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DNA and seroprevalence study of MW and STL polyomaviruses

Melinda Katona[1,2] | Krisztina Jeles[1,2] | Péter Takács[3] | Eszter Csoma[2]

[1]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Debrecen, Hungary

[2]Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

[3]HUN-REN Balaton Limnological Research Institute, Tihany, Hungary

Correspondence

Eszter Csoma, Department of Medical Microbiology, Faculty of Medicine, University of Debrecen, Hungary

Email: csoma.eszter@med.unideb.hu

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Abstract

The clinical importance and the pathogenesis of the MW and STL polyomaviruses (PyVs) remain unclear. Our aim was to study the seroprevalence of MWPyV and STLPyV, and to examine the prevalence of viral DNA in respiratory samples and secondary lymphoid tissues. In total, 618 serum samples (0.8–90 years) were analyzed for seroprevalence. For the DNA prevalence study, 146 patients (2.5–37.5 years) were sampled for adenoids (n = 100), tonsils (n = 100), throat swabs (n = 146), and middle ear discharge (n = 15) in study Group 1. In Group 2, we analyzed 1130 nasopharyngeal samples from patients (0.8–92 years) tested for SARS-CoV-2 infection. The adult seropositivity was 54% for MWPyV, and 81.2% for STLPyV. Both seroprevalence rates increased with age; however, the majority of STLPyV primary infections appeared to occur in children. MWPyV was detected in 2.7%–4.9% of respiratory samples, and in a middle ear discharge. STLPyV DNA prevalence was 1.4%–3.4% in swab samples, and it was detected in an adenoid and in a middle ear discharge. The prevalence of both viruses was significantly higher in the children. Noncoding control regions of both viruses and the complete genomes of STLPyV were sequenced. MWPyV and STLPyV are widespread viruses, and respiratory transmission may be possible.

KEYWORDS

MWPyV, prevalence, respiratory samples, seroprevalence, STLPyV

1 | INTRODUCTION

MW and STL polyomaviruses (MWPyV and STLPyV, respectively) were described from stool samples in 2012 and 2013, respectively.[1,2] Although research on viruses began immediately, few sero- and DNA prevalence studies have been published, and the pathogenesis and clinical significance of these viruses remain unclear. Seroprevalence studies suggest that both MWPyV and STLPyV are widespread in the human population; however, there is a wide range of published data, 68%–98% seropositivity for STLPyV,[3–5] and 41%–99% for MWPyV, which is even controversial.[4,6–11] Consequently, there is a need for additional seroprevalence data from different geographical locations and from different age groups. Furthermore, the transmission route, portal of entry, site of viral replication and possible latency of MWPyV and STLPyV are still unknown.

MWPyV DNA was detected in 0.5%–12.8% of stool samples from healthy and hospitalized children and adults with diarrhea, suggesting a fecal–oral route of transmission.[1,12–17] Viral DNA was detected in 0.7%–9.2% of respiratory samples which were mostly from children.[12,14,15,18] Based on these data, the respiratory

transmission of these viruses may also be possible. In a previous study, we did not detect MWPyV DNA in lung tissue samples.[19] The virus has been detected in the tonsils and adenoids, suggesting that these secondary lymphoid tissues may be the site of viral replication or persistence.[20,21] Blood, urine, and cerebrospinal fluid were also examined to study the possible spreading of the virus within the body,[12–15,21–23] but MWPyV DNA was detected in only one sample from a blood donor.[22] The virus has been detected in a condyloma samples,[24] and skin swab samples.[25,26]

Limited data are available regarding the prevalence of STLPyV. The fecal–oral transmission of STLPyV has also been hypothesized since the virus was detected in 0.26%–4.4% of stool samples from children with diarrhea and from healthy individuals.[13,17,27] Viral DNA was found in the tonsils of children,[20] a respiratory sample[23] and a urine sample[13] of patient with a kidney transplant, a condyloma of an immunocompromised patient,[28] and skin swab samples.[25]

Based on these data, further prevalence studies are required to identify the portals of entry and the routes of transmission of MWPyV and STLPyV.

2 | OBJECTIVE

The primary aim of this study was to examine the seroprevalence of MWPyV and STLPyV in the Hungarian population. The second aim was to investigate whether these viruses could be transmitted via respiratory pathways. Therefore, the DNA prevalence of MWPyV and STLPyV was studied in respiratory samples and secondary lymphoid tissues. We sequenced and analyzed the noncoding control region (NCCR) of both viruses.

3 | MATERIALS AND METHODS

3.1 | Patients and samples for the seroprevalence study

A total of 618 serum samples collected between 2016 and 2021 were analyzed for antibodies against MWPyV and STLPyV. Samples were obtained and sent to Medical Microbiology, University of Debrecen, Hungary for routine serological diagnostic tests (routine occupational health examination and/or herpes-, hepatitis, and SARS-CoV-2 serology). Individuals were excluded from the study if they received blood or blood products within 1 year before blood collection; or received chemotherapy, immunosuppressive therapy, or biological therapy; and/or had basic diseases affecting the immunostatus. Samples were collected from residents of eight counties in eastern Hungary. The serum samples were frozen until further use. The study group included 317 females and 301 males, with ages ranged from 0.8 to 90 years. In total, 383 adults and 235 children were included. The samples were categorized into seven age groups, as shown in Table 1, along with the patient data.

TABLE 1 Data from the seroprevalence study group.

Age groups	Number of samples	Age in years, min–max (median)	Female/male
<6 years	49	0.8–5.9 (3.1)	16/33
6–9 years	41	6.1–9.9 (7.5)	22/19
10–13 years	66	10.1–13.9 (11.9)	37/29
14–20 years	92	14–20 (16)	51/41
21–39 years	122	21–39.5 (30)	62/60
40–60 years	130	40–59.5 (50)	66/64
>60 years	118	60–90 (69)	63/55
Total	**618**	**0.8–90 (30)**	**317/301**
Adults (>18 years)	383	18–90 (48)	198/185
Children (<18 years)	235	0.8–17.9 (11.6)	119/116

This study was approved by the Regional and Institutional Research Ethics Committee, Clinical Centre, University of Debrecen, Hungary (DERKEB/IKEB: 5134-2018).

