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Prevalence and molecular characterization of WU/KI polyomaviruses isolated from pediatric patients with respiratory disease in Thailand

Sunchai Payungporn^a, Thaweesak Chieochansin^b, Chittima Thongmee^b, Rujipat Samransamruajkit^b, Apiradee Theamboolers^b, Yong Poovorawan^{b,*}

^a Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

^b Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Rama IV Road, Patumwan, Bangkok 10330, Thailand

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ABSTRACT

WU and KI polyomaviruses represent novel viruses discovered in respiratory secretions from human patients with acute respiratory tract infection. However, the association between WU/KI polyomaviruses and human disease has remained unclear. In this study, the prevalence of these two novel viruses and occurrence of co-infection with other respiratory viruses were determined in Thai pediatric patients with respiratory disease. Previously described PCR assays were applied to detect WU/KI polyomaviruses as well as other respiratory viruses in 302 nasopharyngeal suction specimens collected from February 2006 through February 2007. The results revealed the annual prevalence of WU and KI polyomaviruses in the Thai population was 6.29% and 1.99%, respectively. The frequency of co-detection of WU and KI polyomaviruses with other respiratory viral pathogens was 42.11% and 33.33%, respectively. Moreover, each of the two complete genome sequences of WU (CU_295 and CU_302) and KI (CU_255 and CU_258) polyomaviruses were genetically and phylogenetically characterized. Sequence analysis showed that they contained features common to those found in previous studies. However, there were several nucleotide variations within the non-coding regulatory regions and various non-synonymous mutations within the coding regions which may influence virulence and pathogenesis of these viruses. Nevertheless, it is still possible that these viruses are not the causative agents of clinical respiratory disease. Therefore, judging the association of WU/KI polyomavirus infections with a particular disease will be challenging and require more comprehensive case control investigations.

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1. Introduction

Globally, respiratory virus infections are responsible for significant morbidity and mortality, especially in children (Mulholland, 2003). The viruses associated with respiratory disease that are well recognized in human populations include influenza viruses, parainfluenza viruses, rhinoviruses, respiratory syncytial viruses, adenoviruses, human coronaviruses, human metapneumoviruses and human bocaviruses. Recently, KI polyomavirus (Allander et al., 2007) and WU polyomavirus (Gaynor et al., 2007) were identified in respiratory secretions from human patients with acute respiratory tract infection.

Polyomaviruses are small (40–50 nanometers in diameter) DNA viruses belonging to the *Polyomaviridae* family. They are non-enveloped icosahedral particles containing a circular dou-

ble stranded DNA genome of approximately 5000 base pairs in length. They can infect a number of hosts of various species such as birds, rodents and primates. Four distinct polyomaviruses – JC (John Cunningham) virus, BK virus, KI (Karolinska Institute) virus and WU (Washington University) virus – have been reported to infect humans. Both JC virus and BK virus were discovered in 1971 (Gardner et al., 1971 and Padgett et al., 1971) and are genetically closely related to each other. They usually cause asymptomatic infections; yet, they can become oncogenic or induce disease in immunosuppressed or immunocompromised patients (Khalili et al., 2006; Hirsch et al., 2006; Fioriti et al., 2005).

Two novel, closely related polyomaviruses (KI and WU) were recently identified in respiratory secretions from children with acute respiratory tract infections (Allander et al., 2007 and Gaynor et al., 2007). Thus, KI and WU polyomaviruses were reported as novel viruses potentially causing respiratory disease in humans. However, both KI and WU polyomaviruses were frequently co-detected with other respiratory viruses. Moreover, a previous study reported that no evidence for an aetiological link between KI

* Corresponding author. Tel.: +66 2256 4909; fax: +66 2256 4929.
E-mail address: Yong.P@chula.ac.th (Y. Poovorawan).

or WU polyomavirus infections and respiratory disease (Norja et al., 2007). Therefore, the clinical significance of KI and WU polyomaviruses requires further investigation. This study was aimed at establishing prevalence and clinical association of KI and WU polyomavirus infections in children with respiratory disease in Thailand. Moreover, KI and WU polyomaviruses isolated in Thailand were subjected to genetic and phylogenetic analysis.

2. Materials and methods

2.1. Clinical samples

At King Chulalongkorn Memorial Hospital, Thailand, nasopharyngeal suction specimens were collected from 302 hospitalized pediatric patients (age range 5 days to 14 years) with respiratory illness from February 2006 through February 2007. NP suction samples were collected in 1 ml of transport medium with antibiotics (Penicillin G 2×10^6 U/l and Streptomycin 200 mg/l) and stored at -70°C until tested. The study was approved by the ethics committee of Chulalongkorn University and informed consent was obtained from the patient's parents.

