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Human polyomaviruses, WU and KI in HIV exposed children with acute lower respiratory tract infections in hospitals in South Africa

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ABSTRACT

Background: The importance of two recently identified polyomaviruses, WUV and KIV, as respiratory pathogens in populations with a high HIV prevalence needs to be defined, since human polyomaviruses can cause significant morbidity and mortality in patients with immunosuppression. Geographic distribution and disease association of WUV and KIV genotypes are not yet clearly defined.
Objectives: To investigate the prevalence of WUV and KIV in HIV-positive and HIV-negative patients with respiratory infections in hospitals in South Africa and determine their genotypes.
Study design: Specimens from patients with acute respiratory infections from hospitals serving Pretoria were screened for WUV and KIV. Positive specimens were sequenced and subjected to phylogenetic analysis.
Results: WUV was identified in (7%) and KIV in (1%) of mainly pediatric patients. Co-infections were common in WUV- and KIV-infected patients (71% and 66.6%, respectively); 57% of patients with WUV and 33% of patients with KIV were HIV-positive while the HIV prevalence in the respiratory virus patient group screened in this study was 33% WUV and KIV patients presented with moderate to severe lower respiratory tract disease. Four distinct and 2 unique WUV strains were identified clustering into 2 of 4 globally identified genotypes. KIV strains were identical to strains from Sweden.
Conclusion: WUV is frequently detected in HIV-infected patients with respiratory disease, but its role as respiratory pathogen remains uncertain.

1. Introduction

WU (WUV) and KI (KIV) polyomaviruses were recently identified in respiratory specimens from patients with acute respiratory infections (ARI).[1–11] WUV was present in respiratory secretions of 1–3% of cases of acute upper and lower respiratory tract infections (ALRI) in children in Australia, the USA,[1] Canada,[4] Great Britain,[5,12] and China[11]; 3.2–8.1% in Germany[10]; 6.2% in Thailand[13]; and 7% in South Korea.[6] KIV was detected in 1% of cases in Sweden.[3] Australia,[2] Great Britain,[5,12] China,[11] and 2% in Thailand.[13] Co-infections were identified in >50% of cases and a similar frequency were identified in hospitalised patients without symptoms in Canada and Scotland.[4,12] A higher frequency of co-infection was noted in symptomatic (7%) as compared with asymptomatic (4.2%) children in South Korea, although this finding was not statistically significant.[6] Although this places the disease association of WUV and KIV in question in immunocompetent children, their role as pathogens in immunocompromised children, especially those with HIV/AIDS remains unknown.

Previously, JC and BK virus (JCV and BKV) were the only polyomaviruses considered as undisputed human pathogens.[14] Both cause latent and asymptomatic infections in the majority of people, but may cause serious disease in immunocompromised individuals.[15,16] Specific JCV genotypes are associated with specific populations and certain genotypes are identified more frequently in progressive multifocal leukoencephalopathy (PML) cases.[17–21]

The HIV sero-prevalence amongst antenatal clinic attendees in the Gauteng province, Republic of South Africa (RSA) was 32.4% by 2005.[22] The important role that JCV and BKV play in mortality in HIV-infected patients raises questions about the role of WUV and KIV in respiratory tract disease in HIV-infected children.

To address this, nasopharyngeal aspirates (NPA) of HIV-positive and HIV-negative patients with respiratory disease from South Africa, were screened for WUV and KIV by PCR over a period of 1 year. Patients with a positive specimen were included in a clinical analysis determine disease association and the amplicons from their specimens subjected to a molecular analysis to determine the

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relationship of prevalent strains to those reported elsewhere in the world.