3.2 | Patients and samples for the DNA prevalence study

To determine whether MW and STL polyomaviruses were present in respiratory samples and secondary lymphoid tissues in the respiratory tract, we studied the presence of the viral DNA in different sample types.

For MWPyV and STLPyV DNA detection, we collected samples during two periods. Tonsils and/or adenoids, throat swabs and middle ear discharge samples were collected from May to November 2016, and the nucleic acids were isolated from each sample, as described previously.[29] Detailed data of the patients in Group 1 are shown in Table 2. Briefly, 146 patients were included, and the throat of each patient was swab-sampled before routine adenectomy and/or tonsillectomy, whereas middle ear samples ($n = 15$) were collected if the patient had serous otitis media at the time of surgery. Adenoids and tonsils were removed simultaneously from 54 patients, whereas either the adenoids or tonsils were obtained from 46 to 46 patients, ensuring 100 samples from both lymphoid tissues. The majority of the patients were children ($n = 126$; 2.5–17 years; median: 4.7 years), and 20 adults were included (18–37.5 years; median: 26.1 years).

Sample collection and analysis were approved by the National Public Health and Medical Officer Service (number: IF-5418-7/2016) and the National Scientific and Ethical Committee of Hungary (ETT TUKEB, 651-3/2016/EKU; 0027/16). All participants or their legal representatives (parents) signed written informed consent forms.

TABLE 2 Patients data and samples for the DNA prevalence study.

Group 1				
	Sample types			
	Adenoids	Tonsils	Throat swab	Middle ear discharge
Number of samples/patients:	100/100	100/100	146/146	15/15
Female/male:	44/56	37/63	60/86	8/7
Age of patients in years: min–max (median):	2.5–12.1 (4.4)	2.5–37.5 (7.1)	2.5–37.5 (5.5)	3.1–8.9 (5.2)

Group 2			
	Sample type: Nasopharyngeal swab		
	Total	SARS-CoV-2 positive	SARS-CoV-2 negative
Number of samples/patients:	1130/1130	565/565	565/565
Female/male:	564/566	283/282	281/284
Age of patients in years: min–max (median):	0.8–92 (30.2)	0.8–89.8 (32.1)	0.9–92 (29.5)

In Group 2, nasopharyngeal swab samples collected from 1130 patients were tested for the presence of MWPyV and STLPyV DNA. Samples were sent for SARS-CoV-2 PCR diagnosis to the Medical Microbiology, University of Debrecen from September 2020 to March 2022. Nucleic acid isolation and detection of SARS-CoV-2 RNA were performed as described previously.[30] Half of the samples ($n = 565$) tested positive for SARS-CoV-2 RNA, of which 180 were obtained from children. The SARS-CoV-2 RNA negative samples were also collected from 180 children and 385 adults. The patients data from Group 2 are detailed in Table 2.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and was approved by the Regional and Institutional Research Ethics Committee, Clinical Centre, University of Debrecen (DERKEB/IKEB: 5770-2021).

3.3 | Expression and purification of recombinant proteins

The expression and purification of recombinant VP1 proteins of MWPyV and STLPyV were carried out as described previously.[30,31] The major capsid protein-encoding genes were from MWPyV strain MA095 (GenBank accession number: JQ898291.1), and STLPyV strain MA138 (GenBank accession number: JX463183.1).

3.4 | ELISA

To detect IgG antibodies against MWPyV and STLPyV VP1 antigens, indirect ELISA was performed. Briefly, each well of a MaxiSorp 96-well plate (Nunc) was coated with 100 ng of antigen in 100 µL of ELISA/ELISPOT Coating Buffer (Thermo Fisher Scientific) for 16 h at 4°C. After washing three times with phosphate-buffered saline (PBS, pH 7.4), blocking was carried out at 37°C for 1 h using 2% casein in PBS (Sigma). Serum samples diluted in blocking buffer (1:100 in 100 µL) were added after washing with PBS three times, and then incubated for 1 h at 37°C. The HRP-conjugated, goat anti-human IgG Fc Highly Cross-Adsorbed Secondary Antibody (Thermo Fisher Scientific) was diluted in blocking buffer (1:10 000) and added after three washes with PBS containing 0.05% Tween-20 (PBS-T), then the plate was incubated at 37°C for 1 h. Following three washing cycles with PBS-T, 100 µL of TMB substrate was incubated in each well for 15 min at room temperature. The enzymatic reaction was stopped by the addition of 1 M H_2SO_4. A Multi-scan Sky Microplate Spectrophotometer (Thermo Fisher Scientific) was used to measure the absorbance at 450 nm. All samples were analyzed in duplicate, and the optical density (OD) of each sample was determined as the average after subtracting the OD value of the blank. The cut-off value was determined without negative or positive controls, as previously described.[30,31] Briefly, the OD values were ranked and plotted, and then the inflection point was calculated based on polynomial regression. The cut-off value for positivity was calculated as the OD value based on the inflection point +10% gray zone. A sample was considered seropositive if the OD values were >0.157 and >0.201 for MWPyV and STLPyV, respectively.

3.5 | Detection of MWPyV and STLPyV DNA using real-time PCR

MWPyV and STLPyV qPCRs were carried out in a final volume of 25 µL using TaqMan™ Universal PCR Master Mix (Applied

Biosystem), 400–400 nM primers, and 250–250 nM probes, as published previously.[1,23] The thermal-cycling parameters were set according to the instructions for the master mix. In the case of tonsils and adenoids, the template DNA amount was 1000 ng, and 10 µL of the nucleic acids was analyzed from the other sample types. Plasmids were used as positive controls, as described previously.[19]

3.6 | Amplification and sequencing of the NCCR of MWPyV and STLPyV

Primer sequences were designed to amplify and sequence parts of the MWPyV and STLPyV genomes, and the complete genomes of which are shown in Table S1. Together with the PCR protocol, the amplifications were carried out in a final volume of 25 µL using Phusion Hot Start II High Fidelity DNA Polymerase (1U) in HF buffer with 500–500 nM primers and 200 µM dNTP mix (Thermo Fisher Scientific). The PCR products were purified after separation using agarose gel electrophoresis, and then amplicons were sequenced using the BigDye Terminator Cycle Sequencing Kit and ABI PRISM 3100-Avant Genetic Analyzer (Thermo Fisher Scientific). AliView software (version 1.28) was used for sequence alignment.

