Complete Genome Characterization and Phylogenetic Analysis of WU Polyomavirus in Thai Pediatric Patients with Respiratory Tract Infections in 2013

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Background: *The WU polyomavirus (WUPyV) is a small DNA virus (family Polyomaviridae) that contains a circular double-stranded DNA genome, approximately 5 kb in length. WUPyV was first discovered in the respiratory tract of children with acute respiratory symptoms.*
Objective: *This study focuses on the complete genome characterization and phylogenetic analysis of WUPyV obtained from Thai patients with respiratory diseases in 2013.*
Material and Method: *DNA was extracted from nasopharyngeal (NP) suction specimens (n = 614) from patients with respiratory tract infections. WUPyV was detected by using semi-nested PCR and then characterized by whole genome sequencing. The nucleotides and deduced amino acid sequences were analyzed by multiple sequences alignment and phylogenetic tree.*
Results: *Analysis revealed that 0.16% (1/614) of the sample was positive for WUPyV. Phylogenetic trees demonstrated that WUPyV (isolate CU_Chonburi 3) was closely related to previously described WUPyV. Moreover, whole genome sequences alignment of WUPyV showed several nucleotide variations within non-coding regions and amino acid changes in VP1 (position S347T); VP2 (positions L40V, G120R, Y121I, P123R, G127S, L137F, Q287R, and A327V); LTAg (positions Q357P, V369E, E377K, D378V, A381T, R382E, R383G, and D389G); and, STAg (positions R139S, K141E, R148K, and W153C).*
Conclusion: *Nucleotide variations within non-coding regions and critical amino acid substitutions in viral proteins may affect the rate of viral replication and viral adaptation; factors that may be linked to the susceptibility and severity of viral infection. Data obtained from this study may be useful in better understanding the genetic characterization and mutation patterns of WUPyV.*

Keywords: *WU polyomavirus, Characterization, Phylogenetic, Thailand*

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Respiratory tract infection is a major cause of hospitalization in infants and young children. The common respiratory viruses include influenza viruses, parainfluenza virus, rhinovirus, respiratory syncytial virus, coronaviruses, adenoviruses, and metapneumovirus(1). Novel viruses that may cause respiratory illness have recently been identified. In 2007, WU (Washington University) polyomavirus (WUPyV) was first discovered in the respiratory tract of children with acute respiratory symptoms(2). WUPyV is a small DNA virus belonging to the *Polyomaviridae* family. It contains a circular double-stranded DNA genome that is 5,229 bp in length(3). The genome consists of three functional regions, including early, late, and non-coding regions. The early region encodes regulatory proteins, including large T antigen (LTAg) and small T antigen (STAg), which are responsible for viral DNA replication and gene expression. The late region encodes three capsid proteins: VP1, VP2, and VP3. The non-coding region encompasses the origin of replication and transcription control elements(3). Accordingly, this virus contains 5 functional proteins: LTAg, STAg, VP1, VP2, and VP3. The large T-antigen is required for viral DNA replication, virion assembly, and transcription. The small T-antigen protein is also able to activate several cellular pathways that stimulate cell proliferation. The three capsid proteins include a major coat protein, VP1, and two minor coat proteins, VP2 and VP3.

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WU polyomavirus was observed in clinical specimens obtained from children with respiratory tract infection in several countries, including Australia (2.97%)[2], United Kingdom (1.02%)[4], France (2.42%)[5], China (2%)[6], Thailand (6.29%)[7], South Korea (6.99%)[8], and Canada (6.41%)[9]. The prevalence and molecular characterization of WUPyV in Thai patients were previously reported in 2008[7]. However, mutations in the viral genome may have occurred since that time. Therefore, this study focused on the complete genome characterization of WUPyV obtained from Thai pediatric patients with respiratory disease in 2013. Those findings were then compared with previously described characterizations of WUPyV. The comparative findings may provide insights into the viral mutation and genetic variability of WUPyV that may be responsible for the virulence and pathogenesis of this virus.