2. Materials and methods

2.1. Specimen collection

Informed consent was obtained from all healthy patients and ethical clearance for retrospective screening was obtained from the University of Pretoria Ethics committee (Protocol number 25/2006). NPA specimens submitted for virus diagnosis to the department of Medical Virology, University of Pretoria/NHLS Tshwane Academic division, were selected randomly from patients with ARI from every month between February 2006 and February 2007 ($n = 300$) from three hospitals serving the Pretoria region: Pretoria Academic-, Kalafong secondary- and 1-Military hospitals. Specimens included 30 NPAs that previously tested positive for respiratory syncytial virus (RSV); parainfluenza viruses (PIV) 1, 2, 3, influenza A and B; cytomegalovirus; or adenovirus by routine antigen detection tests; and 270 that tested negative for these viruses. Healthy control specimens from 50 children attending a vaccine clinic in the same region were included. The control group was only tested for WUV and KIV. Disease severity was classified as: mild (upper respiratory tract infections, seeking medical care at outpatients); moderate (LRTI, requiring hospitalisation); or severe (LRTI, admission to the intensive care unit or oxygen dependent). Negative specimens were selected randomly each month for inclusion in this study.

2.2. HIV testing

HIV-1 was detected by HIV Ag/Ab Combo Assay (Abbott, Santa Clara, CA) followed by either a HIV-1 DNA Amplicor assay version 1.5 (Roche Diagnostics) for patients <18 months of age or a 4th generation ELISA (Roche diagnostics) in older patients.[23] Polyomavirus-positive specimens of unknown status were retrospectively tested by HIV-1 DNA PCR assay on DNA obtained from NPA cell pellets.[24–26] Specimen quality was monitored using an internal control. All specimens in the database were blinded and handled anonymously.

2.3. Extraction and amplification

Nucleic acids were extracted with the MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche Diagnostics (Mannheim, Germany). PCR screening was carried out using the Expand High Fidelity PLUS PCR System, Roche Diagnostics (Mannheim, Germany).[1,3] WUV and KIV amplicons were 250 bp (VP2) and 323 bp (VP1) in size, respectively.

2.4. Sequencing and phylogenetic analysis

DNA sequencing of positive amplicons was carried out as recommended by the supplier using the ABI Prism big dye terminator cycle sequencing kit Version 3.1 on an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, USA). Sequence editing was performed with SequencherTM Version 4.6, Gene Codes Corporation (Ann Arbor, USA). Nucleotide sequences were aligned with Clustal X (1.8).[27] Phylogenetic analysis was performed using the VP1 region for KIV and the VP2 region for WUV.[1–3] Maximum-likelihood trees were created using DNAML version 3.573c of Phylip[28] using optimised transition to transversion ratios and base frequencies. Bootstrap statistics was calculated from a Neighbor-Joining consensus tree using 1000 replicates and the maximum-likelihood model. Trees were midpoint rooted in Figtree V1.1.1 (http://tree.bio.ed.ac.za). Percentage nucleic acid and amino acid differences were calculated using the *P*-distance option of MEGA version 4.[29]

2.5. Detection of co-infections

WUV- and KIV-positive specimens were screened for co-infections by a newly developed multiplex realtime RT-PCR for RSV, human metapneumovirus (hMPV), bocavirus, human coronaviruses NL63 (CoVNL63), CoV229E, CoVOC43, HKU1, PIV 1,2,3, adenovirus, influenza A and B, and rhinovirus (RV) thereby covering both newly identified respiratory viruses and viruses not commonly included in routine respiratory virus tests, as well as viruses included in conventional tests. Screening all specimens by PCR eliminated the bias of immunofluorescence testing, which requires high quality specimens with intact cells. Details of this tests will be reported elsewhere (Lassauniere and Venter, unpublished data).

3. Results

3.1. Prevalence of WUV and KIV

Patients were between 0 days and 77 years old with 98.7% being <2 years (mean age = 3 months) and 45% female. This was representative of the demographics of patient specimens submitted for respiratory virus diagnosis over a one-year period to the virology laboratory. In total 21/300 specimens were positive for WUV (7%) and 3 for KIV (1%). Co-infections were identified in 15/21 (71%) and 2/3 of WUV- and KIV-positive specimens, respectively. Most co-infections were with RV (6/21), followed by bocavirus (3/21), and adenovirus, RSV, PIV3 and influenza A (2/21 each); CoVNL63 and hPMV were each identified in one case. No CoVOC43, −229E or HKU1 co-infections were identified. Bacterial co-infections were identified in three cases (Table 1). KIV-positive specimens were also positive for WUV in 2/3 cases. No seasonality was observed. None of the 50 healthy patients tested positive for WUV or KIV.