3.7 | Statistical analysis

Fisher's exact test, Mann–Whitney *U* test, Kruskal–Wallis Dunn's multiple comparisions test, chi-square test for trend, and Spearman's rank correlation were performed using GraphPad Prism version 9.4.0 (GraphPad Software). Statistical significance was set at $p < 0.05$. Evolutionary analyses were conducted using MEGA X software, and TCS (Templeton-Crandall-Sing) networks were developed uing PopART software.

4 | RESULTS AND DISCUSSION

4.1 | Seroprevalence of MWPyV and STLPyV

IgG antibodies against the VP1 protein of MWPyV and STLPyV were detected using indirect ELISA.

Figure 1 shows the seroresponse of each sample to the MWPyV and STLPyV antigens by age groups. To study whether there was a significant change in OD values in the two consecutive age groups, the Mann–Whitney *U* test was performed. A significant difference in the OD values for the MWPyV ELISA was observed only between the two youngest age groups ($p = 0.027$) and between the 10–13 years and 14–20 years age groups ($p = 0.0025$). Based on Dunn's multiple comparision test, seroreactivity increased with age (statistically significant differences are shown in Figure 1A). In the case of STLPyV seroresponse, the Mann–Whitney *U* test revealed a significant difference between the 10–13 years and 14–20 years age groups ($p = 0.0006$). Statistically significant differences in OD values between the younger and adult age groups were observed by Dunn's post hoc test (statistically significant differences are shown in Figure 1B). Relatively stable intensity was observed at seroreactivity >21 years for both viruses (no significant difference in OD values was detected). These results are in agreement with those of previous publications.[4,8]

Both viruses studied belong to the *Deltapolyomavirus* genus of the *Polyomaviridae* family, and their VP1 proteins share a 56% amino acid-sequence identity. To examine the potential association between the seroresponses to MWPyV and STLPyV, we performed Spearman's rank correlation analysis. A significant ($p < 0.0001$), but weak correlation ($r = 0.293$) was observed between the MWPyV and STLPyV OD values. Selected serum samples were pre-incubated with a 20-fold excess of homologous or heterologous antigens, and then the ELISA measurements were performed. The homologous VP1 preincubation resulted in a decrease in the OD values, therefore, the samples had negative ELISA measurements, while this did not occur for the heterologous VP1 (data not shown). Seroreactivity against MWPyV and STLPyV is thought to be specific to the given virus, consistent with other publications.[5,32]

The overall MWPyV seropositivity was 45.6% (282/618), the adulthood seroprevalence was 54% (207/383; >18 years), and the prevalence was 31.9% (75/235) in children (<18 years). Although the sex ratio (female/male) in the study groups was not markedly different (51.3% vs. 48.7%), seropositivity was significantly higher in females than in males (165/317 vs. 117/301; $p = 0.0012$). This female sex difference was significant among children (51/119 vs. 24/116; $p = 0.0003$), but not among adults (114/198 vs. 93/185; $p = 0.182$). A significant age difference was revealed between the seropositive (3.1–90 years, median: 37 years) and the seronegative individuals (0.8–80 years, median: 22.4 years) within the whole study group ($p = 0.0001$), and among children ($p = 0.0009$; 3.1–17.5 years, median: 13.6 years vs. 0.8–17.9 years, median: 10.6 years), respectively. The antibody-positivity rate significantly increased with age until adulthood, which was shown by the χ^2 test for trend analysis ($p = 0.0042$). There was a statistically significant difference in seropositivity between the age groups <6 years and 6–9 years and between the 10–13 years and 14–20 years age groups. These results agree with the significant increase in seroreactivity. The antibody positivity rate did not show significant changes among adults >21 years old (50%–55.1%). The data for the age groups are shown in Figure 2. The overall seropositivity rates are inconsistent in previous publications, because there is a wide range of 42%–98% seropositivity.[4,6–11] Our data are similar to those from Italy; however, higher seropositivity rates were detected in children.[6] Another Italian publication reported an even higher childhood seroprevalence, reaching 91% in the 8–17 years age group.[10] Compared with our data, the teenagers and young adults from the USA showed nearly the same proportion of antibody positivity (50%), as measured by Berrios et al. Although the trend of seroprevalence increasing with age until young adulthood was somewhat similar, they detected a higher seroprevalence (65.7% for >20 years) in adulthood, with a decreasing tendency in the elderly population.[7] The adulthood (>20 years) seroprevalence in a Czech study was 85.6%,[8] but the published adulthood seropositivity rates in Australia, the USA, and the Netherlands were >97%.[4,9,11] Differences in the study groups, antigens expressed, methods, or even geographic locations may explain these

