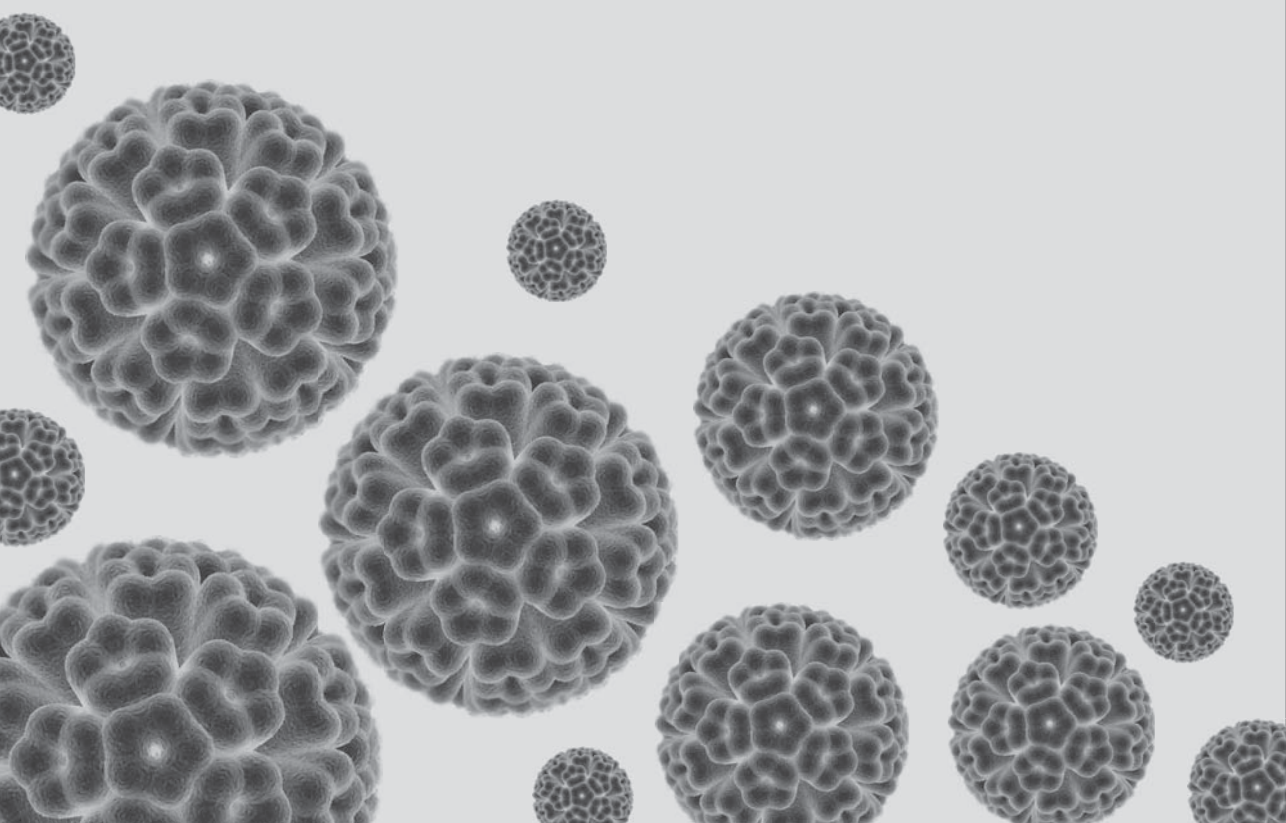

VePyV1 CaPyV JCPyV MPyV SA12 RacPyV GHPyV BKPyV
BatPyV CPyV OraPyV1 FPyV MptV APPyV1 SqPyV LPyV CSLPyV
EPyV PRPyV1 APPyV2 KIPyV MiPyV PtvPyV2c OtPyV1 STLPyV
MFPyV1 HPyV9 SV40 TSPyV CoPyV1 PPPyV CPPyV HPyV12
MXPyV PtvPyV1a PDPyV EiPyV1 AtPPyV1 HaPyV GggPyV1
CdPyV DRPyV MWPyV APyV CAPyV1 HPyV7 ChPyV MasPyV
WUPyV HPyV6 BPyV MCPyV OraPyV2 MMPyV SLPyV HPyV10

Part II

TSPyV Infection and Pathogenesis



Chapter 2

Clinical and viral aspects of trichodysplasia spinulosa

Adapted from:*

The trichodysplasia spinulosa-associated polyomavirus: virological background and clinical implications