Material and Method

Clinical samples

Nasopharyngeal (NP) suction samples (n = 614) were collected from patients with influenza-like illness at King Chulalongkorn Memorial Hospital and Chonburi Hospital, Thailand. The protocol for this study was approved by the Institutional Review Board (IRB No. 457/56), Faculty of Medicine, Chulalongkorn University.

Detection of WUPyV by semi-nested PCR amplification

DNA was extracted from 100 µL of NP suction using a HiYield™ Viral Nucleic Acid Extraction kit (Real Genomics, USA), according to manufacturer protocol. Semi-nested PCR amplification within LTAg of WUPyV was performed using specific primers. The first round of PCR was amplified by WU_4337F: 5′-CATTATTAACWCCTTTACARAATAA-3′ and WU_4585R: 5′-TGTC WCAWGCTGTATTTAGTAA TA-3′; whereas, the second PCR was performed by WU_4390F: 5′-AAG TTATTAAYAGCACTAACTCTA TG-3′ and WU_ 4585R.

The expected semi-nested PCR product of WUPyV was approximately 215 bp. Moreover, amplification of the GAPDH gene (199 bp) using GAPDH_F404: 5′-CTTCACCACCATGGAGAAGG-3′ and GAPDH_R603: 5′-GTTGTCATGGATGACC TTGGC-3′ served as the internal control for detection.

Whole genome amplification of WUPyV

The complete genome of WUPyV was amplified directly from clinical samples, using 4 primer pairs in order to generate overlapping PCR products of the circular genome. All primers used for whole genome amplification were designed from nucleotide conserved regions from different strains of WUPyV, as previously described[7].

PCR conditions

The PCR reaction mixture comprised 1 µl of DNA, 2.5 µl of 10xPCR buffer minusMg, 0.5 µl of 10 mM dNTPs mixture, 0.75 µl of 50 mM $MgCl_2$, 0.5 µM of each primer, and 19.25 µl of distilled water for a final volume of 25 µl. Thermal profiles are described, as follows: initial denaturation at 94°C for 3 minutes; followed by, 40 amplification cycles consisting of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1.30 minutes; and, a final extension at 72°C for 7 minutes. Positive and negative controls were included in each run to ensure suitable detections.

Gel electrophoresis and nucleotide sequencing

PCR products were separated by 2% agarose gel electropholysis and then visualized by ethidium bromide staining under UV transillumination. The PCR amplified products were then purified using HiYield ™ Gel Extraction (RBC Bioscience, Taiwan). Nucleotide sequencing was then performed by First BASE Laboratories Sdn Bhd, Selangor, Malaysia.

Nucleotide sequences analysis

Nucleotide sequences were analyzed using the BLAST analysis tool (http://www.ncbi.nlm.gov/BLAST). Complete genome sequences were assembled using the contig assembly program (CAP) and aligned using ClustalW software, and implemented using the BioEdit Sequence Alignment Editor (version 7.0.4.1). The complete genome sequence of WU polyomavirus obtained from the present study was submitted to the GenBank database and assigned accession number: KJ725028. Other genome sequences of WUPyV analyzed in this study were obtained from the GenBank database, including: WUPyV reference strain (NC_009539); USA (EF444554 and FJ794068); Thailand (EU358768 and EU358769); China (EU296475, GQ926980, and FJ890981); Germany (EU711058); and, Australia (GU296363, GU296405, and GU296408). Nucleotide sequences were selected according to year and country of isolation. Redundant sequences were excluded from this analysis. The similarity of genome sequences between sample and WUPyV reference sequence were analyzed by Simplot version 3.5.1 (http://sray.med.som.jhmi.edu/SCRoftware/simplot).

Phylogenetic analysis

Phylogenetic trees were constructed by neighbor-joining (NJ) method with bootstrap (1000) and Tamura-Nei nucleotide substitution model implemented in Molecular Evolutionary Genetics Analysis (MEGA) program version 5.2 (http://www.mybiosoftware.com/phylogenetic-analysis/2334). All WUPyV genome sequences described above were included in the phylogenetic tree.