Nine of 21 (42%) WUV- and 1/3 KIV-infected patients were HIV-positive. The HIV status was unknown in a majority of cases (47.69%). Retrospective anonymous screening by DNA PCR on NPA cell pellets increased the WUV/HIV-positive group by 14% to 12/21 (57.1%) (Table 1). HIV DNA PCR results on NPAs correlated with known HIV PCR positive blood tests. HIV infection in WUV-positive patients was more common than in the screened patient group (57.1% vs 33%), although not statistically significant (Fisher's exact test; $P = 0.10$ at a 95% confidence interval).

Seventy-one percent of WUV-infected patients were <1-year old, although an infection was detected in an HIV-positive patient of 40 years. No gender association was found for either virus infection. Clinical presentation ranged from mild to severe, presenting as RSV-like disease, pneumonia, bronchiolitis, and broncho-pneumonia (Table 1A). Clinical symptoms similar to pneumocystis jerovecii pneumonia (PCP) were diagnosed in three cases despite negative IFA on NPA's for PCP. Two of these cases had no other infecting agent identified, while the third was co-infected with RV. All three had moderate to severe disease, required hospitalisation, and had AIDS. All HIV-positive patients infected with WUV were classified with moderate and severe disease.

Ten WUV sequence products were of sufficient quality to use for phylogenetic analysis. Nucleotide alignments identified five distinct RSA strains. Comparison of the RSA strains to all available WU partial VP2 sequences on Genbank clustered existing strains into four genotypes that were named genotypes 1–4 for this study on the basis of bootstrap and *p*-distance analysis. Representative sequences of each unique strain in Genbank were included in the tree in Fig. 1. Genotypes differed on average between 1.3% and 3.6% to each other and less than 1.3% within groups (results not shown). Most RSA WU strains clustered with genotype 1, which could be

Table 1
Clinical details of patients with WU and KI infections.

A.) Data from medical files and laboratory analysis

Specimen number	Co-infection	WU	KI	Age of patient	M/F	HIV status	Clinical details	Disease severity	Genetic cluster
SA375167P06	*K. pneumoniae*	Pos	Neg	3 m	M	Sero (+), PCR (−)	Respiratory tract infection, not meriting admission	Mild	
SA692492P06	Adenovirus, RV	Pos	Neg	7 m	M	Sero (−)	Respiratory tract infection, not meriting admission	Mild	
SA691910P06	hBOV	Pos	Pos	20 m	M	PCR (−)	Respiratory tract infection, not meriting admission	Mild	
SA642630P06	hMPV	Pos	Neg	4 m	M	PCR (−)	Broncho-pneumonia	Mild	
SA387137P06	NONE	Neg	Pos	5 m	F	PCR (−)	Respiratory tract infection, not meriting admission	Mild	
SA479959P06	NONE	Pos	Neg	1 m 12 d	F	PCR (−)	RSV-like	Mild	
SA415567P06	RSV; *H. para-influenzae*	Pos	Neg	1 y 5 m	F	PCR (−)	Respiratory tract infection, not meriting admission	Mild	1e
SA403923P06	None	Pos	Neg	0 d	F	Unknown	Respiratory tract infection, not meriting admission	Mild	1e
SA361684P06	CoV NL63, RV	Pos	Neg	92 d	F	PCR (+)	Pneumonia	Moderate	
SA776772P06	hBoV, RV	Pos	Neg	1y4 m	M	PCR (+)	Pneumonia	Moderate	
SA442718P06	RSV; RV	Pos	Neg	56 d	M	PCR (−)	No clinical detail	Moderate	
SA353703P06	NONE	Pos	Neg	6 m	F	PCR (+)	pTB/lobar; clinical diagnosis: PCP, IF(−) for PCP	Moderate	
SA668826P06	PIV-3, RV	Pos	Neg	2 y 2 m	M	PCR (−)	Bronchiolitis	Moderate	1b
SA544993P06	Adenovirus	Pos	Neg	2 y 6 m	M	Sero (−), PCR (−)	Broncho-pneumonia	Moderate	1c
SA526534P06	Influenza A	Pos	Neg	3 y	F	PCR (+)	Broncho-pneumonia	Moderate	1c
SA666528P06	RV	Pos	Neg	7 m	F	PCR (+)	Pneumonia	Moderate	1d
SA675757P06	RV	Pos	Pos	8 m	F	PCR (+)	Stage 4 AIDS, clinical diagnoses PCP, not confirmed	Moderate	1d
SA522896P06	Influenza A MRSA	Pos	Neg	3 d	M	Sero (+), PCR (−)	Broncho-pneumonia	Moderate	1e
SA526524P06	*E. coli*; *Enterobacter cloaca*	Pos	Neg	48 d	F	PCR (+)	Broncho-pneumonia	Moderate	2
SA726527P06	PIV-3	Pos	Neg	40 y	M	PCR (+)	Illegible diagnosis	Severe	
SA442979P06	NONE	Pos	Neg	25 d	M	Sero (+), PCR (−)	Respiratory distress, ICU; clinical diagnosis PCP, IF(−) for PCP	Severe	
SA551007P06	hBoV	Pos	Neg	5 m	F	PCR (+)	RSV-like Bronchiolitis	Severe	1d