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** Note: Adaptation of this chapter from the original published article concerns textual, tabular and figure adjustments. The text has been updated with novel TSPyV data from the literature until mid-2014. The tables were reviewed and updated until mid-2014. In figure 2, the TSPyV genome map is updated with the newly identified ORF5 that encodes ALTO/MT-antigen.*

Abstract

Trichodysplasia spinulosa-associated polyomavirus (TSPyV) is one of the new species of the *Polyomaviridae* family discovered in 2010. TSPyV infects humans and is associated with the development of a rare skin disease called trichodysplasia spinulosa. Trichodysplasia spinulosa is a disease of severely immunocompromized hosts characterized by follicular distention and keratotic spine formation, most notably on the face. Electron microscopy, immunohistochemistry, and viral load measurements suggest an etiological role of active TSPyV infection in the development of this disfiguring disease. This chapter will address some clinical and virological properties of TSPyV, and touches upon epidemiologic, diagnostic, and therapeutic aspects of TSPyV infection.

Pathogenic human polyomaviruses

Human polyomaviruses (HPyVs) are ubiquitous viruses that infect their host without causing apparent disease. After primary infection, they can persist asymptomatically, sometimes producing small quantities of detectable progeny. This state is often referred to as latency. In case of impaired immune function, for example because of AIDS or the use of immunosuppressive drugs, HPyVs can reactivate from latency and cause severe disease. As the number of solid organ transplantations gradually increases, as shown for instance by the United States Organ Procurement and Transplantation network (**Figure 1**) [1], the incidence of HPyV-associated disease in long-term immunosuppressed patients is expected to rise.

So far, at least four out of thirteen HPyVs have been associated with human disease. As pointed out in **Chapter 1**, this includes BKPyV (allograft nephropathy [2, 3]), JCPyV (progressive multifocal leukoencephalopathy [4 - 6]), MCPyV (Merkel cell carcinoma [7 - 10]) and TSPyV. TSPyV, identified in our lab in 2010, is associated with a rare skin disease called trichodysplasia spinulosa (TS) [11]. This disease is observed exclusively in severely immunocompromized hosts [12, 13]. In this chapter, several clinical and virological aspects of TS and TSPyV are presented in more detail.

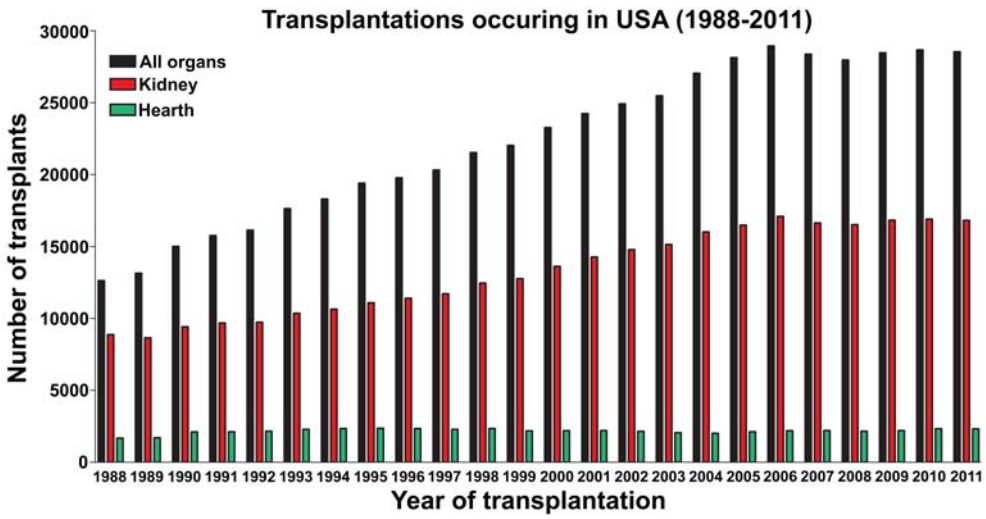

Figure 1. Overview of (solid) organ transplantations in USA between 1988 and 2011. Bars depicted in black represent all solid organ transplantation, i.e., kidney, liver, heart, lung, pancreas, intestine, kidney+pancreas and heart+lung. Red colored bars represent the number of kidney transplantations and green bars the number of heart transplantations (source OPTN, data as of September 2012).