2.2. Nucleic acid extraction and cDNA synthesis

DNA and RNA were extracted using TRI REAGENT[®] LS (Molecular Research Center, Inc., Cincinnati, OH) and the resulting pellets were dissolved in 20 μl of 8 mM NaOH solution or DEPC-treated water for DNA or RNA, respectively. Reverse transcription (RT) was

performed at 37°C for 2 h using the M-MLV reverse-transcription system (Promega, Madison, WI) consisting of 200 units of M-MLV reverse transcriptase, 5 μl of $5\times$ M-MLV reaction buffer, 5 μl of 10 mM dNTP, 25 units of RNasin[®] ribonuclease inhibitor, 0.5 $\mu\text{g}/\mu\text{l}$ of random hexamer, 0.5 μg of RNA and nuclease-free water to a final volume of 25 μl .

2.3. Detection of respiratory viruses by PCR amplification

PCR amplifications of KI and WU polyomaviruses were performed by using specific primer sets described in previous publications (Allander et al., 2007 and Gaynor et al., 2007). In addition, PCR amplifications of other respiratory viruses including human metapneumovirus, human bocavirus, influenza A virus, influenza B virus, parainfluenza virus, adenovirus and respiratory syncytial virus were performed by using specific primers described in previous reports (Samransamruajkit et al., 2006; Allander et al., 2005; Chieochansin et al., 2008; Chutinimitkul et al., 2008; Chi et al., 2005; Krafft et al., 2005; Samransamruajkit et al., 2002). Amplification of the human GAPDH gene served as an internal control of specimen quality and integrity. The limits of detection were approximately 10^3 copies/ μl and 10 copies/ μl for conventional PCR and nested PCR, respectively. All specific primers used in this study are summarized in Table 1.

The conventional PCR reaction mixture comprised 2 μl of DNA or cDNA, 0.5 μM of each primer, 10 μl of $2.5\times$ Eppendorf MasterMix (Eppendorf, Hamburg, Germany), and nuclease-free water to a final volume of 25 μl . The amplification reactions

Table 1
Primers used for PCR amplification of specific viruses

Target	Oligo name	Sequence (5' → 3')	Position	Gene	Product size (bp)	References
WU polyomavirus	WU_925F	5'-GCAGGATACAATCCCCAAGGA-3'	925–948	VP2	656	Modified from Gaynor et al. (2007) Gaynor et al. (2007)
	WU_1580R	5'-GCTGCATAATGGGGAGTACC-3'	1580–1561			
	WU_1331F	5'-TGTTACAAATAGCTGCAGGTCAA-3'	1331–1353	VP2	250	
	WU_1580R	5'-GCTGCATAATGGGGAGTACC-3'	1580–1561			
KI polyomavirus	KI_1534F	5'-ACAAGGCCAAGAAGTCAAGTTC-3'	1534–1555	VP1	329	Allander et al. (2007)
	KI_1862R	5'-TCACACTACTAACTTGATTTGG-3'	1862–1840			
	KI_1613F	5'-CAGTACCCTGTGAGAGAAAC-3'	1613–1634	VP1	208	
	KI_1821R	5'-TTCTGCCAGGCTGTAACATAC-3'	1821–1801			
Human Metapneumovirus	MPVP_F	5'-ACGGGGTAGAGAAGAGCTGG-3'	389–408	N	616	Samransamruajkit et al. (2006)
	MPVP_R	5'-GCAAAGTTGGGACAGTTGGC-3'	1004–985			
	MPVN_F	5'-GCATCAACCATAGAAGTGGGAC-3'	556–577	N	259	
	MPVN_R	5'-GCATTGTTTGACCGGCCCA-3'	814–795			
Human Bocavirus	HBoV_188F	5'-GACCTCTGTAAGTACTATTAC-3'	2351–2371	NP1	354	Allander et al. (2005)
	HBoV_542R	5'-CTCTGTGTTGACTGAATACAG-3'	2704–2684			
	HBoV_VPF2	5'-TTCAGAATGGTCACCTCTACA-3'	3639–3659	VP1	648	
	HBoV_VPR2	5'-CTGTGCTTCCGTTTTGTCTTA-3'	4286–4266			
Influenza A virus	FluA_M.F	5'-RGGCCCTCAAAGCCGA-3'	76–93	M1	160	Chutinimitkul et al. (2008)
	FluA_M.R	5'-ACTGGGCACGGTGAGYGT-3'	235–218			
Influenza B virus	FluB_M.F	5'-ATGTCGCTGTTGGAGACACAAT-3'	25–47	M1	295	Chi et al. (2005)
	FluB_M.R	5'-TCAGCTAGAATCAGRCYTTCTT-3'	320–301			
Parainfluenza virus	Para_F	5'-GCTAAACTACTGCTTMAHTGGAGAT-3'	11254–11278	L	139	Chieochansin et al. (2008)
	Para_R	5'-GTAAGGATCACWCATADAWTGTA-3'	11392–11370			
Adenovirus	Adeno_F	5'-GATGGCCACCCATCGATGMTGC-3'	18227–18249	L3	96	Krafft et al. (2005)
	Adeno_R	5'-GCGAACTGACCCAGACCCGGAC-3'	18324–18303			
Respiratory syncytial virus (type A and B)	RSV_AB-F	5'-GTCTTACAGCCGTGATTAGG-3'	1673–1692	N	838	Samransamruajkit et al. (2002)
	RSV_AB-R	5'-GGGCTTTCTTTGGTACTTC-3'	2510–2491			
Respiratory syncytial virus (type A)	RSV_A.F	5'-GATGTTACGGTGGGGAGTCT-3'	1908–1927	N	343	
	RSV_A.R	5'-GTACACTGTAGTTAATCACA-3'	2242–2223			
Respiratory syncytial virus (type B)	RSV_B.F	5'-AATGCTAAGATGGGGAGTTC-3'	1905–1923	N	183	
	RSV_B.R	5'-GAAATTGAGTTAATGACAGC-3'	2098–2070			
Internal control	GAPDH.F	5'-GTGAAGTCCGGAGTCAACGG-3'	112–131	Human GAPDH	492	Chutinimitkul et al. (2008)
	GAPDH.R	5'-GTTGTCATGGATGACCTTGGC-3'	603–583			

