



**HAL**  
open science

## Secondary lymphoid tissue as an important site for WU polyomavirus infection in immunocompetent children.

Manola Comar, Nunzia Zanotta, Tatiana Rossi, Giorgio Pelos, Pierlanfranco d'Agaro

### ► To cite this version:

Manola Comar, Nunzia Zanotta, Tatiana Rossi, Giorgio Pelos, Pierlanfranco d'Agaro. Secondary lymphoid tissue as an important site for WU polyomavirus infection in immunocompetent children.. Journal of Medical Virology, 2011, 83 (8), pp.1446. 10.1002/jmv.22124 . hal-00652149

**HAL Id: hal-00652149**

**<https://hal.science/hal-00652149>**

Submitted on 15 Dec 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Secondary lymphoid tissue as an important site for WU polyomavirus infection in immunocompetent children.



Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-11-2304.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	04-Apr-2011
Complete List of Authors:	<p>Comar, Manola; UCO Hygiene and Preventive Medicine, University of Trieste, Institute of Child Health IRCCS Burlo Garofolo, Trieste, Italy, Department of Reproductive, Developmental and Public Health Sciences</p> <p>Zanotta, Nunzia; UCO Hygiene and Preventive Medicine, University of Trieste, Institute of Child Health IRCCS Burlo Garofolo, Trieste, Italy, 1Department of Reproductive, Developmental and Public Health Sciences</p> <p>Rossi, Tatiana; UCO Hygiene and Preventive Medicine, University of Trieste, Institute of Child Health IRCCS Burlo Garofolo, Trieste, Italy, Department of Reproductive, Developmental and Public Health Sciences</p> <p>Pelos, Giorgio; Institute of Child Health IRCCS Burlo Garofolo, Pediatric Otolaryngology Unit</p> <p>D'Agaro, Pierlanfranco; UCO Hygiene and Preventive Medicine, University of Trieste, Institute of Child Health IRCCS Burlo Garofolo, Trieste, Italy, Department of Reproductive, Developmental and Public Health Sciences</p>
Keywords:	tonsils, adenoids, nasopharyngeal tract, WUPyV

SCHOLARONE™  
Manuscripts

Table I. Virological analysis of polyomavirus in lymphoid and nasopharyngeal samples.

Specimens	No.	Pathogen detected					Ref.
		<u>Polyomavirus</u>				HHV-6	
		WUPyV	KIPyV	BKV	JCV		
<b>Adenoids</b>	83	23 (27.7%)	-	1 (1.2%)	-	7 (12.7%)	
<b>Tonsils</b>	50	6 (12%)	-	3 (6%)	-	20 (25%)	
<b>PBMCs</b>	50	-	-	-	-	3 (6%)	
<b>Total</b>	183	29 (15.8%)	-	4 (2.2%)	-	30 (16.4%)	

---

	No.	<u>Polyomavirus</u>		<u>Respiratory virus</u>			<u>Co-infection</u>
		WUPyV	KIPyV	FLU	RSV	ADENO	WUPyV/RV
		<b>NP</b>	98	27 (27.5%)	-	30 (30.6%)	19 (19.4%)

\*10 FLU-2 Adeno-7 RSV

(A) The distribution of WUPyV, KIPyV, BKV, and JCV among secondary lymphoid tissues from immunocompetent children. (B) Detection of WUPyV, KIPyV, influenza virus, RSV, and adenovirus in nasopharyngeal secretions from children with influenza-like infection. FLU, influenza virus; ADENO, adenovirus; RV, respiratory virus; NP, nasopharyngeal.

Table II. Nucleotide variation in the VP2 gene of WUPyV (in bold) in comparison with some GenBank registered sequences (nucleotide position is deduced by alignment with the prototype).