The trichodysplasia spinulosa-associated polyomavirus

TSPyV genome and gene products

TSPyV genome consists of a relatively small double-stranded DNA of 5232 basepairs. Its genomic orientation is similar to other known polyomaviruses, with its early T-antigen and late VP products encoded bidirectionally on separate DNA strands. At least five open reading frames can be located, transcription of which is regulated by the non-coding control region (NCCR) and is subject of alternative splicing (**Figure 2**) [11].

In analogy with other polyomaviruses, upon infection probably first the T genes are expressed that code for small (ST) and large T-antigen (LT), and possibly for middle T-antigen (MT) and ALTO as well (van der Meijden, unpublished results). ST-, MT- and LT-antigen initiate at the same start codon, but are a subject of alternative splicing downstream. As a result, ST-, MT- and LT-antigen share N-terminal region of approximately 80 amino acids which contain some highly conserved regions important for polyomavirus replication and cellular transformation (**Figure 2**) [11]. Furthermore, ST-antigen contains a unique putative PP2A-binding motif, MT-antigen a membrane-spanning domain and several putative Tyr, Ser and Thr phosphorylation sites, and LT-antigen a number of motifs that putatively interact with tumor suppressor and cell cycle regulatory proteins like pRB and p53. Preliminary analyses of messengerRNA and protein expression of the T-region confirmed this pattern for TSPyV that includes ST-, MT-, LT-antigen expression including an additional ALTO product (van der Meijden, unpublished results). The function of TSPyV T-antigens, individually and in concert, related to virus replication and possibly cellular transformation, remains to be studied.

The opposing strand of the TSPyV genome encodes the late structural proteins VP1, VP2, and possibly VP3 that together form the viral capsid. VP1 is the major protein of the pentameric viral capsomere that likely incorporates one copy of either VP2 or VP3 [14]. The icosahedral capsid is constructed of 72 of such capsomers. Antigenetically, VP1 is considered the immunodominant polyomavirus protein and therefore TSPyV VP1 represents an important antigen in measuring host antibody seroreactivity against TSPyV.

From the late pre-messengerRNA, complementary to the early coding region, microRNAs can be encoded, which can regulate gene expression by directing cleavage of the targeted T-antigen messengerRNAs and thereby repressing translation. For BKPyV, this mechanism was recently experimentally identified [15]. For MCPyV this mechanism was postulated as well [16, 17]. Preliminary bioinformatics data suggest that TSPyV may code as well for a microRNA that targets the transcripts of both LT-antigen and MT-antigen/ALTO (**Figure 2**).

TSPyV prevalence

For BKPyV and JCPyV it is suggested that the primary infection takes place sometime early in life and transmission probably occurs through the feco-oral, urino-oral, or respiratory route

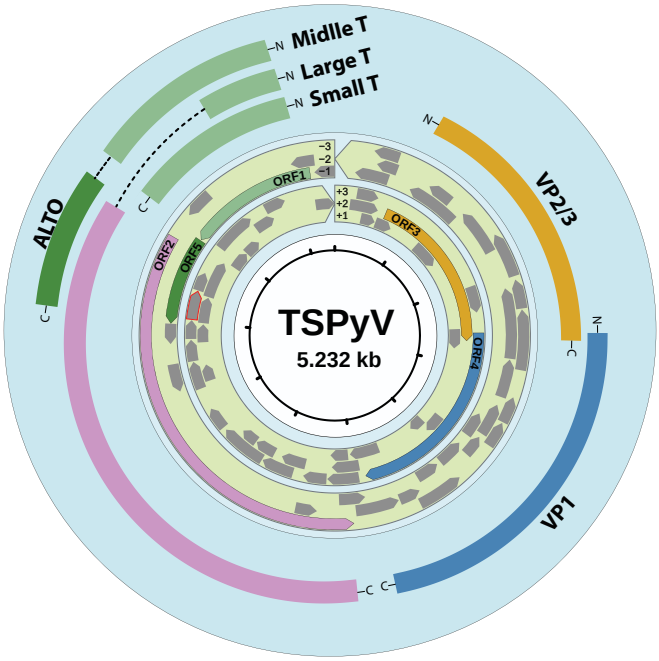

Figure 2. TSPyV genome map. The five large open reading frames (ORFs) in different reading frames are depicted by colored inner arrows (ORF1-ORF5). Additional smaller ORFs are depicted by gray inner arrows. Transcription of the early region results into a pre-messengerRNA that contains ORF1, ORF2 and ORF5. Upon alternative splicing events (introns indicated by dotted lines), small T-antigen (light-green), large T-antigen (light-green + pink) and middle T-antigen (light-green + dark-green) translation of the indicated protein will be putatively initiated at the same start codon and all proteins will share the N-terminal region (light-green). Alternatively, internal start codons could be used in RF-1 that might result into translation of ALTO product(s) (dark green). The late coding region contains ORF3 and ORF5 that encode VP1, VP2, and possibly VP3 viral capsid proteins. Putatively, a microRNA that is complementary to the early coding region targeting ORF2 and ORF4, is transcribed from a late pre-messengerRNA (red-lined arrow). The region before the early and late ORFs contains the non-coding control region and the origin of replication.