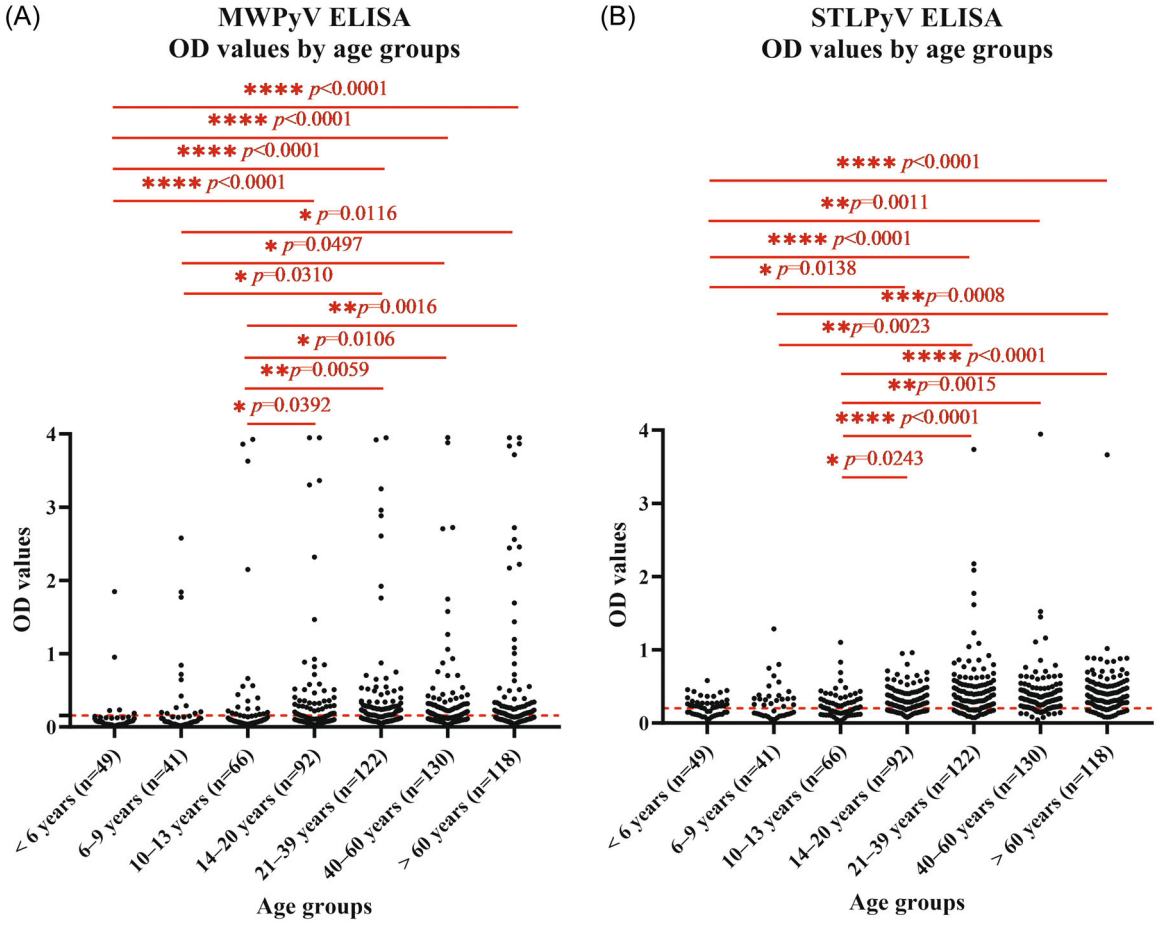

FIGURE 1 Age distributions of seroresponses against VP1 protein of MWPyV (A) and STLPyV (B). Each dot represents the OD value of an individual serum sample measured by ELISA. Dashed red lines represent the thresholds for seropositivity. Significant differences in seroreactivity between age groups are indicated by red lines and asterisks (Mann–Whitney *U* test). OD, optical density; PyV, polyomavirus.

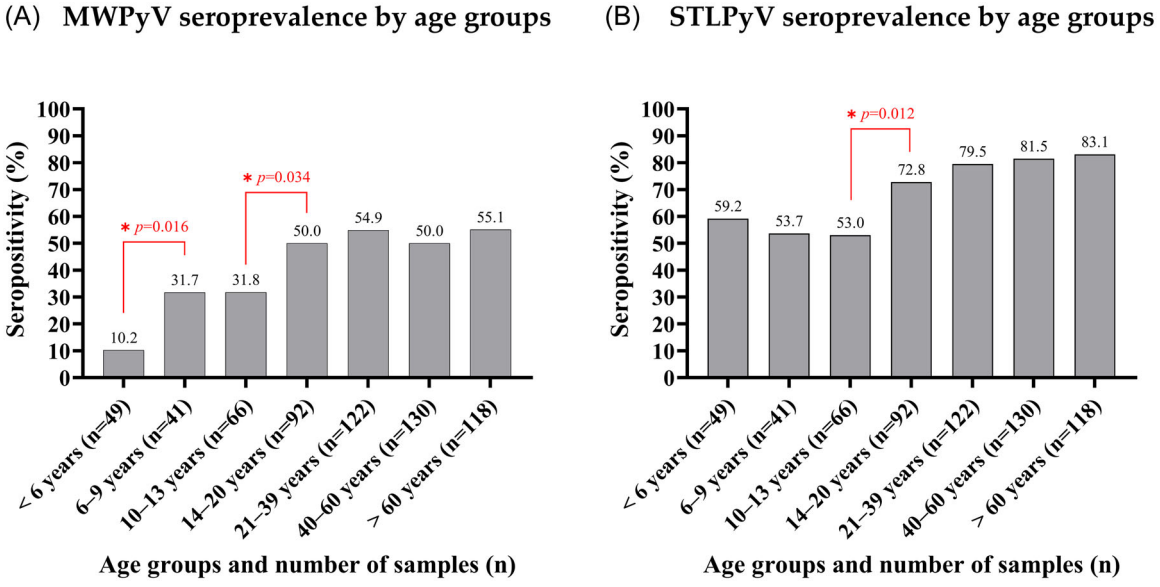

FIGURE 2 Seroprevalence of MWPyV (A) and STLPyV (B) by age groups. Red lines and asterisks represent significant differences between the age groups (Fisher's exact test). PyV, polyomavirus.

TABLE 3 DNA prevalence in samples and data of patients with MWPyV and STLPyV.

Group 1					
		Sample types			
		Adenoids	Tonsils	Throat swab	Middle ear discharge
MWPyV positive	Number of samples/total (%):	0/100	0/100	4/146 (2.7%)	1/15
	Female/male:	–	–	1/3	1/0
	Age of patients in years: min–max (median):	–	–	3.4–7.1 (5)	5.2
STLPyV positive	Number of samples/total (%):	1/100 (1%)	0/100	5/146 (3.4%)	1/15 (6.7%)
	female/male:	1/0	–	3/2	0/1
	Age of patients in years: min–max (median):	4	–	2.7–4 (3.6)	3.6

Group 2				
		Sample type: nasopharyngeal swab		
		Total	SARS-CoV-2 positive	SARS-CoV-2 negative
MWPyV positive	Number of samples/total (%):	55/1130 (4.9%)	14/565 (2.5%)	41/565 (7.2%)
	female/male:	28/27	8/6	20/21
	Age of patients in years: min–max (median):	0.8–38 (3.6)	0.8–16.7 (2.3)	0.9–38 (8.6)
STLPyV positive	Number of samples/total (%):	16/1130 (1.4%)	2/565 (0.4%)	14/565 (2.5%)
	female/male:	5/11	0/2	5/9
	Age of patients in years: min–max (median):	1.3–28.5 (4.5)	0.8–16.7	1.3–28.5 (5)

different data, and further studies with comparable methods are required for clarification.