<u>Strain (GenBank Acc. No.)</u>	<u>VP2 gene - nucleotide position</u>							<u>Country/year</u>
	3	37	50	56	79	100	192	
WU09L (n=27)	A	G	G	C	G	C	G	Italy/2010
WU34L (n=3)	A	<b>C</b>	<b>C</b>	<b>G</b>	<b>C</b>	<b>G</b>	<b>A</b>	Italy/2010
WU35L (n=1)	A	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>S</b>	<b>R</b>	Italy/2010
WU45L (n=2)	A	G	G	C	G	C	A	Italy/2010
WU61L (n=1)	A	G	<b>C</b>	<b>G</b>	<b>C</b>	<b>G</b>	<b>A</b>	Italy/2010
GQ988386_WU709	A	G	G	C	G	C	A	China/2008
EF444550_WU_S1	A	G	G	C	G	C	G	USA/2003
EU711058_WU	<b>G</b>	<b>G</b>	<b>C</b>	<b>G</b>	<b>C</b>	<b>G</b>	<b>A</b>	Germany/2007
FJ464552WUV-RM3	<b>G</b>	<b>C</b>	<b>C</b>	<b>G</b>	<b>C</b>	<b>G</b>	<b>A</b>	Italy/2009

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Secondary lymphoid tissue as an important site for WU polyomavirus infection in immunocompetent children

Manola Comar<sup>1,\*</sup>, Nunzia Zanotta<sup>1</sup>, Tatiana Rossi<sup>1</sup>, Giorgio Pelos<sup>2</sup>, Pierlanfranco D'Agaro<sup>1</sup>.

<sup>1</sup>Department of Reproductive, Developmental and Public Health Sciences, UCO Hygiene and Preventive Medicine, University of Trieste, Institute of Child Health IRCCS Burlo Garofolo, Trieste, Italy

<sup>2</sup>Pediatric Otolaryngology Unit, IRCCS Burlo Garofolo, Trieste, Italy.

\*Correspondence: Manola Comar, Department of Reproductive, Developmental and Public Health Sciences-University of Trieste, IRCCS Burlo Garofolo, Via dell'Istria 65/1, 34137 Trieste, Italy.

Phone: +39 040 773433. Fax: +39 040 7600324.

E-mail: [comar@burlo.trieste.it](mailto:comar@burlo.trieste.it)

**Funded by:** Research Ministry, Italy (R.C. 38/08 IRCCS 'Burlo Garofolo')

**Key words:** adenoids, nasopharyngeal tract, tonsils

**Running title:** Lymphoid tissue and WUPyV in children

### Abstract

The polyomaviruses KI and WU (KIPyV and WUPyV) have been identified in respiratory specimens from children with acute respiratory infections, which suggests the respiratory tract as a possible site of infection. However, the persistence of infection in the lymphoid system is unknown. Fresh samples (n=211) of tonsils, adenoids, and peripheral blood mononuclear cells (PBMCs) from 83 immunocompetent children (mean age 4.8 years) were tested for amplification of the KIPyV VP1 and WUPyV VP2 genes. The known BK and JC polyomaviruses and the lymphotropic human herpesvirus HHV-6 were also investigated by quantitative real-time PCR and direct sequencing. In addition, 98 nasopharyngeal swabs collected from children (mean age 6.2 years) affected by seasonal influenza-like illness were tested. Of the lymphoid tissues, 34.9% were positive for WUPyV, 4.8% for BK virus, and 33.8% for HHV-6. KIPyV and JC virus were not detected in these specimens. None of the polyomaviruses were detected in PBMCs. Among the nasopharyngeal samples, the prevalence of WUPyV was 27.5%, although 70% of the positive samples were co-infected with at least one of the following respiratory viruses: influenza virus, adenovirus, and respiratory syncytial virus. Phylogenetic analysis revealed high sequence homology (99%) between lymphoid- and nasopharynx-derived WUPyV strains. These results suggest that the tonsils and adenoids of immunocompetent children are a reservoir for WUPyV infection; probably due to the respiratory route of transmission. In addition, the prevalence of WUPyV was high among the children, and the virus was identified more frequently in older children than during the first years of life.