[18]. However, for none of the HPyVs the exact route of transmission is known, including the TSPyV and MCPyV pathogenic viruses. Although these latter two HPyVs cause skin disease, large differences in skin viral DNA prevalence suggest different routes of infection, transmission, and/or persistence. MCPyV DNA can be detected on healthy and diseased skin in 30–60% of individuals, regardless of the host immune status [19 - 27]. Studies that have looked at TSPyV prevalence in various samples (e.g., skin swabs, skin biopsies, plucked eyebrows, serum/plasma, tonsils, urine etc.) have found less than 5% DNA-positivity in asymptomatic individuals, regardless of their immune status [11, 13, 28, 29] (**Table 1**). Higher TSPyV prevalence and high viral loads were only reported in TS patients [13], which suggest that TSPyV is the causative agent of TS disease (**Chapter 3**). Hypothetically, at some stage in life TSPyV infects the skin, to cause disease there in a small minority of immunocompromized hosts. If, however, the skin represents the major organ of TSPyV persistence, is far from clear, as

the majority of infected (seropositive) individuals are skin TSPyV-negative. Whether TS is a manifestation of TSPyV reactivation from a yet unidentified (transplanted) organ reservoir [29], or an unfortunately timed primary infection in the midst of immunosuppression, is unknown.

Table 1: TSPyV prevalence

Samples tested	Number of samples	Number of positives (%)	References
Skin biopsy			
TS, Immunocompromized	1	1 (100)	[11]
TS, Immunocompromized	1	1 (100)	[12]
TS, Immunocompromized	11	11 (100)	[13]
TS, Immunocompromized	1	1 (100)	[49]
TS, Immunocompromized	1	1 (100)	[50]
TS, Immunocompromized	2	2 (100)	[51]
Various other skin disease,	193	0 (-)	[52]
Pilomatricoma	10	0 (-)	[53]
Plucked eyebrow hair			
Immunocompromized (TS)	1	1 (100)	This study
Immunocompromized (RTR)	69	3 (4)	[11]
Immunocompromized (RTR)	81	2 (2)	This study
Tonsillar tissue			
Immunocompetent	229	8 (4)	[29]
Nasopharyngeal swabs			
Immunocompromized (TX)	32	1 (3)	[54]
Feces			
Immunocompromized (TX)	32	1 (3)	[54]
Immunocompetent (GE)	38	0 (-)	[28]
Immunocompetent (GE)	160	2 (1)	This study
Kidney biopsy			
Immunocompromized (TS)	1	1 (100)	[49]
Urine			
Immunocompromized (TS)	1	0 (-)	[49]
Immunocompromized (TX)	179	2 (1)	[28]
Immunocompetent	17	0 (-)	This study
Serum/plasma			
Immunocompromized (TX)	88	0 (-)	[28]
Immunocompetent	19	0 (-)	This study
Cerebrospinal fluid			
Immunocompromized (TX)	74	0 (-)	[28]
Immunocompetent	38	0 (-)	This study

Abbreviations: TS, trichodysplasia spinulosa; RTR, renal transplant recipients; TX, transplant patient (not fully specified in the cited study [28]); GE, gastroenteritis.

TSPyV seroprevalence

Epidemiological studies looking at polyomavirus seroprevalence in humans indicate that HPyV infections of the general population are highly prevalent and occur at early age, probably without clinical manifestation. In healthy, immunocompetent adults, seroprevalences of 80-100% for BKPyV [30 - 33], 40-70% for JCPyV [30 - 33], 55-90% for KIPyV [31, 32, 34, 35], 70-100% for WUPyV [31, 32, 34, 35], 40-80% for MCPyV [19, 31, 32, 36 - 38], 60% for HPyV6 [19, 38], 35% for HPyV7 [19, 38], 35-50% for HPyV9 [38 - 40] and 17-23% for HPyV12 [41] have been reported so far.