Results

Detection of WUPyV

The detection of WUPyV from Thai patients with respiratory diseases in 2013 revealed that 0.16% (1/614) of the sample was positive for WUPyV. BLAST analysis confirmed that this positive sample was closely related to WUPyV. This sample was assigned as WUPyV isolate CU_Chonburi 3 and the complete genome was further characterized. Other samples were negative for WUPyV, but were positive for GAPDH internal control; a result indicating appropriate sample collections and DNA extraction processes.

Complete genome analysis of WU polyomavirus

The genome of WUPyV (isolate CU_Chonburi 3) was closely related to the reference sequence of WUPyV (NC_009539), with 99% similarity. The genomes were then further compared by using the SimPlot software program (public domain). The result revealed that WUPyV (CU_Chonburi 3) was slightly different from WUPyV (NC_009539), as follows: non-coding region (positions 100-350); VP2 (positions 850-1,000); LTAg (positions 3,500-3,700); STAg (positions 4,650-4,800). The complete genome of WUPyV (CU_Chonburi 3) was then aligned with several WUPyV whole genome sequences from several countries, including Australia, USA, Germany, China, and Thailand during the timeframe of 2007-2011. Table 1 describes several nucleotide variations within non-coding regions, to include positions 37, 67, 73, 95, 130, 152, 173, 177, 180, 181, 206, 275, 293, 294, 350, 451, 499, 4522, and 5211. Table 2 illustrates amino acid changes in VP1 (position S347T); VP2 (positions L40V, G120R, Y121I, P123R, G127S, L137F, Q287R, and A327V); LTAg (positions Q357P, V369E, E377K, D378V, A381T, R382E, R383G, and D389G); and, STAg (positions R139S, K141E, R148K, and W153C).

Phylogenetic analysis

Phylogenetic trees were constructed based on analysis of nucleotide sequences within VP1, VP2, LTAg, and STAg (Fig. 2). The phylogenetic tree of the VP1 gene (Fig. 2A) demonstrated that WUPyV (isolate CU_Chonburi 3) was closely related to previously described WUPyV, such as WUPyV reference sequence (NC 009539), isolate MN 2726 from Australia in 2010 (GU296405), and isolate CLFF from China in 2008 (EU296475). Conversely, phylogenetic trees obtained from the analysis of VP2 (Fig. 2B), LTAg (Fig. 2C), and STAg (Fig. 2D), revealed that WUPyV (isolate CU_Chonburi 3) was quite unrelated to other strains of WUPyV.

Discussion

According to the previous study, the prevalence of WUPyV in Thailand in 2008 was 6.29% (19/302)[7]. However, the present study observed a prevalence of only 0.16% (1/614) in Thai patients with respiratory disease during 2013. When comparing the current and previous studies, there was no difference in specimen type (NP suction) or detection technique (semi-nested PCR). Moreover, the sample size used in this study was double the sample size used in the previous report. Accordingly, type of specimen, detection method, and sample size were not responsible for the low prevalence of WUPyV observed in the present study. A possible explanation may be a difference in antibody response against WUPyV. WUPyV contains VP1 as a major coated protein to target cell receptors; as such, VP1 protein becomes the main target of host neutralizing antibodies. According to the analysis in the present study, the VP1 gene of WUPyV (isolate CU_Chonburi 3) was relatively conserved (only one amino acid change: S347T), consistent with the previous descriptions of WUPyV (Fig. 1 and 2A). This finding implies that WUPyV in 2013 should be neutralized by antibodies against the VP1 protein. Consequently, the prevalence of WUPyV in 2013 should be very low, as compared to prevalence rates reported in the previous studies[2,4-15].