The STLPyV seropositivity rate within the entire study group was 73.5% (454/618): 60.9% among children (143/235) and 81.2% among adults (311/383). There was a statistically significant ($p = 0.0001$) age difference between the seropositive (0.8–90 years, median: 35 years) and the seronegative groups (0.9–80 years; median: 16.7 years) within the cohort; however, this phenomenon was not detected among children and adults. Seropositivity was detected in a significantly higher proportion of females than that in males in the entire study group (245/317 vs. 209/301; $p = 0.029$), but not in children and adults. Seropositivity rates according to age groups are shown in Figure 2. The proportion of antibody positivity was almost 60% in the <6 years group, consistent with previously reported seroprevalence rates,[3,5] indicating a high rate of primary STLPyV infection during childhood. A significant increase was detected in the 14–20 years age group, and seropositivity increased with age, reaching a maximum of 83.1% in the >60 years age group. Although there is some variability in the previously published seroprevalence data, our data are similar to these, suggesting that STLPyV is also a ubiquitous polyomavirus in humans.

4.2 | DNA prevalence study

DNA prevalence data are presented in Table 3.

We did not detect MWPyV in either the tonsils or adenoids of study Group 1. An Italian research team reported low levels of MWPyV DNA in 1% of adenoids and 6% of tonsils in children.[21] Peng et al. also confirmed the presence of this virus in tonsils, with a prevalence of 2%.[20] Similar to our study, two other teams did not detect the virus in tonsillar tissues.[33,34] We previously detected other polyomaviruses in these samples,[29] therefore, we concluded that the methodology, sample handling, or nucleic acid isolation were not the reasons for the negative results.

In study Group 1, MWPyV was detected in 2.7% of the throat swab samples and one middle ear discharge sample (6.7%). All positive samples were taken from children (3.4–7.1 years). In study Group 2, we detected MWPyV DNA in 4.9% of the nasopharyngeal samples (55/1130), which was not statistically different from the prevalence in study Group 1. In Group 2, the prevalence of MWPyV was significantly ($p < 0.0001$) higher among children (38/360) than that among adults (17/770). A significant age difference ($p = 0.0001$) was revealed between the MWPyV positive (0.8–38 years, median: 3.6 years) and negative patients (1–92 years, median: 32.2 years). No sex difference was detected between the MWPyV positive and negative patients, within the group, or among children and adults. Previously, MWPyV was not found in nasopharyngeal or nose and throat swab samples of renal transplant adults,[13,23] in respiratory samples of symptomatic adults,[14] or in throat gargles of adults.[34] The 2.2% adulthood DNA prevalence that we observed was consistent with our seroprevalence data. Most primary infections occur until young adulthood, however, a high proportion of adults remains susceptible to infection. Our prevalence data among children are consistent with those of previous research. Yu et al. reported a 0.74% MWPyV DNA prevalence in nasal wash samples from hospitalized, Mexican children.[12] In an Australian study, the prevalence in respiratory samples was 1.5% in symptomatic patients and 9.2% in healthy individuals, respectively.

All positive nasopharyngeal aspirates and nose and throat swabs were obtained from children <9 years.[14] In China, 1.47% of the nasopharyngeal aspirate samples obtained from symptomatic, hospitalized children tested positive for MWPyV DNA.[15] The frequency of MWPyV in nasopharyngeal samples from pediatric, Brazilian patients was 2.1%.[18]

In Group 2, although a higher MWPyV frequency was observed among SARS-CoV-2 negative children (24/180) than that among the SARS-CoV-2 positive patients (14/180), the difference was not statistically significant ($p = 0.122$). In addition, MWPyV frequency was significantly higher ($p = 0.0003$) among SARS-CoV-2 negative patients (41/565) than that among SARS-CoV-2 positive patients (14/565) within the entire study group (7.3% vs. 2.5%) and among adults (17/385 vs. 0/385; $p < 0.0001$).

The pathogenesis, the mode of transmission, and the portal of entry of MWPyV remain unknown. The virus was described from fecal samples and later studies revealed a prevalence of up to 12.8% in stool samples, therefore, fecal–oral transmission was hypothesized.[1,12–17] However, the fact that the viral DNA was detected in the nasopharyngeal samples suggests that transmission may occur via the respiratory route. Notably, we detected MWPyV DNA in one middle ear discharge sample, indicating the transmission of the virus into the middle ear. This may indicate the possible transmission of the virus into the middle ear, however, further studies and analysis of several samples are needed to clarify this question. Other infectious agents were not examined from the samples, therefore, we did not investigate the link between the presence of MWPyV and the patient symptoms.

In study Group 1, STLPyV DNA was detected in throat swab samples (3.4%), middle ear discharge (6.7%), and adenoid (1%) samples. All positive samples were obtained from children. Throat swab samples taken at the same time from a patient with an STLPyV positive adenoid sample and a patient with viral DNA in a middle ear discharge sample were also positive. In Group 2, the prevalence of STLPyV was 1.4%. STLPyV prevalence was significantly ($p < 0.0001$) higher among children (13/360, 3.6%) than that among adults (3/770, 0.4%), and there was a significant ($p = 0.0001$) age difference between the virus negative (1.3–28.5 years, median: 4.5 years) and positive (0.8–92 years, median: 30.7 years) patients. No sex differences were observed between STLPyV positive and negative individuals. The viral positivity rate was significantly ($p = 0.0039$) higher in SARS-CoV-2 negative patients than in SARS-CoV-2 positive patients, and the same phenomenon was observed in children (11/385 vs. 2/358; $p = 0.01$). All the three STLPyV positive samples were obtained from SARS-CoV-2-negative adults.