## INTRODUCTION

The recently identified distinct polyomaviruses KI and WU (KIPyV and WUPyV) were identified first in young children with acute respiratory infections, which suggests that these viruses establish infections in the respiratory tract, either transiently or persistently, and are reactivated as a consequence of respiratory disorders [Allander et al., 2007; Gaynor et al., 2007]. Co-infection with one or more respiratory viruses is a common finding in individuals who are positive for KIPyV or WUPyV. However, in a small number of susceptible children, KIPyV or WUPyV was the only virus detected, which suggests that these viruses might cause respiratory disease independently. [Foulongne et al., 2008; Abed et al., 2008]. Nevertheless, unlike in the case of other human and simian polyomaviruses, the aetiopathogenicity of WUPyV and KIPyV remains speculative [Norja et al., 2007].

The widespread nature of WUPyV and KIPyV infection throughout the human population has been confirmed by extensive molecular investigations and supported by recent serological surveys. A pattern of infection that is similar to that of the well-known JC and BK polyomaviruses (JCV and BKV) has emerged, which suggests primary exposure to the virus during childhood, after which infection is sustained by close inter-human contacts [Abedi Kiasari et al., 2008; Kean et al., 2009].

The results of the intensive search for these novel viruses in clinical specimens to determine basic aspects of their natural history, including the sites of viral persistence and the potential routes of spread, have been inconclusive [Bialasiewicz et al., 2009]. Although both

1  
2  
3  
4 viruses have been found sporadically in faecal specimens from patients with acute nonbacterial  
5 gastroenteritis and in some hospitalized infants [Wattier et al., 2008; Ren et al., 2009],  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

viruses have been found sporadically in faecal specimens from patients with acute nonbacterial gastroenteritis and in some hospitalized infants [Wattier et al., 2008; Ren et al., 2009], WUPyV and KIPyV have been detected more frequently in nasopharyngeal secretions [Abed et al., 2007; Feng et al., 2008; Foulongne et al., 2008; Neske et al., 2008]. Recently, DNA sequences from KIPyV have been detected in lung and paranasal biopsies from cancer patients, which suggests a possible tropism of the virus for these tissues [Babakir-Mina et al., 2009]. In a subsequent study, a role for the lymphoid system has been suggested by the recovery of WUPyV and KIPyV DNA from a large series of archived paraffin-embedded tonsils from adult patients with a wide spectrum of benign and malignant conditions [Babakir-Mina et al., 2009]. These data support the hypothesis that, in analogy with BKV and JCV, these novel polyomaviruses can infect and establish a persistent infection in tissues that are rich in B cells [Patel et al., 2008].

The study was designed to evaluate the role of secondary lymphoid tissues, apart from the respiratory tract mucosa, as sites of persistence for KIPyV and WUPyV. The frequency of infection by WUPyV, KIPyV, JCV, and BKV was investigated in fresh tonsils, adenoids and peripheral blood mononuclear cells (PBMCs) from a selected cohort of immunocompetent children. For comparison, a series of nasopharyngeal specimens from children with comparable demographic characteristics, which were collected during the surveillance of seasonal influenza, were also tested.