were performed in a thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 94°C for 3 min, followed by 40 amplification cycles consisting of 94°C for 30s, 50–60°C (depending on the specific primer pair) for 30s, and 72°C for 1 min, and concluded by a final extension at 72°C for 7 min. After 2% agarose gel electrophoresis, the expected amplified products (Table 1) were stained with ethidium bromide and then visualized under UV transillumination.

2.4. Amplification of complete genome sequences of WU/KI polyomaviruses

In order to study the genome characteristics of WU and KI polyomaviruses isolated in Thailand, whole genome sequencing was performed on two specimens of WU polyomaviruses (CU_295 and CU_302) and two specimens of KI polyomaviruses (CU_255 and CU_258). The complete genomes of WU/KI polyomaviruses were amplified directly from clinical samples using four primer pairs to generate overlapping fragments of the circular genome. All primers used for whole genome sequencing were designed from regions conserved among WU and KI polyomaviruses as summarized in Table 2. The reaction mixture was prepared using the Eppendorf MasterMix (Eppendorf, Hamburg, Germany) as described above. The amplification reactions were performed in a thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation at 94°C for 3 min, followed by 40 amplification cycles consisting of 94°C for 30s, 55°C for 30s, and 72°C for 2 min, and concluded by a final extension at 72°C for 7 min. After 2% agarose

Table 2

Conserved primers for whole genome amplification and sequencing of WU/KI polyomaviruses

Oligo name	Sequence (5' → 3')	Position ^a	Product (bp)
WU/KI.4969F	TTGCAGTTTTAAATATAAAGAATTAAG	4969–4995	1395
WU/KI.761R	ACAGTAGYAGCTGCTTCAGATAT	761–739	
WU/KI.1134R	GGCAATTCTATTGAACACGTCTC	1134–1112	
WU/KI.989F	CCCCCTACYCAGGAATGGCA	989–1008	1560
WU/KI.1990R	ATTWGGWATATCAGGGGGAGCAA	1990–1968	
WU/KI.2548R	CCTTCTTTGCTCAAARTGTAGCCTA	2548–2524	
WU/KI.2407F	ACTRTTGGATGAAAATGGCATTGG	2407–2430	1695
WU/KI.2870F	GCAAAYTCAGTAAGGCCTATATA	2870–2892	
WU/KI.4101R	CATAGAGTTAGTGCTRTTAATAACTT	4101–4076	
WU/KI.3780F	TACTAYTGCATTTTTACACTCTTC	3780–3804	1389
WU/KI.4703R	TGTGGATAGAGTGYTACTGCTA	4703–4682	
WU/KI.5168R	AAGAAGGCAAATGGATAAACTTT	5168–5144	

^a Position based on nucleotide sequence of WU polyomavirus (NC.009539).

gel electrophoresis, the expected amplified products were purified and subsequently sequenced using 12 primers as summarized in Table 2.

