Article DOI: https://doi.org/10.3201/eid2409.180829

Trichodysplasia Spinulosa Polyomavirus in Respiratory Tract of Immunocompromised Child

Technical Appendix

Supplementary Information

Nucleic acids (NA) were extracted from respiratory specimens of symptomatic individuals referred to Nottingham University Hospitals Trust, Nottingham, UK by the Nuclisens EasyMAG system (Biomerieux). Routine diagnostic PCR / RT-PCR assays for Human Adenovirus, Influenza (A and B), Human Metapneumovirus, Parainfluenza (1–4), Rhinovirus and Human Respiratory Syncytial Virus (RSV, A and B) were undertaken and surplus NA archived at −72°C. A total of 198 NPA and 20 throat swabs negative for the aforementioned viruses were identified from patients aged 6 months to 5 years old between January 2015 and February 2016 and subjected to extended diagnostic tests. All subsequently analyzed longitudinal samples from the sole TSPyV positive individual identified had been subject to the same diagnostic pathway, with the exception of January 2015 skin swab. This sample was screened by PCR / RT-PCR for Herpes Simplex Virus 1 and 2, Varicella Zoster Virus, Mumps virus, Enterovirus and Parechovirus and found to be negative for all targets.

Five µl NA from 8 –10 samples were combined to make a total of 22 pools and 1 µl of each screened for Polyomaviruses using a previously described degenerate pan-*Polyomavirus* assay (*1*) modified by using only the VP1–2 primer pair (see table below) and HotStarTaq (QIAGEN) in 15 µl reactions with the following PCR cycle conditions: 95°C for 15 minutes then 55 cycles of 95°C for 20s, 57°C for 20s and 72°C for 20s. Constituents of any positive pools were then re-screened individually to isolate the positive sample component(s). All putative positive samples were confirmed by Sanger sequencing (Source Bioscience, Nottingham UK) and BLAST analysis of the resulting nucleotide sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

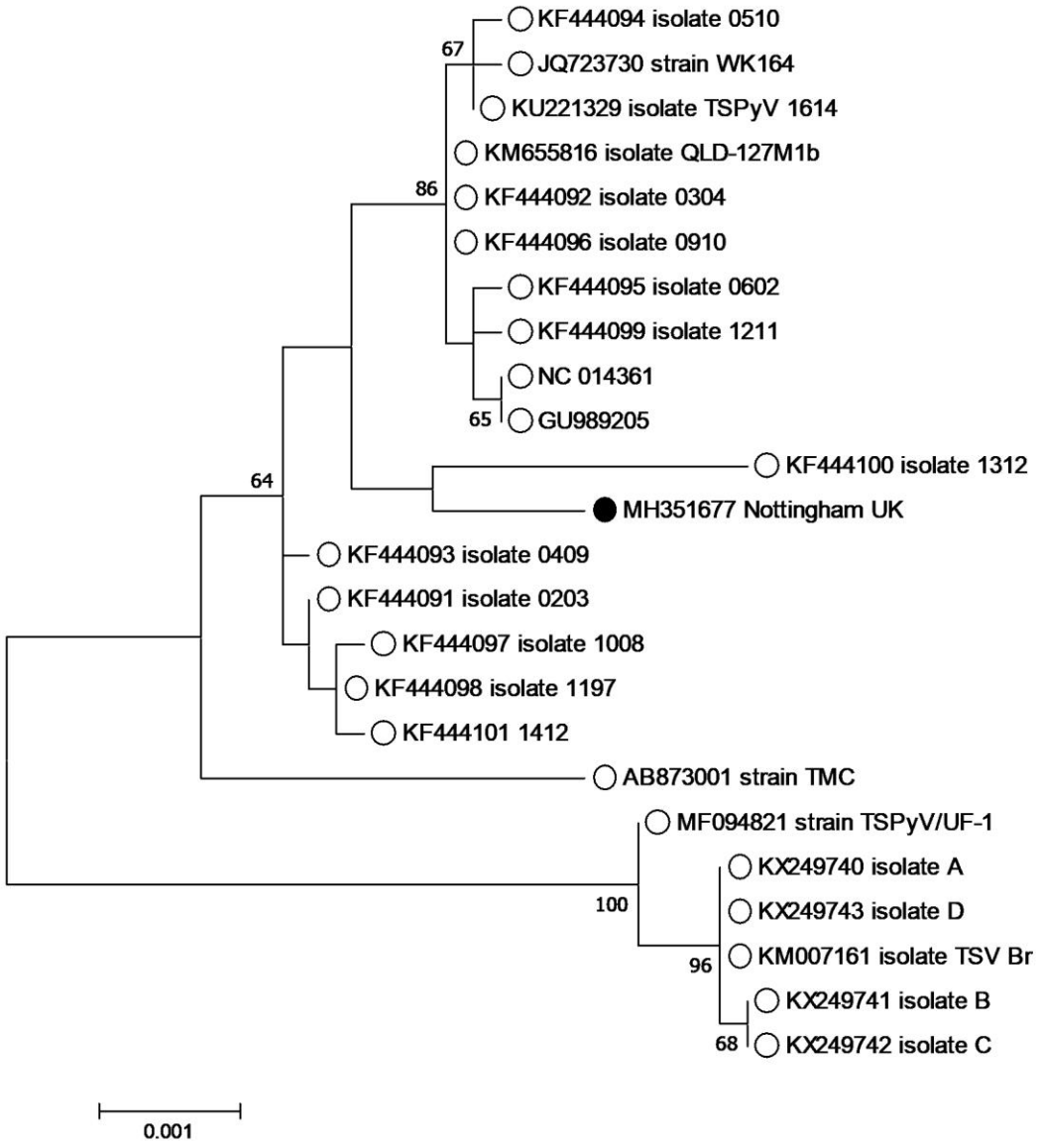
TSPyV-positive samples were quantitatively investigated by SYBR green Realtime PCR (qPCR) using novel in-house primers TSPyV326f and TSPyV510r (see table below, PCR conditions as above, Ct values represent an average of triplicate values) designed in Primer3 (http://primer3.ut.ee/) using an alignment created in MEGA7 (http://www.megasoftware.net/) of all 23 complete reference sequences available on Genbank in March 2018 (https://www.ncbi.nlm.nih.gov/nuccore/?term = txid862909[Organism:noexp]) to target a highly conserved genomic region. The entire circular 5232 nt genome of a single TSPyV-positive sample was amplified in 4 overlapping PCR products using additional primers designed as above (see table below, PCR conditions as above except with a 2 minute extension step and 45 cycles only). Amplicons were Sanger sequenced using both forward and reverse primers and a contiguous sequence representing the complete genome (Genbank ID MH351677) was assembled using Seqman Pro (DNASTAR, Madison WI) and compared to the reference genomes in MEGA7 (see figure below).