Phylogenetic analysis based on the VP1 gene revealed that WUPyV isolated from several countries, including Australia, USA, Germany, China, and Thailand, from 2007 to 2013 were closely related. The phylogenetic trees of WUPyV (isolate CU_Chonburi 3) based on VP2, LTAg, and STAg were located a considerable distance from other strains of WUPyV. However, additional WUPyV genome sequences from several countries should be chronologically investigated in order to understand better the evolution of this virus.

Mutations within viral proteins (LTAg, STAg,

Table 1. Nucleotide variations within non-coding regions

Isolate name	Country	Year	Nucleotide variations within non-coding regions*																			Accession No.
			37	67	73	95	130	152	173	177	180	181	206	275	293	294	350	451	499	4522	5211	
WU Ref strain	Australia	2007	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	a	g	NC_009539
S5	USA	2007	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	EF444554
CU-295	Thailand	2008	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	EU358768
CU-302	Thailand	2008	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	g	EU358769
CLFF	China	2008	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	a	g	EU296475
GD-WU709	China	2009	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	GQ926980
Rochester-7029	USA	2009	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	FJ794068
Wuerzburg	Germany	2009	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	EU711058
O91	Australia	2010	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	GU296363
MN2726	Australia	2010	g	g	g	g	c	g	c	c	a	g	a	a	t	a	c	c	c	g	a	GU296405
O3	Australia	2010	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	GU296408
FZ18	China	2011	g	g	g	g	c	g	c	c	g	g	a	a	t	a	c	c	c	g	a	FJ890981
CU_Chonburi 3	Thailand	2013	c	c	a	c	a	t	a	a	t	a	c	g	g	g	a	t	t	g	t	KJ725028

* Position refers to NC_009539 (WU Ref strain)

Table 2. Amino acid changes in VP1, VP2, LTAg, and STAg

Isolate name	Country	Year	Amino acid changes*																					Accession No.
			VP1	VP2								LTAg								STAg				
			347	40	120	121	123	127	137	287	327	357	369	377	378	381	382	383	389	139	141	148	153	
WU Ref strain	Australia	2007	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	NC_009539
S5	USA	2007	S	L	G	Y	P	G	L	Q	A	Q	V	E	G	A	R	R	D	R	K	R	W	EF444554
CU-295	Thailand	2008	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	EU358768
CU-302	Thailand	2008	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	EU358769
CLFF	China	2008	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	EU296475
GD-WU709	China	2009	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	GQ926980
Rochester-7029	USA	2009	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	FJ794068
Wuerzburg	Germany	2009	T	L	G	Y	P	G	L	E	A	Q	V	E	D	A	R	R	D	R	K	K	W	EU711058
O91	Australia	2010	T	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	K	W	GU296363
MN2726	Australia	2010	S	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	GU296405
O3	Australia	2010	T	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	GU296408
FZ18	China	2011	T	L	G	Y	P	G	L	Q	A	Q	V	E	D	A	R	R	D	R	K	R	W	FJ890981
CU_Chonburi 3	Thailand	2013	T	V	R	I	R	S	F	R	V	P	E	K	V	T	E	G	G	S	E	K	C	KJ725028

* Position refers to NC_009539 (WU Ref strain)

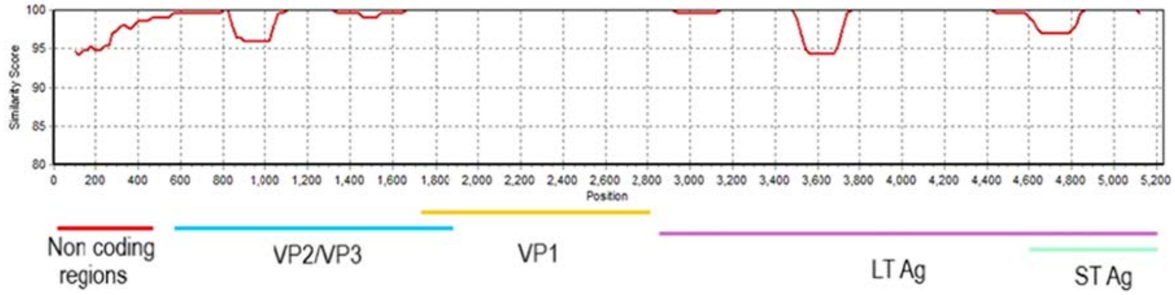

Fig. 1 Similarity plot of complete genome sequences between WUPyV (CU_Chonburi 3) and WUPyV reference sequence (NC_009539).