The prevalence of STLPyV was significantly lower than that of the MWPyV prevalence in the entire study group (4.9% vs. 1.4%; $p < 0.0001$), among children (10.6% vs. 3.6%; $p = 0.0004$) and adults (2.2% vs. 0.4%; $p = 0.0024$). Coinfection with MWPyV and STLPyV was detected in seven samples, and one patient was SARS-CoV-2-infected at the same time. Little is known about STLPyV, as there are only a few publications with prevalence data, and its pathogenesis is completely unexplored. The fact that the viral DNA has been detected in stool samples from different studies suggests that the fecal–oral route is a possible transmission route.[13,17,27] To date, only two studies have reported data regarding the examination of respiratory samples. Lim et al. did not detect STLPyV in nasopharyngeal samples from renal transplant adults,[13] while Bialasiewicz et al., who also investigated renal transplant adults, detected the virus in a nose and throat swab sample.[23] STLPyV was found in 2% of tonsillar samples from children,[20] whereas another research team did not detect it in the tonsils of adults.[33] The presence of the virus in adenoid, throat, and nasopharyngeal swab samples in this study suggests that respiratory transmission of STLPyV may occur, and that the virus may spread to the middle ear.

4.3 | Complete genomes of STLPyV

One explanation for the very different seroprevalence data published by different research groups may be that the viruses studied have different genotypes and serotypes, which may even be geographically distinct from each other. BK and JC polyomaviruses have geographically distinct genotypes and serotypes,[35] which has also been suggested for Merkel cell polyomavirus.[36,37] We sequenced two complete STLPyV genomes (GenBank accession numbers: PP549201 and PP549202), and performed phylogenetic analysis using all available STLPyV genomes from GenBank (Figure 3). The analyses showed that the sequences clustered into two branches. The geographical distribution of the isolates could not be revealed, because isolates from the same geographical regions were identified in both branches. In our opinion, the number of sequence records currently available is too low; therefore, additional sequences from several geographical regions are needed to clarify this issue.

4.4 | NCCR of MWPyV and STLPyV

The complete NCCR regions of the polyomaviruses were amplified from 21 MWPyV positive and 11 STLPyV positive samples. The sequences were uploaded to GenBank, and the accession numbers were PP346854–PP346874 for MWPyV NCCRs, and PP407403–PP407411 for STLPyV NCCRs. The NCCRs were analyzed for mutations by comparing them to all of the available sequences in GenBank, which are 22 NCCRs for MWPyV and eight NCCRs for STLPyV. Among STLPyV NCCRs, three new variants and three new point mutations were identified (isolates H566, T28, and T9). The other eight sequences were identical to those of previously described isolates. For MWPyV NCCRs, eight new sequence variants were identified (isolates W317, P839, H284, 6452, C1049, C1062, H230, and W578, which is identical to C666), whereas the other isolates were identical to one or more previously known sequences. Four new point mutations were described, and one isolate, H230 (GenBank: PP346859), had a 13-bp deletion. The mutations and their frequencies are listed in Table S2. Although the biological importance of sequence variations in the NCCR of MWPyV and STLPyV is still unknown, it is hypothesized that these mutations might alter the

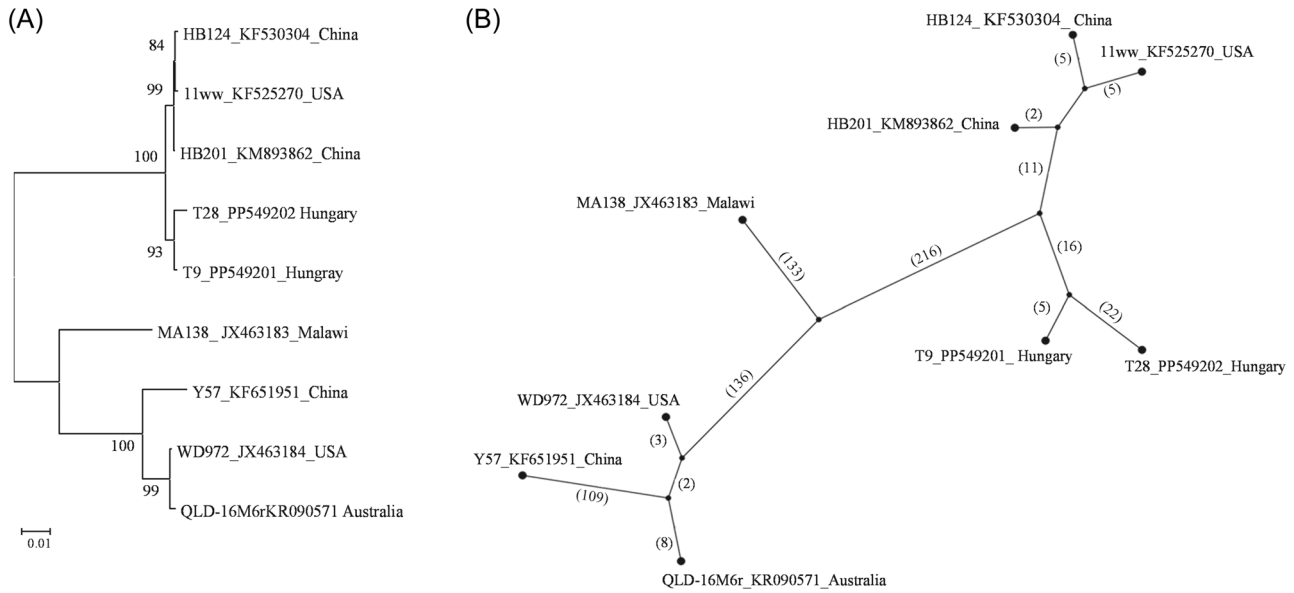

FIGURE 3 Results of phylogenetic analyses using the all available STL genomic sequences. Evolutionary analysis by the maximum likelihood method (A) and TCS network (B) The evolutionary history was inferred by using the maximum likelihood method and the Tamura 3-parameter model. The tree with the highest log likelihood (−10 126.48) is shown. The percentages of trees in which the associated taxa clustered together are shown next to the branches. The initial tree(s) for the heuristic search were obtained automatically by applying the Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with a superior log likelihood value. A discrete gamma distribution was used to model the evolutionary rate differences among sites (five categories (+G, parameter = 0.1159)). The tree was drawn to scale, with branch lengths measured as the number of substitutions per site. The codon positions included were 1st+2nd+3rd+noncoding. There were a total of 4777 positions in the final dataset. In (B), the numbers in parentheses indicate the mutation steps between the two junctions/haplotypes. Evolutionary analyses were conducted using MEGA X, whereas TCS networks were developed using PopART.[38–40]

binding sites for transcription factors, and the function of promoters and enhancers.[41]