B.) Summary of HIV disease severity of patients with WUV and KIV infections. KIV values are given in brackets

HIV data	Mild	Moderate	Severe	Total	%
PCR (+)	0	7 (1)	2	9 (1)	42.85% (33%) 14.3% } 57.1% HIV+ (WUV)
Sero (+) PCR (−)	1	1	1	3	
Sero (−) PCR (−)	5 (2)	3	0	8 (2)	38% (66%)
Unknown	1	0	0	1	4.7%
Total	7 (2)	11 (1)	3	21 (3)	

subdivided into five subgenotypes (1a–1e). Nineteen variable sites were identified across the 250 bp VP2 gene region (Fig. 2). Most nucleotide differences between the main genotypes translated into amino acid changes (2.1–7% average amino acid difference between genotypes) (Fig. 2B). RSA isolates in subgenotypes 1b, 1c and genotype 2 were unique and differed from all other strains. Strain B2 had a deletion of 12 nucleotides (translating into five amino acids) that was not present in the RSA genotype 2 strain (SAW526524) and was not visible in the alignment. RSA genotype 1d strains differed by one amino acid from genotype 1b, 1c, and 1e strains, which were identical at amino acid level. RSA strains in subgenotype 1e and 1d were identical to strains identified in Australia, the USA and Korea (Fig. 2). No correlation seems to exist between disease severity and genotype, but this should be confirmed with larger sample sizes.

Nucleotide sequence of the VP1 region of RSA KIV strains was 100% identical to strains identified in Sweden and Australia.

4. Discussion

This study confirms the presence of WUV and KIV in respiratory secretions of children with mild to severe respiratory infection symptoms in Africa. The frequencies of WUV (7%) was higher than those detected in Scotland[12]; Australia, the USA and Canada,[1,4] but similar to levels in Thailand[13] and Germany.[10] The only study including immunocompromised patients, which was performed in Scotland, had very limited numbers of patients with advanced HIV disease. HIV infection was more prevalent in WUV-positive patients (57%) as compared with the overall respiratory virus patient group screened in the present study (33%), although not yet statistically significant due to the small sample size. Too few KIV-positive patients were identified to draw any conclusion about HIV prevalence. The low number of detected KIV-positive cases (1%) does not suggest a significant role in either HIV-positive or HIV-negative patients. Although immunocompromised patients were identified among WUV-positive patients from Australia, and the USA, very few HIV-infected patients were included. The immune status of children in Thailand or Germany was not clear.[10,13] Fifty healthy volunteers were all negative for WUV and KIV suggesting an absence of these viruses in healthy immunocompetent children. Despite the small number of specimens in this control group, they represent truly healthy children rather than hospitalised patients that may be more susceptible to infections. WUV-positive control patients in previous studies were all immunosuppressed or hospitalised for other reasons.[4,12] Larger control groups of children without respiratory disease that are immunocompetent and immunosuppressed may shed further light on WUV disease association.

