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Short Communication

Detection of TS polyomavirus DNA in tonsillar tissues of children and adults: Evidence for site of viral latency

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ABSTRACT

Background: The trichodysplasia spinulosa-associated polyomavirus (TSPyV), a recently discovered species of the family *Polyomaviridae*, is associated with development of trichodysplasia spinulosa (TS), a rare follicular skin disease of immunocompromised individuals. The viral seroprevalence in the general population is ~70%, with little known of its route of transmission, latency, or primary infection site.
Objectives: We aimed to determine whether the viral DNA is detectable in tonsillar tissue of constitutionally healthy individuals, and what the corresponding antiviral seroreactivities are.
Study design: We tested 229 matched pairs of tonsillar tissue biopsies and serum samples from asymptomatic donors for TSPyV DNA by real-time quantitative PCR with primer pairs and Taq-Man probes targeting the VP1 and LT genes. The sera were studied by enzyme immunoassay (EIA) for TSPyV-VP1-IgG and the PCR-positive individuals also for -IgM and -IgG-avidity.
Results: TSPyV DNA was detectable in 8 (3.5%) of 229 tonsillar tissues, and in none of the corresponding sera. TSPyV IgG seroprevalence among children was 39% and among adults 70%. Each of the 8 PCR-positive subjects had antiviral IgG of high avidity but not IgM.
Conclusions: TSPyV PCR positivity of tonsillar samples of individuals with long-term immunity provides the first evidence of TSPyV in tonsils and suggests lymphoid tissue as a latency site of this emerging human pathogen.

1. Background

The human polyomavirus (HPyV) family today tentatively comprises 12 members [1–13], the infections by which are widespread and initially apparently asymptomatic [14]. The eighth HPyV discovered is the TS polyomavirus (TSPyV), infecting approximately 70% of the world's population [15–17]. Primary infection generally occurs during childhood, followed by the development of antiviral antibodies [17,18]. Vigorous replication of the virus in the inner root sheath cells of hair follicles accompanies trichodysplasia spinulosa (TS) [7], a rare skin disease of severely immunocompromised hosts including organ transplant recipients [19,20].

Data for TSPyV transmission and prevalence are scarce. TSPyV occurrence in TS lesions at striking abundance, high prevalence, and specific VP1 expression, imply that TSPyV replication is linked to TS pathogenesis [7,19,20]. The viral DNA has also been encountered at low prevalence and very low copy number in plucked eyebrows [7], skin swabs [19], and nasopharyngeal [21] as well as faecal and urine samples from asymptomatic individuals [8,21].

HPyVs in general establish persistent infections and undergo periodic reactivations, causing disease in susceptible hosts [22]. Evidence suggests that the lymphoid system plays a role in polyomavirus infections and persistence [23–26]. The previously identified HPyVs BKV and JCV or their DNA have occurred in tonsillar tissue in children, and JCV DNA also in the spleen and lymph nodes. We and others [24,25,27–31] have reported MCPyV DNA in tonsillar tissue (Table 1), suggesting lifelong persistence in lymphoid tissue or mucosa [28].

2. Objectives

Despite the ubiquitous distribution of TSPyV, the incidence of TS is very low, and the cases that occur usually have underlying immunosuppression. Our objective was to determine whether TSPyV can infect the lymphoid tissue, as do the BK, JC, KI, WU,

Abbreviations: HPyV, human polyomavirus family; TSPyV, trichodysplasia spinulosa-associated polyomavirus; TS, trichodysplasia spinulosa; VLP, virus like particle; qPCR, quantitative PCR.

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Table 1
Prevalences of PyV DNA in various cohorts with tonsillar tissue analysed by PCR-based methods.

HPyV	No. investigated samples	Prevalence (%)	Method	References	Country
JCPyV	70 children and adults	27 (39%)	PCR	Monaco et al. [26]	USA
	32 donors	14 (44%)	PCR	Kato et al. [24]	Japan
BKPyV	91 children and adults	5 (5.5%)	PCR	Babakir et al. [29]	Italy
	80 children	4 (5.0%)	qPCR	Comar et al. [30]	Italy
	50 children	3 (6%)	qPCR	Comar et al. [31]	Italy
KIPyV	91 children and adults	11 (12%)	PCR	Babakir et al. [29]	Italy
	29 children and adults	2 (6.9%)	qPCR	Astegiano et al. [32]	Italy
WUPyV	91 children and adults	4 (4.4%)	PCR	Babakir et al. [29]	Italy
	229 children and adults	5 (2.2)	PCR	Kantola et al. [28]	Finland
	50 children	6 (12%)	qPCR	Comar et al. [31]	Italy
MCPyV	229 children and adults	8	PCR	Kantola et al. [28]	Finland
TSPyV	ND	ND	ND	ND	ND
HPyV6	ND	ND	ND	ND	ND
HPyV7	ND	ND	ND	ND	ND
HPyV9	ND	ND	ND	ND	ND
HPyV10	ND	ND	ND	ND	ND
STLPyV	ND	ND	ND	ND	ND
HPyV12	ND	ND	ND	ND	ND