## MATERIALS AND METHODS

### Patients and the Collection and Processing of Samples

1  
2  
3  
4 The study involved a total of 309 selected clinical samples that had been submitted to the  
5  
6 Diagnostic Virology Laboratory of the Burlo Garofolo Children's Hospital (Trieste, Italy).  
7  
8 Specimens of tonsils (n=50), adenoids (n=83), and peripheral blood lymphocytes (n=78) were  
9  
10 collected between April and September 2005 from 83 children with tonsillar and/or adenoidal  
11  
12 benign lymphoid hyperplasia. In addition, 98 nasopharyngeal samples were collected during  
13  
14 November 2007 and March 2008 from children with influenza-like illness. To assess the  
15  
16 demographic and clinical factors that might be associated with polyomavirus infection, approval  
17  
18 was obtained from the Institutional Scientific Board of Burlo Garofalo Children's Hospital to  
19  
20 conduct a review of medical records to select children who met the following general criteria:  
21  
22 Caucasian origin; living in the urban area of North-eastern Italy; age 1–10 years; no history of  
23  
24 blood transfusion; no hospitalization within the previous 2 years; no systemic medication for a  
25  
26 chronic condition ; normal haematological data, admission and discharge diagnosis. Additional  
27  
28 criteria for children who were undergoing elective tonsillectomy and/or adenoidectomy were: no  
29  
30 respiratory tract disease, fever or acute infectious processes diagnosed at the time of tissue  
31  
32 sampling.  
33  
34  
35  
36  
37  
38

39 Aliquots of each specimen were stored at  $-80^{\circ}\text{C}$  in viral transport medium (veal infusion  
40  
41 broth in distilled water plus gentamicin and amphotericin) until molecular testing was complete.  
42  
43 Strict measures to avoid cross-contamination were maintained throughout the study, including the  
44  
45 use of separate rooms for the processing and molecular analysis of the samples.  
46  
47  
48

49 DNA was extracted using a commercial kit (QIAamp Tissue Kit; Qiagen, Mannheim,  
50  
51 Germany) and used immediately for PCR analysis. The Rnase P gene (Roche Applied Science,  
52  
53 Mannheim, Germany) was chosen as the cellular target to test the integrity of the DNA sample.  
54  
55  
56  
57  
58  
59  
60

### Molecular Evaluation

Ten microlitres of each DNA sample were used as a template in a conventional PCR amplification, with primers that targeted the WUPyV VP2 and KIPyV VP1 genes. The conditions for PCR amplification and the primer sequences have been described previously [Allander et al., 2007; Gaynor et al., 2007]. The WUPyV VP2 and KIPyV VP1 products were 250 bp and 207 bp, respectively. Sequences of WUPyV (AP-p002 and AP-p003) and KIPyV (pUC18-KIPyV) that had been cloned in plasmid vectors were used as positive controls. JCV and BKV were detected by real-time PCR using the ABI PRISM 7000 Sequence Detection System (Applied Biosystem, Milan, Italy) as described previously [McNees et al., 2005]. In brief, 10 µl of DNA sample and 10 µl of each specific standard scale dilution were added in duplicate to a final volume of 50 µl of reaction mix, which contained 900 µM primers against sequences in the conserved N-terminal region of the large T-Ag gene, 100 µM TaqMan FAM (6-carboxy-fluorescein)-MGB probe, and 25 µl of 2× TaqMan Universal PCR Master Mix (Applied Biosystems, Milan, Italy). JCV-Mad1 and BKV-Dunlop-1 plasmid vectors were included separately as positive controls. Reactions without DNA template were used as negative controls. Each clinical sample was tested twice. In addition, the genomic DNA of the reference lymphotropic human herpesvirus (HHV)-6 was investigated in the same series, as described previously [Allard et al., 1990].

PCR products of the expected sizes (207 bp for WUPyV and 250 bp for KIPyV), as determined by visualization on 0.8% acrylamide gels stained with ethidium bromide, were sequenced bidirectionally (BigDye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems, Milan, Italy) with an ABI PRISM 310 sequencer, in accordance with the manufacturer's guidelines. Contiguous sequences were assembled using the Sequencer

1  
2  
3  
4 software version 4.6 (Gene Codes Corp, Ann Arbor, MI, USA), and aligned with the  
5  
6 polyomavirus sequences deposited in GenBank using the Blast algorithm.  
7  
8