To study whether the sequence variations in NCCRs were related to sample types, possible sites of replications, and/or geographical distributions, TCS haplotype network analyses were performed for the MWPyV and STLPyV NCCR sequences. Based on this analysis, STLPyV NCCR sequences were also clustered into two groups. We did not observ clustering of MWPyV and STLPyV sequences, either by geographic origin or sample types. Figure S1. (for MWPyV) and Figure S2. (for STLPyV) show the TCS networks. The codes for the haplotypes are detailed in Tables S3 and S4.

Although the pathogenesis and clinical importance of MWPyV and STLPyV are still unknown, it is important to collect sequence data and identify the sequence differences, as the NCCR of a polyomavirus plays a pivotal role in the control of viral replication and transcription, as well as in determining permissive cells.[41] These findings may help develop in vitro cell culture models to identify permissive cells and study viral replication.

5 | CONCLUSION

Our seroprevalence data confirmed that both MWPyV and STLPyV are widespread viruses in humans. There are differences in the published data, therefore, further studies are required to reveal the underlying explanations. Based on previously published data, respiratory, oral, and fecal/oral transmission of these polyomaviruses has been suggested. The DNA prevalence data from this study strengthens the hypothesis that respiratory transmissions of these viruses is possible. Although viruses have been detected in one middle ear discharge sample, further studies are required to investigate their spread to the middle ear. To date, the sites of viral replication and the target cells are unknown, but these viruses may replicate in the respiratory tract. Secondary lymphoid tissues, adenoids, and tonsils may be sites of replication; even persistent viral infections may occur in these tissues. The DNA prevalence data of this study were consistent with the seroprevalence, as both viruses were also detected in respiratory samples from adults, but a significantly higher prevalence was observed in children. Seroprevalence increased with age for both MWPyV and STLPyV, but there were still individuals susceptible to primary infection in the adult population, especially for MWPyV. Collection of sequence data from different samples and geographical areas is essential for studying the variability of these viruses.

AUTHOR CONTRIBUTIONS

Conceptualization: Eszter Csoma. *Investigation*: Eszter Csoma, Melinda Katona, and Krisztina Jeles. *Methodology*: Eszter Csoma, Melinda Katona, Krisztina Jeles, and Péter Takács. *Resources*: Eszter Csoma. *Supervision*: Eszter Csoma. *Validation*: Eszter Csoma, Melinda Katona, and Péter Takács. *Visualization*: Eszter Csoma, Melinda Katona,

Krisztina Jeles, and Péter Takács. *Writing—original draft and revision*: Eszter Csoma; Melinda Katona, Krisztina Jeles, and Péter Takács.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper and within the supplementary materials.

