]) and adenovirus (80; 8.1%) infections in the study group. A subset of 913 samples had been previously screened for the human parvoviruses, HBoV and B19, of which 53 (5.8%) and 4 (0.4%) were positive, respectively.

Table 1
Frequency of co-detection of WUV/KIPyV with other respiratory viruses

	WUV/KIPyV+	WUV/KIPyV−	Probability[a]
No virus detected	9 [5/4][b] (2.6%)	357 (97.4%)	
Other virus detection—Total	10 [4/6] (5.3%)	179 (94.7%)	$p = 0.09$
Adenovirus	6 [4/2] (8.8%)	61 (92.2%)	$p = 0.02$
Others (4 RSV, 1 B19, 2 HBoV)	7 [1/6] (3.9%)	173 (96.1%)	$p = 0.42$

[a] Fisher's Exact Test.
[b] Frequencies of WUV and KIPyV in parentheses.

Table 2
Presentation and demographics of WUV/KIPyV infected study subjects

Respiratory disease	Total [WU/KI]	Mean age (range)	M/F	Immunosuppressed	Co-detected viruses
LRTI	8 [5/3]/114	1 (0.3–1.5)	5/3	0/8	RSV (n = 2), AdV (n = 3), HBoV (n = 1)
URTI	5 [2/3]/75	14 (0.7–34)	4/1	3/5[a]	AdV (n = 2), HBoV (n = 1)
None	6 [3/3]/56	5 (1.5–15)	4/2	5/6[b]	AdV (n = 1), B19 (n = 1)

[a] The three immunosuppressed study subjects had acute lymphoblastic leukaemia (ALL), bone marrow transplant, and neutropoenic; none showed virus co-infections.
[b] Three with ALL, one with Gaucher's disease, one immunosuppressed for unspecified reasons, one showed B19 co-infection.

The 983 study samples were screened for WUV and KIPyV by combined PCR using set A and set B primers to test pools of 10 specimens. The 22 initially reactive pools were split into individual components, yielding a total of 24 repeatedly positive samples. All but one sample was positive with both set A and set B primers, the exception was positive for set A primers only. Of the positive samples, 14 were identified as KIPyV by direct nucleotide sequencing of the amplicon, while 10 were WUV. All sequences were identical in the amplified region to published sequences of these viruses (EF127906–127908 and EF444549–444554, respectively). Positive samples originated from 19 different individuals (10 WU, 9 KIPyV), yielding an overall case prevalence of WU/KI infection of 3.1%. This compares with case frequencies of infection by RSV of 14.7% ($n = 90$), by influenza A and B of 0.7% and 5.0% ($n = 4, 31$), by PIV1-3 of 0.7%, 2.8% and 0.3% ($n = 4, 17, 2$), by adenovirus of 10.9% ($n = 67$), by B19 of 0.7% ($n = 4$) and by HBoV of 8.1% ($n = 46$).

Among the 19 individuals in whom WUV or KIPyV DNA sequences were detected, a total of 10 showed evidence for co-infection with other respiratory viruses (4 WUV, 6 KIPyV); Table 1. Six of the co-infections involved adenovirus, with a higher frequency in WUV/KIPyV+ samples (8.8%) than observed in the polyomavirus-uninfected group (2.6%; $p = 0.02$ by Fisher's Exact Test). No over-representation of WUV/KIPyV detection was observed among samples positive for other respiratory viruses, combined ($p = 0.4$) or individually (statistics not shown).

3.2. Clinical associations of WUV/KIPyV infections

Anonymised sample and study subject information was used for analysis of the epidemiology and clinical associations of the newly described polyomaviruses. Approximately one half (9/19) of WUV and KIPyV infections were found in study subjects aged between 1 and 2 years (13% positivity in this age group). Frequencies of infection in other age ranges were consistently lower (0%–3.3%). Infections with WUV and KIPyV were commonest in early winter months (18/19 October to December, 1/19 January to March).