9 Nasopharyngeal secretions were also evaluated for the presence of respiratory viruses,  
10 including adenovirus, respiratory syncytial virus (RSV), and influenza virus, by standardized  
11 molecular tests [Allard et al., 1990; Zhang and Evans, 1991; Valle et al., 2006]. For the  
12  
13 molecular tests [Allard et al., 1990; Zhang and Evans, 1991; Valle et al., 2006]. For the  
14 analysis of RSV and influenza virus, RNA was purified with an RNeasy Mini Kit (Qiagen,  
15 Chatsworth, CA, USA). To detect RSV, 10  $\mu$ l of RNA were amplified by real-time PCR using  
16 specific primers and probes (RSVA and RSVB) for the F protein, whereas, for influenza virus,  
17  
18 10  $\mu$ l of the extracted RNA were amplified using specific primer sets for the type A and B  
19 matrix protein (MP) regions and for the 1 HA and 3 HA genes.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

### 30 **Statistical Analysis**

31  
32 Data are presented as numbers and proportions tabulated in contingency tables. The  $\chi^2$  test was  
33 used to evaluate the difference between proportions.  $P < 0.05$  was considered to be significant.  
34  
35  
36  
37  
38

## 39 **RESULTS**

40  
41 Table I shows the rates of detection for the pathogens in lymphoid and nasopharyngeal samples  
42 from the enrolled children. RNaseP gene sequences were amplified successfully in all 309  
43 samples tested.  
44  
45  
46  
47  
48

49 Amplicons of the expected size that were shown to correspond unequivocally to WUPyV  
50 sequences by DNA sequence determination were detected in 29/83 (34.9%) of the children who  
51 underwent tonsillectomy or adenoidectomy. The frequency of infection with WUPyV was similar  
52 to that found for the reference lymphotropic virus HHV-6 in the same series [27/83 (33.8%),  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  $P=0.74$ ]. The mean age of the children infected with WUPyV was 4.0 years (range, 2–6 years),  
5  
6 showing a prevalence of infection of 51.7% (15/29). Conversely, BKV was detected rarely (4/83;  
7  
8  $P=0.001$  vs. HHV-6) and no sequences from JCV or KIPyV were identified.  
9

10  
11 WUPyV was detected more frequently in samples of adenoids (23/83, 27.7%) than in  
12  
13 tonsils (6/50, 12%) ( $P<0.001$ ). WUPyV was not detected in both the adenoids and the tonsils in  
14  
15 any of the WUPyV-positive subjects. BKV was found only in 1/83 of the samples of adenoids  
16  
17 and 3/50 of the tonsil samples.  
18  
19

20  
21 WUPyV was the only one of the newly discovered polyomaviruses that was found in  
22  
23 nasopharyngeal samples from the children with respiratory symptoms; WUPyV was identified in  
24  
25 27/98 of these samples (27.5%). The mean age of the children who excreted WUPyV was 3.8  
26  
27 years (range, 1–10 years). Of the positive samples, 19/27 (70%) were co-infected with at least one  
28  
29 of the respiratory viruses considered in this study. Conversely, BKV and JCV sequences were not  
30  
31 detected in the nasopharyngeal samples and none of the polyomaviruses were found in PBMC  
32  
33 samples.  
34  
35  
36

37  
38 To assess the sequence variation in WUPyV among the 29 lymphoid tissue samples that  
39  
40 were positive for this virus, the sequences obtained for the 250-bp region of the VP2 gene were  
41  
42 compared with all available WUPyV VP2 sequences deposited in GenBank (Table II). The new  
43  
44 isolates clustered into five groups, designated WU9L, WU34L, WU35L, WU45L, and WU61L,  
45  
46 on the basis of nucleotide sequence. Phylogenetic analysis revealed a high level of identity  
47  
48 (>99%) among the 29 viral strains. Of these, 23/29 (79.3%) showed 100% homology with  
49  
50 WU9L, and the nucleotide sequences differed from each other on average of 0.1%.  
51  
52

53  
54 Direct sequence analysis was possible for five of the 27 WUPyV-positive nasopharyngeal  
55  
56 specimens. These were designated WU16NP (three strains), WU237NP (one strain), and  
57  
58  
59  
60