Detection of serum antibodies against TSPyV VP1 have been described in several studies using either a multiplex serology method based on Glutathione-S-transferase (GST)-VP1 fusion proteins [38, 42, 43] or VP1 VLP-based antibody detection by ELISA [44 - 46]. All these studies suggest that TSPyV circulate widely in humans, with seroprevalences of about 75% in adults in all populations tested, including a total of 528 Dutch, 371 and 394 Finnish, 829 Italian, and 799 Australian healthy individuals [38, 42, 44 - 46]. Furthermore, age distribution analysis of TSPyV VP1 seroprevalences revealed an increase from about 41% in children aged 0–9 years to 75% in adults aged 30 years and older, and wane later in life comparable to BKPyV [38, 42]. This distribution pattern is in agreement with other HPyVs, although modest differences in age distribution patterns are observed sometimes [31, 39, 41].

The seroreactivity of TSPyV VP1 antibody responses of seropositive individuals shows a decline in the median seroreactivity from 40 to 49 years of age onwards [38, 42], similar to what was reported before for BKPyV [47]. This waning of antibody levels in the elderly might be due to immunosenescence [45]. Serum cross-reactivity between TSPyV and other (related) polyomaviruses, as shown for instance between HPyV9/LPyV [39, 40] and MCPyV/ChPyV [48], and to a lesser extent between HPyV6/HPyV7 [19, 38] and BKPyV/JCPyV [31], was not observed [48].

Earlier BKPyV serological studies have revealed increased VP1 antibody and seropositivity levels in immunocompromized transplant patients, in accordance with intensity of BKPyV infection or reactivation post-transplantation [55 - 57]. Whether this pattern is also true for TSPyV remains to be seen. So far, only one study has shown a higher (89%) TSPyV VP1 seroprevalence in a renal-transplant patient population compared to healthy individuals (75%) [42]. How to interpret the putative increase in TSPyV seropositivity concomitant with immunosuppressive treatment is not fully understood. Hypothetically, this may be explained by an increase in humoral immunity in response to TSPyV reactivation, possibly viremia, because of iatrogenic suppression of cellular immunity [11].

Altogether, seroepidemiological data indicate that TSPyV infection is common and occurs primarily at young age with a deterioration of antibody levels at the later stages of life. Knowledge about cellular immunity against TSPyV is scarce. A Finnish study suggested that TSPyV Th-cell responses correlate with TSPyV serological responses, but can sometimes also be detected in TSPyV-seronegative individuals [58].

Trichodysplasia spinulosa

Clinical description and epidemiology

TS is a rare skin disease first reported by Izakovic and colleagues in 1995 [59]. In 1999, Haycox and colleagues fully described the disease and introduced the term “trichodysplasia spinulosa” [60]. They showed for the first time the presence of virus particles and suggested a viral etiology for TS. Ever since, approximately 30 comparable cases were published (**Table 2**). Many of these reports adopted the term “trichodysplasia spinulosa”, whereas others used different terms for the same condition, such as ”trichodysplasia”, “pilomatrix dysplasia of immune suppression”, “cyclosporine-induced folliculodystrophy” or “viral-associated trichodysplasia” [11 - 13, 49 - 51, 61 - 74].

So far, TS has been observed in immunosuppressed organ transplant patients and occasionally in chronic and acute lymphocytic leukemia patients. Despite the disparity in TS terminology, in all cases the disease was clinically characterized by spiny follicular papules distributed largely on the face and ears, and to a lesser extent on extremities, trunk, and scalp. In most patients, non-scaring alopecia of the eyebrows was observed, upon which small hyperkeratotic white-yellowish spicules started to protrude the skin. At the same time, these features manifested also on the nose and ears (**Figure 3A**). As the disease progressed, the skin of eyebrows, ears, and nose thickened to cause disfigurement of the facial appearance, sometimes resulting in a “leonine facies” [70], in combination with increased conspicuous spine expression [11, 71].

Histology and viral pathology

Histological analysis of TS skin biopsies reveals acanthosis of the epidermis in most cases. In addition, enlargement of the hair follicles with excessive number of proliferative cells is observed [60]. Compared with normal skin, TS follicles are absent of hair shafts, and papilla with abnormal corneocytes filling the infundibula of the follicles (**Figure 3B**). Sometimes a subtle perifollicular lymphocytic infiltrate is seen.