There was no evidence for higher frequencies of WUV/KIPyV infection among those with respiratory disease (Table 2). Frequencies of WU/KIPyV infection in those with lower respiratory tract infections (LRTIs, typically bronchiolitis, pneumonia) were 8/114 (7%), remarkably similar to those found in upper respiratory tract infections (URTIs, wheeze, sore throat, rhinitis) of 5/75 (6.7%) and in the no respiratory disease control group of 6/56 (11%). Infected study subjects with LRTIs ($n = 8$) were young (almost invariably less the 2 years of age), immunocompetent, and showed frequent co-infections with other viruses (RSV, adenovirus) that may have accounted for their respiratory disease. A second group comprised those with URTIs or with no respiratory symptoms/signs ($n = 5, 6$), who were typically older and frequently immunosuppressed (8 from 11) from ALL, BMT transplants and with neutropoenia, one with persistent B19 infection.

4. Discussion

This study describes the development of a combined PCR for the amplification of the recently described closely related WU and KI polyomaviruses, and its detection in association with immunosuppression. Although the combined PCR for the two viruses required nucleotide sequencing to identify WUV and KIPyV sequences, separately labelled WUV- and KIPyV-specific probes might be used in a real time PCR for larger scale screening and virus identification.

Overall frequencies of detection of WUV in 10 from 612 cases (1.6%) were comparable to frequencies of WUV

infection recorded in Brisbane (37 from 1245 samples; 3.0%) and St Louis (5 from 410; 1.2%) (Gaynor et al., 2007), while the frequency of KIPyV infection (9 from 612 cases in the current study; 1.5%) was comparable to that in the original Swedish study (6/637; 1.0%) (Allander et al., 2007) and a second study from Brisbane (24 from 951; 2.5%) (Bialasiewicz et al., 2007). While some of the authors speculated potential aetiological roles for these viruses in respiratory disease, the absence of defined non-respiratory disease control group in both studies weakened such conclusions. Indeed, our finding of an actually higher frequency on WUV and KIPyV infections in our control group compared to those with URTIs and LRTIs (Table 2) provides evidence against such an association.

Epidemiologically, infections with WUV and KIPyV differed in many respects from respiratory viruses. These include the virtual absence of infections in January to March when RSV, HBoV and parainfluenza were at their peak prevalences, observations comparable to the wide scatter of detection dates of KIPyV in the Australian study (Bialasiewicz et al., 2007). WUV and KIPyV also seemed to specifically target the 1–2 age group where one in seven respiratory samples were positive, an association consistent and more marked than recorded in previous studies for WUV and KIPyV (Bialasiewicz et al., 2007; Gaynor et al., 2007).

WUV/KIPyV infected study subjects fell into two distinct groups. One group were immunosuppressed, without respiratory symptoms or with URTIs (that may indeed be caused by viruses not in the current diagnostic screen, such as rhinoviruses or coronaviruses). This group was significantly older than the group with LRTIs, who, without exception, were immunocompetent, but who were almost universally infected with other respiratory viruses that might account for their respiratory disease (RSV, HBoV and AdV). Frequent co-infections with respiratory viruses (72%) have previously been observed among predominantly non-immunosuppressed WUV-infected study subjects (Gaynor et al., 2007), further indicating a potential "innocent bystander" role of these polyomaviruses in respiratory disease.

Overall, frequencies of detection and sample distribution of WUV and KIPyV were remarkably similar, occurring at approximately equal frequencies in respiratory samples from each age range, sample month and study subject group. These observations indicate at least partly overlapping biological properties and host interactions of the two viruses. Higher frequencies of WUV/KIPyV infection in immunosuppressed individuals may represent a similar phenomenon to the reactivation of BKV and JCV in leukaemia and in bone marrow transplant patients (both represented in Table 2) (Boubenider et al., 1999; Behzad-Behbahani et al., 2004; Shah et al., 1997). Future studies of the disease associations and pathogenesis of these new polyomaviruses and their relationship with immunosuppression are required to understand their biology and host interactions.

Acknowledgements

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