1  
2  
3  
4 WU32NP (one strain), and showed a high degree of homology with the most representative  
5  
6 lymphoid-derived strains (WU9L).  
7  
8  
9

## 10 11 **DISCUSSION**

12  
13  
14 The presence of polyomaviruses WUPyV and KIPyV in respiratory tract specimens from children  
15  
16 with acute respiratory infection suggests that the respiratory tract is a site of viral persistence.  
17  
18 Although not proven, the notion that the respiratory tract, apart from the mucosa, is a site of  
19  
20 persistent infection seems to be supported by the recent recovery of WUPyV and KIPyV  
21  
22 sequences from archival tonsillar transformed tissues [Babakir-Mina et al., 2009].  
23  
24

25  
26 To clarify whether secondary lymphoid tissues act as sites of viral persistence, fresh  
27  
28 specimens of tonsils, adenoids, and PBMCs from a selected group of asymptomatic  
29  
30 immunocompetent children were screened. At the same time, to evaluate the prevalence of  
31  
32 infection with these polyomaviruses within the same geographic area, an independent series of  
33  
34 nasopharyngeal swabs from children diagnosed with influenza-like illness was investigated. This  
35  
36 survey demonstrated that WUPyV was the only one of the newly discovered polyomaviruses that  
37  
38 could be detected in the tonsils (12%) or adenoids (27.7%) of this healthy paediatric population.  
39  
40 The overall prevalence rate of WUPyV (34.9%) was similar to that of the lymphotropic reference  
41  
42 virus HHV-6, which was detected in 33.8% of children. These findings emphasized the  
43  
44 unexpectedly high prevalence of WUPyV in the secondary lymphoid system, which suggested the  
45  
46 possible involvement of these tissues as a site for WUPyV persistence.  
47  
48  
49

50  
51 To the best of our knowledge, this is the first report demonstrating that the secondary  
52  
53 lymphoid organs of immunocompetent children play an important role in WUPyV infection; a  
54  
55 role that is favoured possibly by the respiratory route of transmission of the virus. The validity of  
56  
57  
58  
59  
60

1  
2  
3  
4 these findings was supported by the timing of collection of the lymphoid samples. The samples  
5  
6 were collected during a period that was unrelated to the season of respiratory epidemic diseases,  
7  
8 and processed for virological evaluation before the influenza-like cases were enrolled.  
9  
10

11 In the study described above, the frequency of WUPyV in lymphoid tissues was  
12 significantly higher than that reported for other human polyomaviruses [Patel et al., 2008].  
13  
14 Among the well-known human polyomaviruses, only BKV was recovered (in 4.8% of the  
15  
16 children tested). This reinforced the notion that, with the exception of WUPyV, the presence of  
17  
18 polyomaviruses in tonsils and adenoids was probably transient, and that different routes of viral  
19  
20 transmission were involved [Comar et al., 2010]. These data were consistent with a high  
21  
22 frequency of recovery of WUPyV (27.5%) from the nasopharyngeal secretions of children  
23  
24 affected by influenza-like illness, which were collected during a recent influenza season.  
25  
26 Nevertheless, the low level of sequence variation that was observed between lymphoid and  
27  
28 nasopharynx-derived strains was consistent with a high level of conservation among the WUPyV  
29  
30 strains circulating in this population.  
31  
32  
33  
34  
35  
36

37 In this study, the frequency of detection of WUPyV in children with influenza-like illness  
38 differed (27.5% vs 1.4%) from that established by Debiaggi et al [2010]. In addition, the previous  
39  
40 study showed that primary infection occurred during the first months of life (1–24 months)  
41  
42 suggesting that WU/KIPyVs have a limited circulation in Italy and a low pathogenic potential in  
43  
44 young children [Debiaggi et al., 2010]. In contrast, in the present study, WUPyV was found  
45  
46 frequently in older children (mean age 3.8 years), as shown recently for KIPyV [Wattier et al.,  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
2010].

