Specific Detection of Trichodysplasia Spinulosa–Associated Polyomavirus DNA in Skin and Renal Allograft Tissues in a Patient With Trichodysplasia Spinulosa

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Background: Trichodysplasia spinulosa (TS) is a rare, disfiguring skin condition that affects immunosuppressed patients, universally involving the central face. New data point to the recently discovered TS-associated polyomavirus (TSPyV) as the causative agent.

Observations: We report a case of TS in a 48-year-old African American man after renal transplant; via polymerase chain reaction and sequencing, confirm the detection of TSPyV DNA in renal allograft tissue. Results of polymerase chain reaction analysis were negative for Merkel cell polyomavirus in lesional skin. Fifteen months later, urine cytologic findings showed morphologic evidence of a urinary tract polyomavirus infection. Results of SV40 immunohistochemical analysis were negative in lesional skin, renal allograft, and urine specimens.

Conclusions: To our knowledge, this is the first reported case in which TSPyV DNA has been detected in extracutaneous tissues and the third with combined ultrastructural and molecular confirmation of the presence of TSPyV in lesional skin. Lack of detection of other pathogenic human polyomaviruses in this patient’s skin supports the specific role of this polyomavirus in the genesis of TS. Further basic science studies are needed to determine the exact pathomechanisms of this polyomavirus and to explore possible tumorigenic roles in other skin diseases.

from the left nasolabial fold and an ultrasonography-guided renal allograft core needle biopsy specimen were obtained from the patient described herein. A formalin-fixed, paraffin-embedded (FFPE) tissue block was prepared. Diagnosis was made using hematoxylin-cosin–stained sections and additional stains as appropriate for the assessment of the renal allograft. Voided urine specimens were obtained at the time of the skin biopsy and 15 months later as part of routine posttransplant screening for polyomavirus. Diagnoses were made using Papanicolau-stained cytopsins.

Immunohistochemical staining of sections of FFPE tissue and cytopsin of a urine specimen were performed using a biotin-streptavidin–amplified method and an enhanced diaminobenzidine detection kit (Ventana) and commercially available antibody against SV40 (mouse monoclonal, 1:200 dilution; Cell Marque), with appropriate FFPE tissue and urine cytospin specimens used for positive controls.

For TEM, FFPE tissue was deparaffinized, dehydrated through graded alcohols, fixed overnight in 4% formaldehyde and 1% gluteraldehyde, postfixed in osmium tetroxide, and embedded in epoxy resin. Ultra-thin sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined on a transmission electron microscope (1200 EX; JEOL).

The DNA extraction from residual FFPE skin and renal allograft biopsy tissues was performed using a commercially available tissue extraction kit (Genta Puregene kit; Qiagen). The quality of the extracted DNA was assessed by means of β-globin reference gene polymerase chain reaction (PCR) analysis and proved to be positive (data not shown).31

For TSPyV detection, PCR technology was used to detect the entire small T antigen viral gene region.7 The forward PCR primer sequence was 5’ATGAGATAAGTTTTAAGTAGAGA’, and the reverse primer sequence was 5’TTACT-TACCCTGTTAAAGGCGTG3’. These primers were expected to generate a 597-base pair (bp) TSPyV-PCR product (NCBI-GenBank GU989205; region 4438..5034). The PCR steps included 1 minute at 94°C followed by 35 cycles (skin) or 40 cycles (renal allograft) of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 1 minute, and a final extension of 10 minutes at 72°C. The PCR products were run on 2.0% agarose gel electrophoresis and visualized on a UV transilluminator. The obtained TSPyV-PCR fragment was excised from agarose gel, electrophoresis and visualized on a UV transilluminator. The PCR products were run on 2.0% agarose gel electrophoresis and visualized on a UV transilluminator.

For MCPyV detection, the forward PCR primer sequence was 5’GCCTGATAGCTGTAAGTTG3’, and the reverse primer sequence was 5’ACACCTAGAATCTCCAGAAGTAGA’. These primers were derived from the small T antigen viral gene region of MCC350 or MCC399 MCPyV isolates and were expected to generate a 150-bp MCPyV-PCR product.24

The PCR steps were similar to those described for TSPyV detection in lesional skin except the annealing temperature was 64°C. The PCR products were run on 2.0% agarose gel electrophoresis and visualized on a UV transilluminator.

RESULTS

A 48-year-old African American man with type 1 diabetes mellitus, hypertension, and end-stage renal disease who had undergone a living-donor renal transplant 1 year earlier was admitted to the University of Maryland Medical Center with progressive shortness of breath due to a recurrent pericardial effusion. At the time, the patient was receiving mycophenolic acid and tacrolimus (FK-506) for immunosuppression. Approximately 2 to 3 months after the kidney transplant, the patient had begun to develop a gradual onset of extensive asymptomatic papules, which at the time of presentation involved his entire central face and ears. Results of a physical examination revealed multiple 1- to 2-mm flesh-colored papules over the glabella, nose, nasolabial folds, philtrum, chin, and ears. Near the center of the face, the papules demonstrated central white spiny excrescences (Figure 1). In addition, patchy alopecia involved both eyebrows. Diffuse skin thickening resulted in a leonine face appearance. Although no other similar skin lesions were noted involving other parts of the body, perifollicular papules with associated postinflammatory hyperpigmentation were present on the patient’s back, most consistent with the clinical impression of acneiform folliculitis.