2.5. Nucleotide sequencing

For identification of nucleotide sequences, the PCR amplified products were purified using the Perfectprep Gel Cleanup kit (Eppendorf, Hamburg, Germany) according to the manufacturer's specifications. The resulting purified DNAs served as templates for DNA sequencing in the ABI PRISM® 310 automated DNA sequencer with the Big Dye Terminator V.3.0 Cycle Sequencing Ready Reac-

Table 3
Clinical characteristics of WU/KI polyomavirus positive patients

Patient No.	Sex	Age	Date ^a (d/m/y)	Hospitalized days	Result	Co-infected viruses	Clinical signs and symptoms	Accession number
CU.4	M	7 months	16/02/06	15	WU	Adenovirus	Severe bronchopneumonia	EU358745
CU.5	F	3 years	22/02/06	4	WU	–	Pneumonia	EU358746
CU.12	M	1 year	08/03/06	2	WU	–	Pneumonia	EU358747
CU.32	F	3 years	13/04/06	6	WU	Flu A	Pneumonia	EU358748
CU.38	F	1 year	28/04/06	3	WU	Adenovirus	Pneumonia	EU358749
CU.50	M	1 year	29/05/06	14	WU	–	Pneumonia	EU358750
CU.62	M	1 year	16/06/06	3	WU	–	Pneumonia	EU358751
CU.76	M	2 years	10/07/06	4	WU	–	Pneumonia (congenital musculoskeletal deformities)	EU358752
CU.84	M	3 months	14/07/06	4	KI	–	Pneumonia	EU358762
CU.93	M	5 months	31/07/06	4	WU	RSV	Pneumonia	EU358753
CU.104	F	9 years	27/07/06	45 (dead)	KI	MPV	Pneumonia, Germinoma of brain	EU358763
CU.141	M	1 year	29/08/06	12	WU	Adenovirus	Pneumonia (gastro-esophageal reflex)	EU358754
CU.182	F	2 years	04/10/06	4	WU	–	Pneumonia, Growth retardation	EU358755
CU.187	F	9 months	04/10/06	5	WU	Adenovirus	Croup	EU358756
CU.199	M	3 years	17/10/06	10	WU	–	Pneumonia, Down's syndrome	EU358757
CU.205	M	1 year	17/10/06	2	WU	Bocavirus	Acute bronchiolitis	EU358758
CU.214	M	2 years	27/10/06	6	WU	–	Asthmatic bronchitis	EU358759
CU.255	M	1 year	17/12/06	2	KI	–	Acute bronchiolitis	EU358766
CU.257	F	1 year	22/12/06	14	KI	–	Pneumonia	EU358764
CU.258	M	1 year	15/12/06	79	KI	–	Pneumonia (cirrhosis)	EU358767
CU.262	M	3 years	03/01/07	3	WU	Flu A	Pneumonia	EU358760
CU.265	M	1 year	10/01/07	5	KI	Bocavirus	Neuroblastoma pneumonia	EU358765
CU.276	M	1 year	18/01/07	8	WU	–	Bronchopneumonia	EU358761
CU.295	F	1 year	17/02/07	3	WU	–	Acute bronchiolitis	EU358768
CU.302	M	1 year	28/02/07	5	WU	–	Pneumonia	EU358769

^a Date of specimen collection.

tion kit (ABI, Foster City, CA). Determination of the nucleotide sequences were performed in duplicate and analyzed by both direction using forward and reverse primers to ensure that variations of nucleotide sequences were not due to sequencing errors. When a difference was observed, triplicate sequences were determined in order to confirm the consistency of the sequencing result.

2.6. Sequencing analysis and phylogenetic tree construction

Nucleotide sequences were analyzed and assembled using the Lasergene 6 Package® (DNASTAR, Inc., Madison, WI, USA) and BLAST analysis tool (<http://www.ncbi.nlm.gov/BLAST>). Complete genome sequences were prepared and aligned using Clustal W implemented in the BioEdit program (version 7.0.4.1). Phylogenetic trees were constructed by neighbor-joining analysis with the Tamura-Nei model implemented in the MEGA3® program (Kumar et al., 2004). All nucleotide sequences of WU/KI polyomavirus obtained from this study were submitted to the GenBank database and assigned accession numbers EU358745–EU358769 (Table 3).