1  
2  
3  
4 In agreement with previous studies, neither KIPyV nor WUPyV DNA was detected in  
5  
6 peripheral lymphocytes, which indicated the absence of any systemic involvement by PBMCs in  
7  
8 healthy subjects, as observed in general for other members of the polyomavirus family.  
9

10  
11 JCV and KIPyV sequences were not detected in the tonsils or adenoids of the children  
12  
13 enrolled in this study. Although the lack of JCV was not unexpected in young children, the  
14  
15 absence of KIPyV was a rather surprising finding that was in contrast with previous data that  
16  
17 demonstrated the presence of a subclade of KIPyV in the tonsils of Italian adults [Babakir-Mina  
18  
19 et al., 2009]. Although the absence of KIPyV in lymphoid specimens suggests that host  
20  
21 susceptibility for KIPyV differs from that for WUPyV, as for JCV and BKV, these conflicting  
22  
23 results were resolved when the presence of KIPyV was investigated in the nasopharyngeal  
24  
25 secretions of immunocompromised subjects. In this North–Eastern Italy area, although infection  
26  
27 with KIPyV seemed to be a rare event in immunocompetent children, this polyomavirus was  
28  
29 detected in 0.5% of hospitalized children aged 15–36 months (data not shown).  
30  
31  
32  
33  
34

35 In summary, the high prevalence of WUPyV in tonsils and adenoids from  
36  
37 immunocompetent children and the high degree of homology of these lymphoid-derived strains  
38  
39 with the most representative nasopharynx-derived strains suggests that lymphoid tissues serve as  
40  
41 an important site of viral persistence. This finding provides support for the hypothesis that  
42  
43 WUPyV is the only known human polyomavirus that has a predilection for respiratory  
44  
45 transmission among children. Nevertheless, the number of patients with respiratory symptoms  
46  
47 who were positive for WUPyV and/or KIPyV was low, and there was no strong evidence for an  
48  
49 aetiological role of these viruses in respiratory disease. Furthermore, the frequent co-detection of  
50  
51 other known respiratory pathogens and the lack of control groups make it difficult to establish a  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 causal link with clinical symptoms. It might be that KIPyV and WUPyV could play a role in  
5  
6  
7 respiratory disease in at least a subset of immunocompromised patients.  
8  
9  
10

### 11 12 **Acknowledgements**

13  
14 The technical assistance of Ms S. Morassut is acknowledged gratefully. We thank Dr A. M.  
15  
16  
17 Gaynor and Dr T. Allander for kindly providing the WUPyV and KIPyV plasmids and Dr J. Butel  
18  
19  
20 for her assistance with the manuscript.  
21  
22

### 23 **REFERENCES**

- 24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- Abed Y, Wang D, Boivin G. 2007. WU polyomavirus in children, Canada. *Emerg Infect Dis* 13:1939–1341.
- Abedi Kiasari B, Valley PJ, Corless CE, Al-Hammadi M, Klapper PE. 2008. Age-related pattern of KI and WU polyomavirus infection. *J Clin Virol* 43:123-125.
- Allard A, Girones R, Juto P, Wadell G. 1990. Polymerase chain reaction for detection of adenoviruses in stool samples. *J Clin Microbiol* 28:2659–2667.
- Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T, Andersson B. 2007. Identification of a third human polyomavirus. *J Virol* 81:4130–4136.
- Babakir-Mina M, Ciccozzi M, Bonifacio D, Bergallo M, Costa C, Cavallo R, Di Bonito L, Perno CF, Ciotti M. 2009. Identification of the novel KI and WU in human tonsils. *J Med Virol* 46:75–79.