Most HIV-positive RSA patients with WUV had moderate to severe disease with symptoms ranging from pneumonia, broncho-

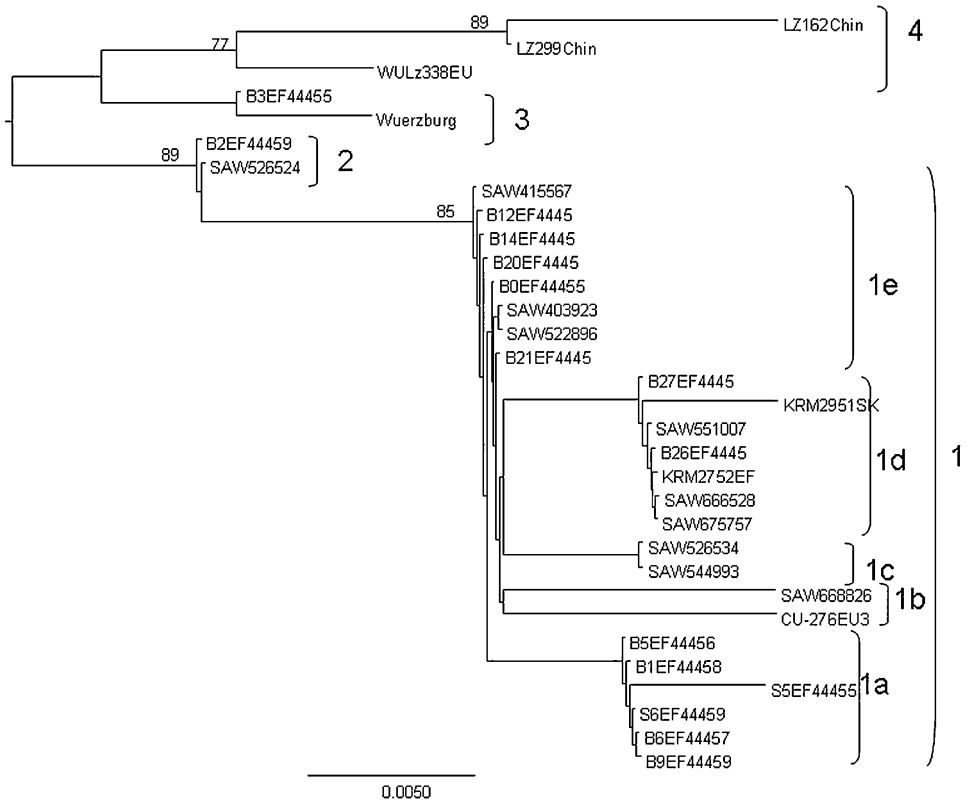

Fig. 1. Midpoint rooted maximum-likelihood tree of the VP2 partial gene sequence of all unique WU strains identified to date world wide. The South African strains are indicated with the prefix SA and the specimen number. Genotypes are indicated on the right and bootstrap statistics greater than 70% on the branch nodes. The scale indicates 0.005 substitutions per base per indicated horizontal distance. Other strains are from Brisbane, Australia (B) St. Louis (S) (1) and Canada (Can) (4), South Korea (KRM), China (Chin- and CU) and Germany (Wuerzburg and WULz) and were represent unique strains as identified through Blastsearch and additional phylogenetic analysis. Accession numbers: EF444592; EF444593; EF444583, EF444578, EF444554, EF444585, EF444562 EF444561, EF444557, EF444555; EU358761, EF444567,EF639269, EF444569, EF639271 EF444591, EF444558; EU711055, EU304323, EU678902.