2.7. Statistical analysis

Statistical data were analyzed using SPSS for Windows version 11.5 software package. Statistical analyses were performed using Pearson χ^2 for determination of seasonal distribution of WU and KI polyomaviruses. The results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Prevalence of WU polyomavirus in Thailand

PCR amplification and subsequent nucleotide sequencing demonstrated that 19 of the 302 nasopharyngeal suction specimens were positive for WU polyomavirus, amounting to a prevalence of 6.29% in this sample population. Single infection with WU polyomavirus was detected in 11 of the 19 positive cases (57.89%), whereas 8 cases (42.11%) showed evidence of co-infection with other respiratory viruses. Four cases were co-infected with adenovirus, two with influenza A virus, one with respiratory syncytial virus and one with human bocavirus (Tables 3 and 4).

3.2. Prevalence of KI polyomavirus in Thailand

KI polyomavirus was detected in 6 of the 302 respiratory specimens, indicating a prevalence of 1.99% in the Thai population. Single infection with KI polyomavirus was detected in four of six positive specimens (66.67%), whereas the remaining two cases

Table 4
Co-infection of WU/KI polyomavirus with other respiratory viruses

	Positive cases (% of positive)	
	WU polyomavirus	KI polyomavirus
Single infection	11 (57.81%)	4 (66.67%)
Co-infection with		
Adenovirus	4 (21.05%)	0
Influenza A virus	2 (10.53%)	0
Influenza B virus	0	0
Parainfluenza virus	0	0
Human Metapneumovirus	0	1 (16.67%)
Human Bocavirus	1 (5.26%)	1 (16.67%)
Respiratory syncytial virus	1 (5.26%)	0
Total	19	6

(33.33%) were co-infected with human metapneumovirus and human bocavirus, respectively (Tables 3 and 4).

3.3. Clinical presentation in WU/KI polyomavirus positive patients

Patient positive for WU or KI polyomavirus suffered from a wide range of respiratory symptoms including bronchopneumonia, croup, bronchiolitis, bronchitis and pneumonia (Table 3). Most of the patients (11 of 15) only infected with WU or KI polyomaviruses showed clinical signs associated with pneumonia. However, associations between the presence of WU/KI polyomaviruses and respiratory diseases remain highly speculative due to the lack of comprehensive case control studies. Although WU/KI polyomavirus positive specimens were detected in subjects ranging in age from 3 months to 9 years, the majority were found in approximately 1-year-old babies (Table 3). There was no significant seasonal variation for WU polyomavirus infection ($P = 0.11$), but a predominance of infection was detected during the winter months (November–January in Thailand) for KI polyomavirus ($P = 0.02$), respectively (Fig. 1).

3.4. Complete genome analysis of WU polyomavirus in Thailand

Whole genome sequences of WU polyomaviruses found in Thai patients (isolates CU.295 [EU358768] and CU.302 [EU358769]) were aligned with Australian isolates [EF444549–EF444554] (Gaynor et al., 2007). Analysis of WU polyomavirus nucleotide sequences showed 99.6–99.9% similarity among the Thai and Australian isolates. Phylogenetic analysis revealed that WU polyomaviruses isolated in Thailand (CU.295 and CU.302) were closely related to the reference strain of WU polyomavirus [NC_009539 or EF444549] (Fig. 2A). Additionally, there were numerous variations of the nucleotide sequences within non-coding regions including

Table 5
Variations of nucleotide sequences within non-coding regions and amino acid sequences within coding regions of WU polyomaviruses

Accession no./isolate name	Variations of nucleotide sequences in non-coding regions						Variation of deduced amino acid in coding sequences									
							VP2				VP1		LTAg			
	4	32	286	2797	4522	4559	239	250	284	256	79	360	378	440	520	594
NC.009539/WU Ref strain	t	g	c	a	a	t	E	E	D	T	T	D	D	V	G	I
EF444549/WU strain B0	t	g	c	a	a	t	E	E	D	T	T	D	D	V	G	I
EF444550/WU strain S1	t	g	c	a	g	t	E	Q	D	T	T	D	D	V	G	L
EF444551/WU strain S2	t	g	c	a	g	t	E	Q	D	T	T	D	D	V	E	L
EF444552/WU strain S3	t	g	c	t	g	t	E	Q	D	T	T	G	D	V	G	L
EF444553/WU strain S4	t	c	c	a	g	t	E	Q	D	T	T	D	D	V	G	L
EF444554/WU strain S5	c	g	c	a	g	t	E	Q	H	T	T	D	G	I	G	L
EU358768/WU Thailand CU.295	t	g	c	a	g	t	E	E	D	T	T	D	D	V	G	I
EU358769/WU Thailand CU.302	t	g	g	a	g	a	Q	E	D	I	G	D	D	V	G	I