- 1  
2  
3  
4 Babakir-Mina M, Ciccozzi M, Campitelli L, Aquaro S, Lo Coco A, Perno CF, Ciotti M. 2009.  
5  
6 Identification of the novel KI polyomavirus in paranasal and lung tissues. *J Med Virol* 81:558–  
7  
8 561.  
9  
10  
11 Bialasiewicz S, Whiley DM, Lambert SB, Nissen MD, Sloots TP. 2009. Detection of BK, JC,  
12  
13 WU, or KI polyomaviruses in faecal, urine, blood, cerebrospinal fluid and respiratory samples. *J*  
14  
15 *Clin Virol* 45:249–254.  
16  
17  
18 Comar M, Zanotta N, Bovenzi M, Campello C. 2010. JCV/BKV and SV40 viral load in lymphoid  
19  
20 tissues of young immunocompetent children from an area of north-east Italy. *J Med Virol*  
21  
22 7:1236–1240.  
23  
24  
25 Debiaggi M, Canducci F, Brerra R, Sampaolo M, Marinozzi MC, Parea M, Arghittu M,  
26  
27 Alessandrino EP, Nava S, Nucleo E, Romero E, Clementi M. 2010. Epidemiology of KI and WU  
28  
29 Polyomavirus in infants with acute respiratory disease and in adult hematopoietic stem cell  
30  
31 transplant recipients. *J Med Virol* 82:153–156.  
32  
33  
34 Lin F, Zheng M, Li H, Zheng C, Li X, Rao G, Zheng M, Wu F, Zeng A. 2008. Wu polyomavirus  
35  
36 in children with acute lower respiratory tract infection, China. *J Clin Virol* 42:94–102.  
37  
38  
39 Foulongne V, Brieu N, Jeziorski E, Chatain A, Rodière M, Segondy M. 2008. KI and WU  
40  
41 polyomaviruses in children, France. *Emerg Infect Dis* 14:523–525.  
42  
43  
44 Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA,  
45  
46 Sloots TP, Wang D. 2007. Identification of a novel polyomavirus from patients with acute  
47  
48 respiratory tract infections. *PLoS Pathog* 3:e64.  
49  
50  
51 Kean JM, Rao S, Wang M, Garcea RL. 2009. Seroepidemiology of human polyomaviruses. *PLoS*  
52  
53 *Pathog* 5:e1000363.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS. 2005. Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. *J Clin Virol* 34:52–62.

Neske F, Blessing K, Ullrich F, Protzel A, Kreth HW, Weissbrich B. 2008. Wu polyomavirus infection in children, Germany. *Emerg Infect Dis* 14:680–681.

Norja P, Ubillos I, Templeton K, Simmonds P. 2007. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. *J Clin Virol* 40:307–311.

Patel NC, Vilchez RA, Killen DE, Zanwar P, Sroller V, Eldin KW, López-Terrada D, Butel JS. 2008. Detection of polyomavirus SV40 in tonsils from immunocompetent children. *J Clin Virol* 43:66–72.

Ren L, Gonzalez R, Xu X, Li J, Zhang J, Vernet G, Paranhos-Baccalà G, Jin Q, Wang J. 2009. Wu polyomavirus in fecal specimens of children with acute gastroenteritis, China. *Emerg Infect Dis* 15:134–135.

Valle L, Amicizia D, Bacilieri S, Banfi F, Riente R, Durando P, Sticchi L, Gasparini R, Esposito C, Icardi G, Ansaldi F. 2006. Performance testing of two new one-step real time PCR assays for detection of human influenza and avian influenza viruses isolated in humans and respiratory syncytial virus. *J Prev Med Hyg* 47:127–133.

Wattier RL, Vázquez M, Weibel C, Shapiro ED, Ferguson D, Landry ML, Kahn JS. 2008. Role of human polyomavirus in respiratory tract disease in young children. *Emerg Infect Dis* 14:1766–1768.

Zhang WD, Evans DH. 1991. Detection and identification of human influenza viruses by the polymerase chain reaction. *J Virol Methods* 33:165–189.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review