REFERENCES

1. Siebrasse EA, Reyes A, Lim ES, et al. Identification of MW polyomavirus, a novel polyomavirus in human stool. *J Virol*. 2012;86(19):10321-10326.
2. Lim ES, Reyes A, Antonio M, et al. Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. *Virology*. 2013;436(2):295-303.
3. Lim ES, Meinerz NM, Primi B, Wang D, Garcea RL. Common exposure to STL polyomavirus during childhood. *Emerging Infect Dis*. 2014;20(9):1559-1561.
4. Kamminga S, van der Meijden E, Feltkamp MCW, Zaaijer HL. Seroprevalence of fourteen human polyomaviruses determined in blood donors. *PLoS One*. 2018;13(10):e0206273.
5. Gaboriaud P, Ferté M, Arnold F, et al. Age-specific seroprevalence of human polyomavirus 12 and Saint Louis and New Jersey polyomaviruses. *Emerg Microbes Infect*. 2018;7(1):22.
6. Nicol JTJ, Leblond V, Arnold F, et al. Seroprevalence of human Malawi polyomavirus. *J Clin Microbiol*. 2014;52(1):321-323.
7. Berrios C, Jung J, Primi B, et al. Malawi polyomavirus is a prevalent human virus that interacts with known tumor suppressors. *J Virol*. 2015;89(1):857-862.
8. Šroller V, Hamšíková E, Ludvíková V, Musil J, Němečková Š, Saláková M. Seroprevalence rates of HPyV6, HPyV7, TSPyV, HPyV9, MWPyV and KIPyV polyomaviruses among the healthy blood donors. *J Med Virol*. 2016;88(7):1254-1261.
9. Antonsson A, Neale RE, O'Rourke P, et al. Prevalence and stability of antibodies to thirteen polyomaviruses and association with cutaneous squamous cell carcinoma: a population-based study. *J Clin Virol*. 2018;101:34-37.
10. Cason C, Monasta L, Zanotta N, et al. Antibody response to polyomavirus primary infection: high seroprevalence of Merkel cell polyomavirus and lymphoid tissue involvement. *J Neurovirol*. 2018;24(3):314-322.
11. Gossai A, Waterboer T, Nelson HH, et al. Seroepidemiology of human polyomaviruses in a US population. *Am J Epidemiol*. 2016;183(1):61-69.
12. Yu G, Greninger AL, Isa P, et al. Discovery of a novel polyomavirus in acute diarrheal samples from children. *PLoS One*. 2012;7(11):e49449.
13. Lim ES, Reyes A, Antonio M, et al. Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. *Virology*. 2013;436:295-303.
14. Rockett RJ, Sloots TP, Bowes S, et al. Detection of novel polyomaviruses, TSPyV, HPyV6, HPyV7, HPyV9 and MWPyV in feces, urine, blood, respiratory swabs and cerebrospinal fluid. *PLoS One*. 2013;8(5):e62764.
15. Ma F, Li D, Wei T, Li J, Zheng L. Quantitative detection of human Malawi polyomavirus in nasopharyngeal aspirates, sera, and feces in Beijing, China, using real-time TaqMan-based PCR. *Virol J*. 2017;14(1):152.
16. Bergallo M, Galliano I, Montanari P, et al. MW polyomavirus in diarrheal Italian infants. *Minerva pediatr*. 2023;75(3):327-330.
17. Pinheiro MS, Mendes GS, Santos N. Human polyomaviruses 10 and 11 in faecal samples from Brazilian children. *Braz J Microbiol*. 2020;51(2):585-591.
18. da Silva AS, de Campos GM, Giovanetti M, et al. Viral metagenomics unveils MW (Malawi) polyomavirus infection in Brazilian pediatric patients with acute respiratory disease. *J Med Virol*. 2023;95(4):e28688.
19. Csoma E, Bidiga L, Méhes G, Katona M, Gergely L. Survey of KI, WU, MW, and STL polyomavirus in cancerous and non-cancerous lung tissues. *Pathobiology*. 2018;85(3):179-185.
20. Peng J, Li K, Zhang C, Jin Q. MW polyomavirus and STL polyomavirus present in tonsillar tissues from children with chronic tonsillar disease. *Clin Microbiol Infect*. 2016;22(1):97.e1-97.e3.
21. Papa N, Zanotta N, Knowles A, Orzan E, Comar M. Detection of Malawi polyomavirus sequences in secondary lymphoid tissues from Italian healthy children: a transient site of infection. *Virol J*. 2016;13:97.
22. Kamminga S, van der Meijden E, de Brouwer C, Feltkamp M, Zaaijer H. Prevalence of DNA of fourteen human polyomaviruses determined in blood donors. *Transfusion*. 2019;59(12):3689-3697.
23. Bialasiewicz S, Rockett RJ, Barraclough KA, et al. Detection of recently discovered human polyomaviruses in a longitudinal kidney transplant cohort. *Am J Transplant*. 2016;16(9):2734-2740.
24. Buck CB, Phan GQ, Raiji MT, Murphy PM, McDermott DH, McBride AA. Complete genome sequence of a tenth human polyomavirus. *J Virol*. 2012;86(19):10887.
25. Bopp L, Wieland U, Hellmich M, Kreuter A, Pfister H, Silling S. Natural history of cutaneous human polyomavirus infection in healthy individuals. *Front Microbiol*. 2021;12:740947.
26. Wieland U, Silling S, Hellmich M, Potthoff A, Pfister H, Kreuter A. Human polyomaviruses 6, 7, 9, 10 and trichodysplasia spinulosa-associated polyomavirus in HIV-infected men. *J Gen Virol*. 2014;95(pt 4):928-932.
27. Li K, Zhang C, Zhao R, et al. The prevalence of STL polyomavirus in stool samples from Chinese children. *J Clin Virol*. 2015;66:19-23.
28. Pastrana DV, Fitzgerald PC, Phan GQ, et al. A divergent variant of the eleventh human polyomavirus species, saint louis polyomavirus. *Genome Announc*. 2013;1(5):e00812-13.
29. Csoma E, Lengyel G, Bányai K, et al. Study of Karolinska Institutet and Washington University polyomaviruses in tonsil, adenoid, throat swab and middle ear fluid samples. *Future Microbiol*. 2018;13(16):1719-1730.
30. Katona M, Jeles K, Kovács R, Csoma E. KI and WU polyomaviruses: seroprevalence study and DNA prevalence in SARS-CoV-2 RNA positive and negative respiratory samples. *Microorganisms*. 2022;10(4):752.
31. Jeles K, Katona M, Csoma E. Seroprevalence of four polyomaviruses linked to dermatological diseases: new findings and a comprehensive analysis. *Viruses*. 2022;14(10):2282.
32. Kamminga S, van der Meijden E, Wunderink HF, Touze A, Zaaijer HL, Feltkamp MCW. Development and evaluation of a broad

bead-based multiplex immunoassay to measure IgG seroreactivity against human polyomaviruses. *J Clin Microbiol*. 2018;56(4):e01566-17.
33. Herberhold S, Hellmich M, Panning M, et al. Human polyomavirus and human papillomavirus prevalence and viral load in non-malignant tonsillar tissue and tonsillar carcinoma. *Med Microbiol Immunol*. 2017;206(2):93-103.
34. Kourieh A, Combes JD, Tommasino M, et al. Prevalence and risk factors of human polyomavirus infections in non-malignant tonsils and gargles: the SPLIT study. *J Gen Virol*. 2018;99(12):1686-1698.
35. Zhong S, Randhawa PS, Ikegaya H, et al. Distribution patterns of BK polyomavirus (BKV) subtypes and subgroups in American, European and Asian populations suggest co-migration of BKV and the human race. *J Gen Virol*. 2009;90(pt 1):144-152.
36. Martel-Jantin C, Filippone C, Tortevoye P, et al. Molecular epidemiology of merkel cell polyomavirus: evidence for geographically related variant genotypes. *J Clin Microbiol*. 2014;52(5):1687-1690.
37. Hashida Y, Higuchi T, Matsui K, et al. Genetic variability of the noncoding control region of cutaneous merkel cell polyomavirus: identification of geographically related genotypes. *J Infect Dis*. 2018;217(10):1601-1611.
38. Tamura K. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Mol Biol Evol*. 1992;9(4):678-687.
39. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):1547-1549.
40. Leigh JW, Bryant D. POPART: full-feature software for haplotype network construction. *Methods Ecol Evol*. 2015;6(9):1110-1116.
41. Moens U, Prezioso C, Pietropaolo V. Genetic diversity of the non-coding control region of the novel human polyomaviruses. *Viruses*. 2020;12(12):1406.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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