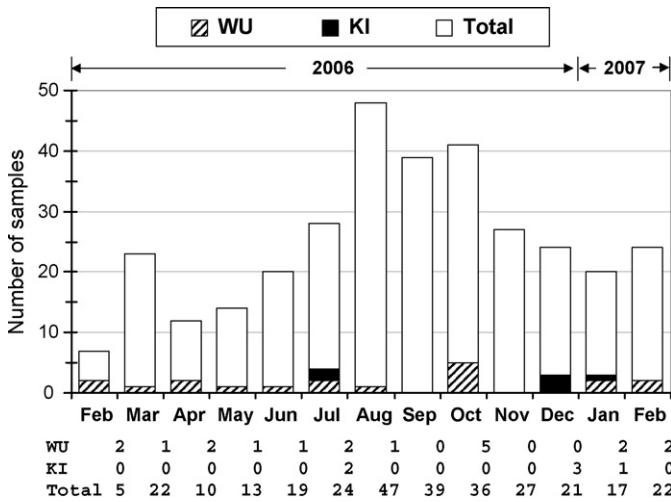


Fig. 1. Seasonal variation and WU/KI polyomavirus infection. Bar graph shows number of WU or KI positive samples as well as total number of clinical specimens collected from 302 pediatric patients with respiratory disease in each month from February 2006 through February 2007.

nucleotide positions 4, 32, 286, 2797, 4522 and 4559 (Table 5). Furthermore, analysis of deduced amino acids within the coding sequences revealed that several variations had occurred within coding sequences including the VP2 protein (amino acid positions 239, 250, 284 and 356), VP1 protein (amino acid position 79), and Large T Antigen (amino acid positions 360, 378, 440, 520 and 594) as shown in Table 5.

3.5. Complete genome analysis of KI polyomavirus in Thailand

The complete genome sequences of KI polyomaviruses isolated from Thai patients (isolates CU.255 [EU358766] and

CU.258 [EU358767]) were aligned with Swedish sequences [EF127906–EF127908] (Allander et al., 2007) and Queensland sequences [EF520287–EF520289] (Bialasiewicz et al., 2007). Nucleotide sequence analysis of KI polyomaviruses revealed 99.4–99.9% identity among the Thai, Queensland and Swedish isolates. Phylogenetic analysis showed that one of the KI polyomavirus isolated from Thailand (CU.258) was closely related to the reference strain of KI polyomavirus [NC.009238 or EF127906] whereas the second one (CU.255) was more closely associated with KI polyomavirus isolated from Queensland (isolate Brisbane 001 [EF520287]) (Fig. 2B). The latter similarity was largely due to a 10 nucleotide insertion (AGGCGCTGCG) at positions 60–69 within the non-coding region which the Thai isolate CU.255 and Queensland isolate Brisbane 001 shared (Table 6). Moreover, there were several variations of the nucleotide sequences within non-coding regions as for example, at nucleotide positions 76, 107, 110, 233 and 4656 (Table 6). Analysis of deduced amino acids within the coding sequences showed a variety of variations within coding sequences for the VP2 protein (amino acid positions 246 and 316), the VP1 protein (amino acid positions 181, 267 and 368), the Large T Antigen (amino acid positions 124, 199, 337, 365 and 494) and the small T Antigen (STAg) (amino acid position 104) as shown in Table 6.

3.6. Characterization of the non-coding regulatory region

Alignment of the non-coding regulatory regions of WU and KI polyomaviruses revealed AT-rich sequences on the downstream of the putative replication origin. Both Thai WU polyomavirus isolates (CU.295 and CU.302) contained an A/T rich region (TTTTTTTATTATAATAA) common to the Australian isolates [EF444549–EF444554] whereas both Thai KI polyomavirus isolates (CU.255 and CU.258) contained an A/T rich region (TTTTTTTCTATATTACA) similar to the Swedish sequences [EF127906–EF127908] and the Queensland sequences