Electron microscopical analyses of TS lesions repeatedly revealed intranuclear, crystalloid-organized, regularly spaced 38 - 45-nm virus particles (**Figure 3E**). The identity of TSPyV remained unknown for many years [12, 60, 63, 64, 66, 67], until in 2010 when the double-stranded DNA viral genome was revealed with the help of rolling-circle amplification, cloning, primer-genome-walking, and sequencing [11]. By now, several research groups have confirmed the presence of TSPyV DNA in lesional TS samples (**Table 2**) [12, 13, 50, 51]. The given name of TSPyV was accepted by the international polyomavirus study group [79], while awaiting official recognition by the ICTV.

Development of TS may be caused by uncontrolled proliferation of TSPyV-infected inner root sheath (IRS) cells. The irregular IRS cells show enlarged, dystrophic and prominent eosinophilic perinuclear globules that probably represent the accumulation of trichohyalin protein (**Figure 3C** and **D**) [13, 60]. Ki-67 staining of the affected cells suggests that the IRS cells, or a subpopulation thereof, are hyperproliferating [60].

Table 2A: Overview of reported TS cases

Publication year	Gender	Age	Medical history	Country	Reference
1995	Male	31	Kidney transplant	GER	[59]
1999 **	Male	44	Kidney-pancreas transplant	USA	[13, 60]
2000	Female	13	Lung transplant	USA	[61]
2004	Female	34	Kidney transplant	USA	[62]
2004	Female	13	Kidney transplant	USA	[63]
2005 (case 1) **	Male	19	ALL (Pre-B cell)	USA	[13, 64]
2005 (case 2)	Male	8	Kidney transplant	USA	[64]
2006	Female	48	Kidney transplant	USA	[65]
2007 (case 1) **	Male	8	ALL (T-cell)	AUS	[13, 67]
2007 (case 2)	Male	6	ALL (T-cell)	AUS	[67]
2007	Male	68	non-Hodgkin's lymphoma	USA	[66]
2008	Male	70	CLL	AUS	[69]
2008 **	Female	37	Heart transplant	USA	[13, 68]
2010 **	Female	5	Heart transplant	CAN	[13, 71]
2010 **	Male	5	Heart transplant	USA	[13, 70]
2010 *	Male	15	Heart transplant	NLD	[11]
2011 **	Female	7	ALL (Pre-B cell)	USA	[12]
2011	Female	27	Kidney transplant	USA	[72]
2011	Female	9	ALL (Pre-B cell)	CAN	[73]
2012 (case 1) **	Male	5	Kidney transplant	USA	[13]
2012 (case 2) **	Female	63	Heart transplant	USA	[13]
2012 (case 3) **	Male	62	Lung transplant	USA	[13, 51]
2012 **	Male	48	Kidney transplant	USA	[49]
2012 **	Female	57	CLL	USA	[50]
2012	Female	46	Kidney transplant	USA	[74]
2013 **	Female	49	Kidney transplant (Lupus)	AUS	[75]
2013 **	Female	20	Lupus glomerulonephritis***	FRA	[76]
2013	Male	5	ALL (Pre-B cell)	LBN	[77]
2013	Female	14	Lung transplant	USA	[78]

Table 2B: Summery of reported TS cases shown in **Table 2A**

Total Number of TS cases	Male:Female ratio	Mean age	Transplant type (TX) or underlying disease (n, relative %)	Relative percentage of Countries reporting TS cases (n, relative %)
29	14:15	29	Kidney TX: (10, 34%) Kidney-pancreas TX: (1, 3%) Heart TX: (5, 17%) Lung TX: (3, 10%) ALL (Pre-B cell): (4, 14%) CLL: (2, 7%) ALL (T-cell): (2, 7%) NHL: (1, 3%) LGN: (1, 3%)	USA: (19, 66%) AUS: (4, 14%) CAN: (2, 7%) NLD: (1, 3%) GER: (1, 3%) FRA: (1, 3%) LBN: (1, 3%)

* Identification of TSPyV [11]

** Presence of TSPyV DNA confirmed

*** Lupus glomerulonephritis condition was treated with immunosuppression, shortly after pt died

Abbreviations: USA, United states of America; AUS, Australia; CAN, Canada; NLD, The Netherlands; GER, Germany; FRA, France; LBN, Lebanon; ALL, Acute lymphocytic leukemia; CLL, Chronic lymphocytic leukemia; M, Male; F, Female; TX, Transplant type; NHL, non-Hodgkin's lymphoma; LGN, Lupus glomerulonephritis