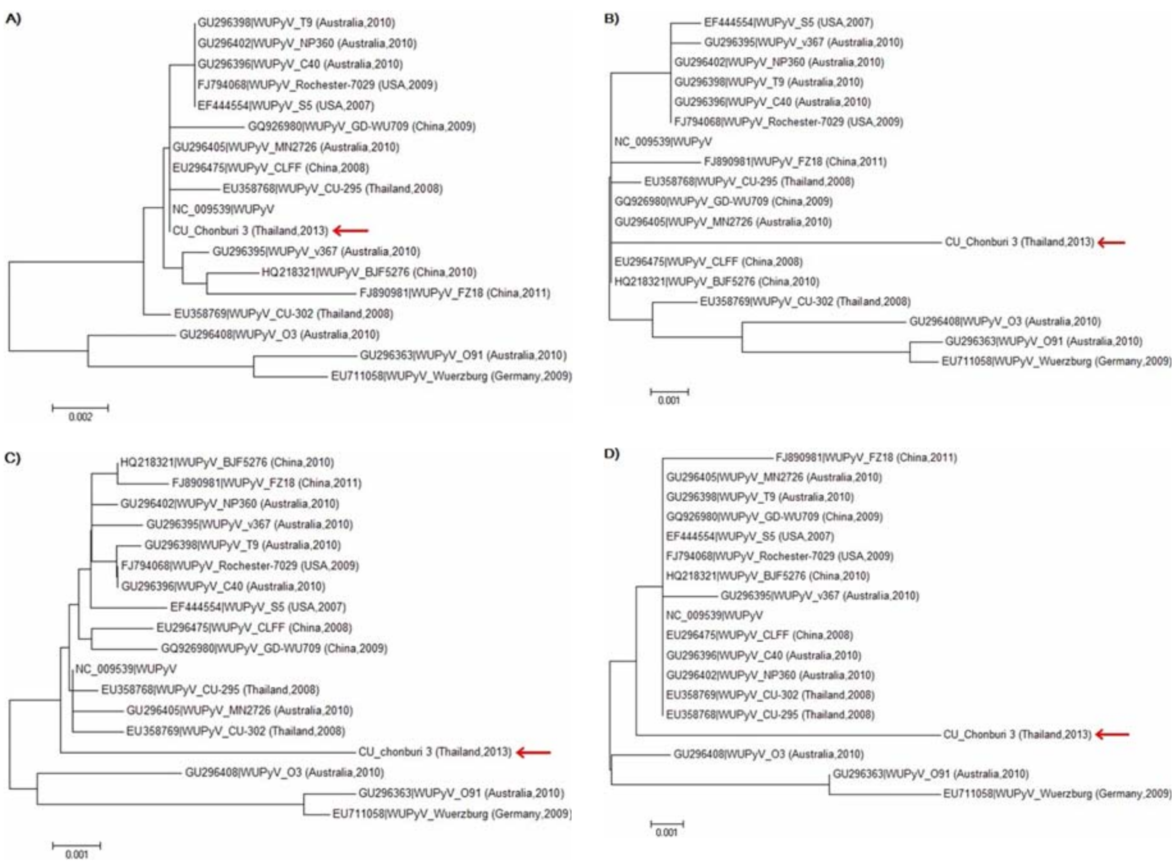

Fig. 2 Phylogenetic analysis of WUPyV based on (A) VP1, (B) VP2, (C) LTAg, and (D) STAg genes.