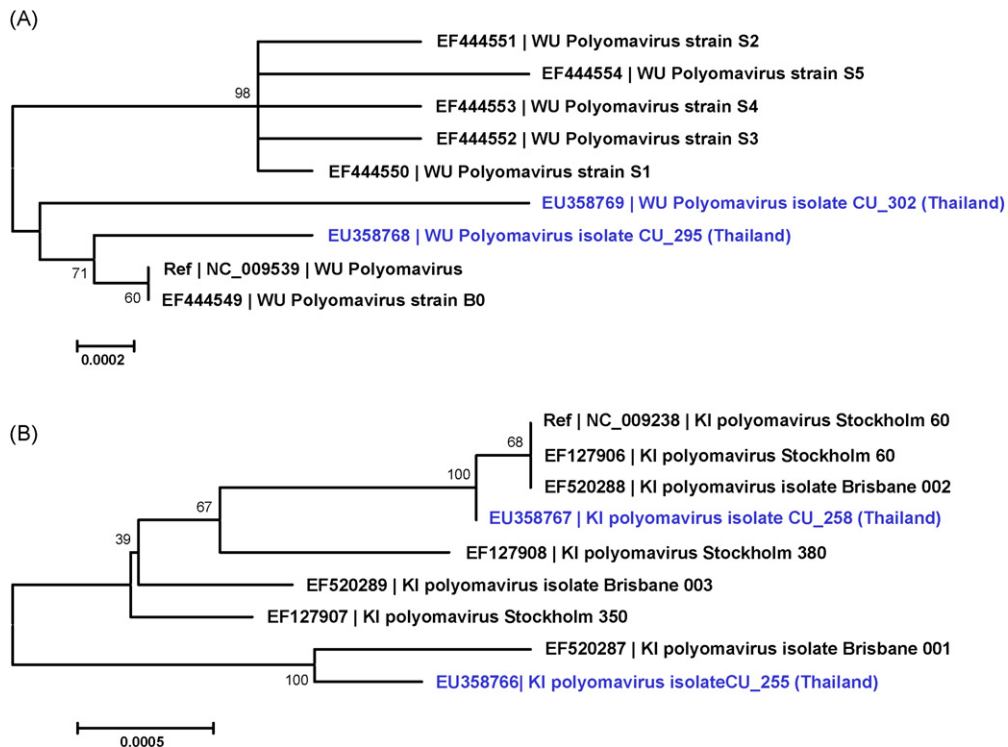


Fig. 2. Phylogenetic tree constructed by neighbor-joining analysis with the Tamura-Nei model implemented in the MEGA3[®] program (version 3.1). Nodal confidence values indicate the results of bootstrap re-sampling (n = 1000). (A) Phylogenetic tree of whole genome sequences of WU polyomaviruses. (B) Phylogenetic tree of whole genome sequences of KI polyomaviruses.

Table 6
Variations of nucleotide sequences within non-coding regions and amino acid sequences within coding regions of KI polyomaviruses

Accession no./isolate name	Variations of nucleotide sequences in non-coding regions						Variation of deduced amino acid in coding sequences										
	60	76	107	110	233	4656	VP2	316	VP1	267	368	124	199	337	365	494	STAg
NC-009238/KI Ref strain	-	t	t	t	c	a	T	P	K	Q	A	E	Y	V	L	Q	D
EF127906/KI Stockholm 60	-	t	t	t	c	a	T	P	K	Q	A	E	Y	V	L	Q	D
EF127907/KI Stockholm 350	-	c	c	g	a	t	S	P	K	Q	T	E	Y	V	V	Q	E
EF127908/KI Stockholm 380	-	a	c	g	a	a	T	P	K	Q	A	E	Y	V	V	H	D
EF520287/KI isolate Brisbane 001	aggcgtgctg	t	c	a	a	t	S	S	R	Q	A	E	Y	L	V	Q	E
EF520288/KI isolate Brisbane 002	-	t	t	t	c	a	T	P	K	Q	A	E	Y	V	L	Q	D
EF520289/KI isolate Brisbane 003	-	t	a	g	a	t	S	P	K	H	A	E	Y	V	V	Q	E
EU358766/KI Thailand CU.255	aggcgtgctg	t	c	a	a	t	S	S	R	Q	A	K	H	L	V	Q	E
EU358767/KI Thailand CU.258	-	t	t	t	c	a	T	P	K	Q	A	E	Y	V	L	Q	D

[EF520287–EF520289]. The origin of replication of WU/KI polyomaviruses contains three potential pentanucleotide Large T Antigen (LTag) binding sites, as opposed to the four found in most polyomaviruses. Two of the LTag binding sites have the typical sequence “GAGGC” found in all WU/KI polyomaviruses analyzed, except for the Australian WU polyomavirus strain S5 [EF444554] that contains “GGGGC” at the second LTag binding site. The third consensus LTag binding site found in all isolates of WU polyomaviruses has the sequence “GCCTC” whereas all isolates of KI polyomaviruses display the sequence “CCCCC”.