A 3-mm punch biopsy of one of the lesions with a central spine was taken from the left nasolabial fold skin. Microscopic examination revealed striking dilatation of anagen and telogen hair follicles with an expanded inner root-sheath cell population replacing the follicular lumina (Figure 2A). Hair shafts were absent in the affected follicles, with plugging of the infundibula (Figure 2A). Focal mild perifolliculitis and a sparse lymphocytic interface dermatitis were noted in the infundibular portions of intervening vellus hair follicles (Figure 2B). The inner root-sheath keratinocytes contained enlarged, deeply eosinophilic trichohyaline granules (Figure 2C). The epidermis, outer root-sheath epithelium, sebaceous lobules, and eccrine structures were unremarkable. Results of SV40 immunohistochemical analysis were repeatedly negative in the altered follicles.

Transmission electron microscopy confirmed the presence of intranuclear viral inclusions within affected inner root-sheath keratinocytes composed of nonenveloped, icosahedral viral particles measuring 33 to 38 nm in diameter (Figure 2D), morphologically consistent with polyomavirus infection. No intracytoplasmic or extracellular viral particles were identified.

Concurrent urine cytologic findings were negative for viral cytopathic changes, although a subsequent voided urine specimen more than 1 year later (Figure 3A) was positive for polyomavirus cytopathic changes (decoy cells), whereas casts, as often seen in nephropathy, were notably absent. Results of SV40 immunohistochemical analysis failed to reveal evidence of BKPyV or JCPyV infection in these cells (Figure 3B). There were no associated urologic symptoms or significant changes in serum creatinine level, and all subsequent urine cytologic specimens were negative for viral cytopathic effects. A renal allograft biopsy near the time of the diagnostic skin biopsy yielded morphologically unremarkable findings, also with negative findings on SV40 immunohistochemical analysis. Viral cultures performed on a bronchial wash specimen at the time of dermatologic presentation were negative for adenovirus; influenza A and B viruses; parainfluenza viruses 1, 2, and 3; and respiratory syncytial virus.
The expected 597-bp putative TSPyV-PCR fragment was generated in the DNA sample extracted from this TS lesion (Figure 4A). The BLAST analysis of the cloned sequences obtained from the putative TSPyV-PCR product revealed a 99% identity to the prototype TSPyV sequences deposited in the NCBI-GenBank (GU989205.1). The applied PCR assay did not detect MCPyV in this TS lesion.

On further analysis, the expected 597-bp putative TSPyV-PCR fragment was also generated in the DNA sample extracted from the patient’s renal allograft biopsy specimen (Figure 4B). The BLAST analysis of the DNA sequences obtained from the putative TSPyV-PCR product again revealed a 99% identity to the prototype TSPyV sequences deposited in the NCBI-GenBank (GU989205.1).

After the skin biopsy, the patient was followed up as an outpatient by the transplant medicine service and continued his baseline immunosuppressive therapy regimen with stable renal allograft function through 3 years of follow-up. No additional dermatologic consultations were obtained; therefore, no antiviral medication therapy was initiated to treat his skin lesions.

**COMMENT**

Trichodysplasia spinulosa is being increasingly recognized among immunosuppressed patients. Cases that had been attributed to an adverse effect of cyclosporine treatment in which TEM was not performed are now believed by multiple authors to represent the same
**Figure 3.** Urine cytologic and immunohistochemical analysis findings in the patient with trichodysplasia spinulosa. A, Isolated polyomavirus (PyV)–infected cells with enlarged, round, hyperchromatic, smudgy nuclei (decoy cells) seen on urine cytologic examination more than 1 year after the patient’s dermatologic presentation (Papanicolaou, original magnification ×400). B, An SV40 immunostain fails to reveal evidence of BKPyV or JCPyV infection; the positive control (inset) shows strong nuclear staining (SV40, original magnification ×400).