and MC polyomaviruses [24–26,28–32]. To this end, we screened archived tonsillar tissue and the corresponding sera from our recent study for TSPyV DNA by qualitative real-time PCR [28]. We also quantified the single-copy gene *RNase P*, such that the DNA copy numbers could be normalised to cell numbers. We determined the sensitivity, specificity, accuracy, and reproducibility of these assays and also studied the corresponding sera for TSPyV-specific antibodies, by newly established virus-like particle (VLP)-based assays (Chen et al., EID, in revision) [16,17].

3. Study design

3.1. Patients, tissues and sera

The clinical material comprised matched pairs of tonsillar tissue and sera from 229 subjects: 80 asymptomatic children and 149 adults (Table 2). Paediatric donors ranged in age from 1.5 to 15 years (average, 7.5), and adult donors from 16 to 72 (average, 30.6). Of the specimens tested, 105 were from male and 124 from female subjects. The tonsillar tissues were from tonsillectomies mostly for chronic tonsillitis or tonsillar hypertrophy.

3.2. DNA extraction from tonsillar tissues and sera

Total nucleic acid extraction from the tonsillar tissues was done by the DNA Mini kit (Qiagen, Crawley, UK) according to manufacturer's instructions, and from the sera by lysis buffer/proteinase *K* treatment and phenol–chloroform extraction [16,28,33].

Table 2
The tonsillar tissues in this study were obtained during tonsillectomy mostly due to chronic tonsillitis.

	Diagnosis	No. samples tested
1	Hyperplastic palatine tonsils	43
2	Chronic tonsillitis	108
3	Acute tonsillitis of unknown causes	9
4	Sleep apnea	2
5	Peritonsillar abscess	19
6	Hyperplastic palatine and adenoid tonsils	25
7	Oral breathing	8
8	Foul breathe	1
9	Chronic pansinuitis	3
10	Unknown	11
	Total	229

3.3. DNA screening

Samples were first screened for the single-copy human *RNase P* gene by real-time quantitative PCR (qPCR). The plasmid containing this gene was a generous gift from Dr Janet S. Butel (Baylor College of Medicine, Houston, Texas, USA) [34]. Each sample was subjected to Taq Man PCR for the control *RNase P* gene using as forward primer 5′-GAGGGAAGCTCATCAGTGGGG-3′, corresponding to nt 9–29, as reverse primer 5′-CTTGGGAAGGTCTGAGACTAGGG-3′, corresponding to nt 70–92, and fluorogenic probe 5′-FAM-AGTGCGTCCTGTCACTCCACTC-TAMRA-3′ corresponding to nt 40–61 of *Homo sapiens ribonuclease P* RNA component H1 (RPPH1), *RNase P* RNA (GenBank accession no. NR_002312.1). Standard curves were obtained for the *RNase P* gene plasmid with serial 10-fold dilutions, and copy numbers ranging from 10^8 to 10^0. PCR amplification reactions were set up in a reaction volume of 17 μl using 10 μl TaqMan universal PCR master mix (Applied Biosystems), and amplifications were performed with the Stratagene Mx3005p (Stratagene, Foster City, CA, USA). Reactions were considered positive if >10 viral genome copies/reaction were detectable. This strategy would detected potential PCR inhibitors in the DNA preparations and determined the human cell equivalents in each DNA sample, as well as normalised TSPyV viral loads to human cell numbers.

3.4. TSPyV PCR

For TSPyV detection, qPCR was performed by previously described LT3 primers and probe [7,16]. All samples were reanalysed with the VP1 PCR primer set [7], and positive samples were sequenced.

3.5. TSPyV serology

TSPyV IgG antibodies in 197 serum samples available from the 229 patients were measured by in-house EIA based on VP1 VLPs as described [17]. The PCR-positive individuals were also tested for IgM by μ-capture EIA and for IgG avidity by the corresponding EIA (Chen et al., EID, in revision).

4. Results

4.1. DNA recoveries

DNA was extracted from the specimens and screened for suitability for qPCR analysis by amplification of the cellular *RNase P*