Patients at risk and diagnosis

TS is exclusively observed in immunocompromized patients, especially kidney(-pancreas) (37%), heart (17%) and lung (10%) transplant recipients (**Table 2B**). It can also develop in otherwise immunocompromized patients, e.g., in pre-B cell (14%) and T-cell (7%) acute lymphocytic leukemia patients, chronic lymphocytic leukemia patients (7%), non-Hodgkin's lymphoma patient (3%), and Lupus glomerulonephritis patient treated with immunosuppression (3%). The solid organ transplant group, especially the kidney graft recipients, seem the most hit by TSPyV. If this reflects the numbers of susceptible immunosuppressed hosts (**Figure 1**), or rather is related pathogenically to the transplanted organ is unknown. For BKPyV it is known that the course and dose of immunosuppression is associated with polyomavirus reactivation and accompanying complications [80]. Whether this is also true for TSPyV remains to be seen.

Diagnosis of TS is primarily established on clinical features, such as visual detection of the spiny follicular papules on the face (**Figure 3A**). The clinical diagnosis can be confirmed by histopathologic analysis of a biopsy of the lesional skin showing enlarged dystrophic hair follicles with eosinophilic perinuclear globules of the IRS cells (**Figure 3C**). TSPyV VP1 staining, as illustrated in **Figure 3D** can be added to the immunohistochemical diagnosis.

Electron microscopy has proven useful for visualization of TSPyV particles in nuclei of IRS cells of affected hair follicles and provided the first clue of TS having a viral etiology.

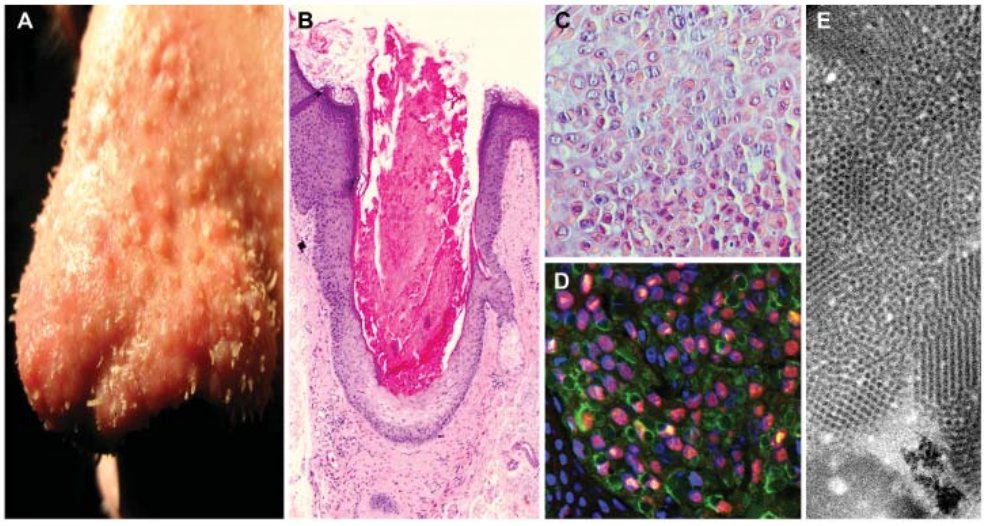

Figure 3. Clinical and (immuno)histopathological characteristics of TS disease. (**A**) Clinical features of the TS disease seen on the nose with hard small hyperkeratotic white-yellowish spicules protruding the skin. (**B**) H&E characteristics of lesional skin showing the conspicuous papule and spicule protruding from the epidermis. (**C**) A magnified picture of an affected hair follicle showing irregular inner root sheath cells with enlarged, dystrophic eosinophilic, and perinuclear globules. (**D**) Immunohistochemistry staining of an enlarged hair follicle showing colocalization of trichohyalin (green) and TSPyV VP1 (red). (**E**) Electron microscopy of an inner root sheath cell with intranuclear, crystalloid-organized, regularly spaced 38 - 45-nm virus particles.

Low sensitivity and specificity, however, as well as its cumbersomeness make this method less suitable for TSPyV detection in a clinical setting. Virus culture from fresh materials, in an attempt to identify the nature of the virus particles, was so far unsuccessful [64].

TSPyV PCR

In addition to the clinical and histopathological approach, TSPyV DNA detection and load measurement by quantitative PCR can be included in the TS diagnosis work-up. All lesional TS samples analyzed so far for TSPyV DNA content, were shown positive with high average viral loads, as described in **Chapter 3** [11, 13]. Normal skin samples of TS patients, as well as skin swabs and plucked eyebrow hairs from unaffected individuals were occasionally shown to be TSPyV DNA-positive, but with low viral loads [11, 13]. Up until now, four primer and probe sets for (quantitative) PCR have been described for TSPyV, one located in the NCCR, one in VP1 and two in the T-antigen coding regions [11, 49].