(A)

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                                  11112222
                          333555778 900691123
                          5029248121 324445636
          B9(1a)          AGCGGGCAAG GCACGGCCT
          S6(1a)          .......... .........
          S5(1a)          .......... C........
      CU-276 (1b)         .......... ..C.AA...
 SAW668826K06 (1b)        ..T....... ...TA....
 SAW526534K06 (1c)        .......... ....A...A
 SAW544993P06 (1c)        .......... ....A...A
 KRM2951SKorea(1d)        ...C...... ....A..A.
          B27 (1d)        ...C...... ....A....
 SAW551007P06 (1d)        ...C...... ....A....
 SAW666528K06 (1d)        ...C...... ....A....
 SAW675757K06 (1d)        ...C...... ....A....
       KRM2752(1d)        ...C...... ....A....
 SAW522896K06 (1e)        .......... ....A....
 SAW403923K06 (1e)        .......... ....A....
 SAW415567K06 (1e)        .......... ....A....
           B0(1e)         .......... ....A....
 SAW526524K06 (2)         .........C .G..A....
  B2 EF444591 (2)         ..--.....C .G..A....
  B3 EF444558 (3)         ...CC.G..C .G..A....
 Wuerzburg0106(3)         G..CC.G..C .G..A....
   LZ162China (4)         .T..C.GGGC .GG.A.T..
   LZ299China (4)         ....C.GGGC .GG.A....
      WULz338 (4)         ....CTG..C .GG.A....
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(B)

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1111122337 77
0378947141 24
DERGTNEDQM PA
.......... ..
.......H.. ..
........HI ..
.......... ..
.......... ..
.......... ..
.Q........ .D
.Q........ ..
.Q........ ..
.Q........ ..
.Q........ ..
.Q........ ..
.......... ..
.......... ..
.......... ..
.......... ..
......Q.E. ..
--....Q.E. ..
.QT.R.Q.E. ..
.QT.R.Q.E. ..
Y.T.RDQ.E. S.
..T.RDQ.E. ..
..TCR.Q.E. ..
```

Fig. 2. Nucleotide (A) and amino acid (B) variable sites on the VP2 partial sequence used for genotyping of WUV. All South African strains and representative sequences of all unique WU virus strains identified to date in Australia, North America, Europe and Asia are included. The genotype is indicated in brackets next to the sequence name as indicated on Genbank and the position above the sequence. Accession numbers correspond to Fig. 1.

pneumonia, and PCP-like pneumonia. This may be due to the immunosuppression as seen in HIV disease, but may also be linked to the increased incidence of co-infections, compounding clinical severity. Patients with mild disease were mostly HIV-negative, but again due to limited patient numbers, this finding was not statistically significant. Co-infections were identified in 71% of WUV-infected patients in our study group, relative to Germany (54.8%), Thailand (43%), Australia (79.7%), and the USA (71%). Two patients had co-infections with both WUV and KIV, one that was HIV-negative with a bocavirus co-infection had mild disease, while the other had stage 4 AIDS with moderate disease, and a co-infection with rhinovirus and symptoms similar to PCP-like pneumonia. Further investigations are needed to determine if these polyomaviruses contribute to respiratory syndromes in young patients with AIDS.

Comparison of our data to populations without significant HIV burden does not indicate a greater infection rate in immunocompetent relative to immunosuppressed children. However, 71% of cases were associated with ALRI in RSA vs 56% in Germany which may possibly be due to the higher HIV sero-prevalence. The range of co-infections was also similar, but may increase the severity of disease in immunosuppressed patients although this should be confirmed in larger studies.

Identification of WUV in a one-day old child complements similar findings in the USA.[7]

Phylogenetic analysis of strains from 5 continents has identified at least 4 major WUV genotypes. WUV genotype 1 is the most widely distributed and is present in Australia, Africa, Europe and Asia, while genotype 4 is specific to China. Genotype 1 was the most abundant in our study group and was identified in patients with mild to severe disease. Larger study cohorts will determine if a statistical significant disease association can be attributed to specific genotypes.

The frequent identification of WUV with co-infections in pediatric HIV patients with lower respiratory tract infections in hospitals in RSA warrant further investigations to determine if WUV contribute to disease in AIDS patients.

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