Reference

1. Johne R, Enderlein D, Nieper H, Müller H. Novel polyomavirus detected in the feces of a chimpanzee by nested broad-spectrum PCR. J Virol. 2005;79:3883–7. PubMed http://dx.doi.org/10.1128/JVI.79.6.3883-3887.2005

Technical Appendix Table. Primers for TSPyV PCR amplification

Assay	Primer	Genome position KU221329	Sequence	Product size	Source
Pan-polyoma screen	VP1–2f	2068–2097	ATGAAAATGGGGTTGGCCCNCTNTGYAARG	274bp	Johne et al 2005 (*1*)
	VP1–2r	2313–2341	CCCTCATAAACCCGAACYTCYTCHACYTG		
TSPyV specific PCR	TSPyVf326	326–345	TAACAACACATCCTCCCGCT	185bp	This study
	TSPyVr510	491–510	GCTGCTTCTCCTGCTAACAC		
Full genome amplification	TSPyVf326	as above	as above	1577bp	This study
	TSPyVr1902	1883–1902	GATTGAGGCCTTGTGTGCTC		
Full genome amplification	TSPyVf1774	1774–1793	ATATGTTTGCAGTGGGTGGG	1403bp	This study
	TSPyVr3176	3156–3176	TGCTCTGATGAATTTCCTGG		
Full genome amplification	TSPyVf3045	3045–3065	AGTGCTATTTGACCCTTGACA	1404bp	This study
	TSPyVr4448	4428–4448	GGGGTAAGTAAMAGGTGGGGA		
Full genome amplification	TSPyVf4255	4255–4274	AGAGRTCTGGGTCCTGCATG	1488bp	This study
	TSPyVr510	as above	as above		



Technical Appendix Figure. Phylogenetic tree of the complete 5232 bp Trichodysplasia Spinulosa-associated Polyomavirus genome. The tree was constructed using the maximum likelihood method based on Tamura-Nei model and 1000 bootstrapping replications. Twenty-three available complete TSPyV genomes (open circles) were retrieved from Genbank in March 2018 and compared with the strain identified in this study (filled circle).