Preferably, a lesional skin biopsy or plucked spicule is used for TSPyV detection in TS patients [11 - 13]. Proteinase-K treatment and total DNA extraction of the material prior to PCR analysis is indicated to remove excessive protein and protective capsid structures. A non-invasive method to obtain clinical sample would be swabbing of the patient's face, for instance of the forehead that proved to bear high loads of TSPyV DNA in one TS patient sampled in this way [11, 13], suggesting equal sensitivity of skin swab and biopsy to detect viral DNA. However, for viral load calculations normalized for cellular DNA content and the number of cells, only a biopsy or plucked spicule can be regarded a reliable specimen.

PCR analysis of plucked eyebrow hairs has revealed the presence of TSPyV DNA in a small proportion (4%) of asymptomatic renal transplant recipients [11]. How forehead swabs and plucked eyebrow hairs compare with respect to the number of obtained cells, viral DNA detection and load measurement is unknown. Interestingly, in a study a 13-year-old immunocompromized heart transplant patient without TS symptoms sampled 1 month after his immunosuppression, TSPyV DNA was detected in his stool and in a nasopharyngeal swab. Repeated analysis in the following months remained negative [54]. Occasionally also urine and kidney samples of immunocompromized patients were found to be positive for TSPyV DNA [49]. The meaning of these findings, in particular their role in TS diagnosis, remains unclear.

Altogether, skin swabs, plucked hairs, and fresh or fixed lesional biopsies of the affected skin can serve as a proper (diagnostic) sample for viral detection and load measurements by (quantitative) PCR to confirm TSPyV infection and TS diagnosis, and to monitor disease progression and/or treatment efficacy.

Antiviral treatment

Improving the patient's immunity usually leads to complete resolution of TS symptoms. In case of TS in (organ) transplant recipients, reducing the dose of immunosuppressive drugs

should be considered, obviously without endangering the grafted organ by immune rejection. This strategy has been shown as the best option at the moment to improve the outcome of BKPyV-associated nephropathy [81, 82].

Next to reduction of immunosuppression, antiviral therapy should be considered a treatment option in controlling viral replication and TS progression without jeopardizing the transplanted organ. A number of TS patients treated with topical cidofovir 1 - 3% cream, which serves as a cytosine analogue inhibiting human polymerase activity needed for polyomavirus replication, have demonstrated significant reduction in symptoms [11, 50, 63, 64, 66]. Nephrotoxicity after topical use of this drug was monitored in some patients, but this effect was not reported. Furthermore, oral valganciclovir, a guanosine analogue also inhibiting polymerase activity, has been reported to have modest in some and strong activity in other TS patients [68, 70, 71]. The putative mechanism of action of this drug in the treatment of TS remains unclear at the moment, since valganciclovir requires (viral) thymidine kinase-mediated modifications to exert its polymerase-inhibiting effect, which are not expected to occur upon polyomavirus infection [83].

Concluding remarks

TSPyV is a ubiquitous virus, similar to most other HPyVs, as concluded from its 75% seroprevalence. When looking at its DNA prevalence in TS-asymptomatic individuals, however, the virus appears difficult to detect with a prevalence not exceeding 5% (**Table 1**). Whether TSPyV persists in human skin and replicates at undetectable levels, or alternatively has its latent reservoir in an extracutaneous site, like for instance the tonsils, remains to be seen. It is also not known how people acquire TSPyV infection, for instance by direct contact or by respiratory transmission, and whether TS symptoms are caused by reactivation as a result of poor immunity or by primary infection later in life in the midst of immunosuppression. In addition, the pathogenic mechanisms used by TSPyV are unknown, but likely include induction of hyperproliferation of IRS cells through its T-antigens.

With the identification of TSPyV as the probable causative infectious agent, improvement of TS diagnosis and clinical care can be achieved. This includes TSPyV-specific DNA detection and quantification, and immunohistochemistry or immunofluorescence in the course of the diagnostic process, and antiviral therapy as a treatment option. Because of the increasing number of immunocompromized patients and the high TSPyV seroprevalence in healthy populations, it is expected that TS will be diagnosed and reported more often in immunocompromized patients than it is at this moment. For this reason, clinicians, in particular nephrologists, dermatologists, and pathologists, should become more aware of this condition, knowing that appropriate viral diagnosis and treatment is at hand.

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