VP1, and VP2) were determined by whole genome comparison. Amino acid changes can be divided into 2 groups, including non-critical and critical amino acid changes. Changes in non-critical amino acids may not affect the function of the proteins, because the properties of amino acids are similar (including: VP1 (position S347T); VP2 (positions L40V, Q287R, and A327V); and, STAg (position R148K)). On the other hand, changes in critical amino acids may influence the function of the proteins by triggering properties specific to individual amino acids, to include: charge, ring structure, glycosylation site, and disulfide linkage. The critical amino acid changes observed in this study include: VP2 (positions G120R, Y121I, P123R, G127S, and L137F); LTAg (positions Q357P, V369E, E377K, D378V, A381T, R382E, R383G, and D389G); and, STAg (positions R139S, K141E, and W153C).

Conclusion

Nucleotide variations within non-coding

regions and critical amino acid substitutions in viral proteins may affect the rate of viral replication and viral adaptation, which may be linked to susceptibility and severity of viral infection. However, further experimental investigation is required in order to confirm the impact of each non-synonymous mutation on the characteristics of WUPyV and the specific immune response to this particular virus.

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Potential conflicts of interest

None.

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การศึกษาจีโนมและการจำแนกสายพันธุ์ของ WU polyomavirus ในผู้ป่วยติดเชื้อระบบทางเดินหายใจในประเทศไทยปี พ.ศ. 2556

ปรางวลัย จันทร์แจ่ม, ยง ภู่วรวรรณ, สัญชัย พยุงภร

ภูมิหลัง: *เชื้อ WU polyomavirus (WUPyV) เป็นดีเอ็นเอไวรัสที่มีขนาดเล็กจัดอยู่ใน family Polyomaviridae ซึ่งมีสารพันธุกรรมเป็นดีเอ็นเอสายคู่ ขนาดประมาณ 5 kb ถูกค้นพบครั้งแรกจากเด็กที่มีการติดเชื้อในระบบทางเดินหายใจ*

วัสดุและวิธีการ: *งานวิจัยนี้ศึกษาลักษณะทางพันธุกรรมของเชื้อ WU polyomavirus ในประเทศไทยปี พ.ศ. 2556 โดยตรวจสอบเชื้อ WU polyomavirus ด้วยวิธี semi-nested PCR และหาลำดับสารพันธุกรรมทั้งจีโนมจากนั้นทำการวิเคราะห์ multiple sequences alignment และ Phylogenetic tree*

ผลการศึกษา: *จากการศึกษาระบาดวิทยานั้นพบว่ามีการระบาดของ WU polyomavirus (WUPyV) คิดเป็น 0.16% (1/614) เท่านั้น จากการศึกษา Phylogenetic tree พบว่า WUPyV (isolate CU_Chonburi 3) มีความสัมพันธ์ใกล้เคียงกับ WUPyV ที่มีรายงานก่อนหน้านี้ (ความเหมือน 99%) นอกจากนี้สารพันธุกรรมทั้งจีโนมของ WUPyV (isolate CU_Chonburi 3) นั้นมีการเปลี่ยนของลำดับนิวคลีโอไทด์บริเวณ non-coding regions หลายตำแหน่งและมีการเปลี่ยนของกรดอะมิโนของโปรตีน VP1 (ตำแหน่ง S347T), VP2 (ตำแหน่ง L40V, G120R, Y121I, P123R, G127S, L137F, Q287R และ A327V), LTAg (ตำแหน่ง Q357P, V369E, E377K, D378V, A381T, R382E, R383G และ D389G) และ STAg (ตำแหน่ง R139S, K141E, R148K และ W153C)*

สรุป: *ข้อมูลที่ได้จากการศึกษานี้สามารถใช้เป็นข้อมูลพื้นฐานทางด้านระบาดวิทยาและการจำแนกสายพันธุ์ระดับโมเลกุลของเชื้อฮิวแมนโพลีโอมาไวรัส ซึ่งอาจมีประโยชน์ต่อไปในอนาคต*