3.7. Characterization of the Large T Antigen

Analysis of the deduced amino acid sequences of the Large T Antigen revealed domains common to both WU and KI polyomaviruses, including the DnaJ domain, Rb binding domain, zinc finger motif and ATPase-p53 binding domain. All WU/KI polyomaviruses analyzed contained the DnaJ domain at the N terminus with the highly conserved hexapeptide motif “HPDKGG” (amino acid positions 42–47) and the Rb binding domain “LRCNE” at amino acid positions 108–113. The zinc finger motif was found in WU polyomaviruses at amino acid residues C321, C324, H336 and H339 whereas KI polyomaviruses displayed it at amino acid residues C312, C315, H327 and H331 of the LTag protein. Conserved motifs “GPINSGKT” (amino acid positions 443–450) and “GSVKVNLE” (amino acid positions 520–527) were detected in all WU polyomaviruses analyzed whereas the motifs “GPINSGKT” (amino acid positions 436–443) and “GCVEVNLE” (amino acid positions 513–520) were found in all KI polyomaviruses analyzed in this study (Pipas, 1992).

4. Discussion

Resulting from this research, we present here preliminary data on the role of WU/KI polyomaviruses as potential respiratory pathogens in Thai pediatric patients. The prevalence of WU polyomavirus in Thailand (6.29%) was higher than that reported in Australia (4.5%) (Bialasiewicz et al., 2008), the United States (1.2%) (Gaynor et al., 2007) and United Kingdom (1%) (Norja et al., 2007) but comparable to the prevalence reported in a previous study from South Korea (7%) (Han et al., 2007). This may be due to particular racial predispositions or different geographical distributions of the virus. In contrast, the incidence of KI polyomavirus infection in Thailand (1.99%) was comparable to that established in Sweden (1%) (Allander et al., 2007), Australia (2.6%) (Bialasiewicz et al., 2008) and the United Kingdom (1.4%) (Norja et al., 2007).

WU polyomavirus infection does not appear subjected to significant seasonal variation; yet, a predominance of infection was detected during the winter months for KI polyomavirus. These findings were not in perfect agreement with previous studies conducted in Australia (Bialasiewicz et al., 2007, 2008) which reported no apparent seasonal variation for KI polyomavirus, but a predominance of infection with WU polyomavirus during late winter to early summer. This discrepancy may be due to seasonal differences in each country.

The genome of WU (5229 bp in length) and KI (5040 bp in length) polyomaviruses comprises an early coding region on one strand for the Small T Antigen and the Large T Antigen, and a late coding region on the opposite strand for the 3 capsid proteins VP1, VP2, and VP3. These two regions are separated by a regulatory non-coding region that contains typical polyomavirus characteristics (Allander et al., 2007 and Gaynor et al., 2007). Based on complete genome analysis, the WU/KI polyomaviruses isolated from Thai patients were closely related to the isolates described in previous reports (Gaynor et al.,

2007; Allander et al., 2007 and Bialasiewicz et al., 2007). This may be due to the fact that both WU and KI polyomaviruses are DNA viruses which are less error prone in the DNA replication process. However, there were numerous nucleotide variations within the non-coding regulatory regions as well as several non-synonymous mutations within coding regions.

No other respiratory virus was detected in 57.81% of the WU-positive specimens and 66.67% of the KI-positive specimens in our study, hinting at an association between WU/KI polyomaviruses and respiratory disease. However, these viruses may not be the causative agents of clinical respiratory disease and their presence in the respiratory tract may simply reflect their mode of transmission similar to other polyomaviruses in humans such as JCV and BKV. Nevertheless, most known human viruses are pathogenic in certain conditions and thus, novel viruses must be considered as potential pathogens. Various degrees of clinical symptoms ranging from asymptomatic to mild and severe illness depend on several factors, such as the host's immune status and age, the virus strain, viral load and mode of transmission. The 10-nucleotide insertion and several nucleotide sequence variations within non-coding regulatory regions as well as various non-synonymous mutations within the coding regions should be taken into consideration as factors potentially involved in the pathogenesis of these novel viruses. The difficulty with persisting viruses is that they are often discovered out their symptomatic context, so that judging the association of WU/KI polyomavirus infections with a particular disease will be challenging and require further extensive case control investigations.

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