**Figure 4.** Results of DNA analysis in the patient with trichodysplasia spinulosa (TS). A, Detection of TS-associated polyomavirus (TSPyV) DNA and absence of Merkel cell PyV (MCPyV) DNA by means of polymerase chain reaction (PCR) analysis in a TS lesion. For TSPyV detection, lane M contains 1X174RF DNA marker (Promega Corporation); lane 1, TS lesion; lane 2, TSPyV negative control DNA extracted from peripheral blood mononuclear cells (PBMCs) (Promega Corporation); and lane 3, reagent control. In lane 1, an expected 597–base pair (bp) TSPyV-PCR product can be seen. For MCPyV detection, lane M contains 1X174RF DNA marker (Promega Corporation); lane 1, TS lesion; lane 2, MCPyV positive control (plasmid with MCPyV DNA insert from small T antigen viral gene); lane 3, MCPyV negative control DNA extracted from PBMCs (Promega Corporation); and lane 4, reagent control. No MCPyV-PCR product was detected in lane 1 (TS lesion). In lane 2 (positive control), the expected 150-bp MCPyV-PCR fragment can be seen. B, Detection of TSPyV DNA by PCR in a renal allograft biopsy specimen from the TS patient. Lane M contains 1X174RF DNA marker (Promega Corporation); lane 1, kidney biopsy specimen; lane 2, TSPyV negative control DNA extracted from PBMCs (Promega Corporation); lane 3, positive control, cloned DNA of TSPyV small T gene; and lane 4, reagent control. In lanes 1 and 3, the expected 597-bp TSPyV-PCR product can be seen.
entity as TS on the basis of common clinicopathologic features. Twenty-five cases of TS, including the present case and cases reported under different names, were identiﬁed from peer-reviewed publications and conference proceedings. The clinical, histopathologic, ultrastructural, and molecular findings are summarized in Table 1. The median age at diagnosis was 27 years (range, 5-70 years). There was no sex predilection, with 13 male and 12 female patients. Clinical findings were similar across all represented racial and ethnic groups, with all patients experiencing follicular papules of the central face, followed in frequency by the extremities, with infrequent involvement of the scalp. Most of the patients demonstrated spiny excrescences protruding from some of the papules. The papules were asymptomatic in almost two-thirds of cases, whereas others experienced mild pruritus.

In regard to certain cases of TS associated with leukemia or lymphoma, it has been postulated that the onset or worsening of TS lesions may predict impending relapse of the underlying hematolymphoid malignant neoplasm, although this remains unclear owing to a paucity of cases, lack of long-term follow-up, and uncertainty about the latency period of infection. Although no correlation has been suggested among patients with TS who underwent solid-organ transplant in terms of allograft function or secondary malignant tumors, the initial onset of lesions in one patient occurred in temporal proximity to renal allograft rejection.

Treatment options are summarized in Table 2. Oral valganciclovir hydrochloride has shown efficacy in TS in all 4 informative reported cases. Topical cidofovir has been used with success in 4 of 5 cases in which response to treatment was described. One patient’s lesions were controlled by shaving to the level of uninolved skin followed by treatment with tazarotene gel, and another patient’s lesions improved with a topical compound of acyclovir, 2-deoxy-D-glucose, and epigallocatechin (green tea extract). Modifications in immunosuppressant regimen among transplant patients with TS have demonstrated improvement in half these cases. The overall disease course of TS is somewhat unpredictable owing to the lack of reported long-term follow-up, although lesions may persist or recur for years, and the virus is known to perpetuate in hair follicles in the absence of visible lesions. Moreover, TSPyV DNA has been identified in a small subset of immunosuppressed patients without clinical evidence of TS.

Most published cases of TS have closely matched the histopathologic and ultrastructural descriptions of Haycox et al. Less typical histologic findings include multiple small hair shafts or hair shaft–like material within affected follicles, vacuolated keratinocytes with pyknotic nuclei and coarse keratohyalin granules in the upper layers of the perifollicular epithelium, gray-blue cytoplasmic material in the inner root sheath cells, and, as demonstrated in this case, focal perifolliculitis and a sparse lymphocytic interface dermatitis. Lee et al characterized the pathologic findings in the plucked spicules, which may be used to validate a diagnosis of TS at another anatomic site in patients with historically confirmed TS and to use for molecular detection of TSPyV. Positive findings on polyoma middle T antigen immunohistochemical analysis have recently been described in a patient with TS.

![Table 1](attachment:Table1.png)
Ultrastructural detection of viral particles may vary in localization,\(^1\) and this modality may not be adequately sensitive; in 4 of 13 cases in which TEM was successfully performed, no viral particles were identified.\(^4,9,13,18\) This is likely the manifestation of differing phases of infection within the inner root-sheath keratinocytes in the areas sampled for TEM. During the replication cycle of BKPyV within renal tubular cells in polyomavirus allograft nephropathy, localization of virions supports the specific role of TSPyV in the viral genesis of TS.\(^1,7,14,16-18\) Seven of the 9 human polymaviruses have not yet been performed in TS skin lesions.\(^7\) Molecular confirmation of the presence of TSPyV in a TS lesion. Despite a difference in measured virion diameter of 33 to 38 nm in this case (obtained from deparaffinized FFPE tissue) and 39 to 45 nm in their published case (obtained specifically for TEM), both showed 99% to 100% sequence homology to the results of van der Meijden et al., favoring the first hypothesis.\(^7\)

The consistent lack of detection of other HPys with known disease associations in TS skin lesions further supports the specific role of TSPyV in the viral genesis of TS.\(^1,7,14,16-18\) Seven of the 9 human polymaviruses have been identified in human skin,\(^33\) whereas only TSPyV and MCPyV are currently believed to be linked to cutaneous diseases.\(^7,16,18\) (Table 3). Although our results essentially rule out active infection of the skin with MCPyV, BKPyV, and JCPyV in our patient with TS, assays for the other recently identified HPys—KIPyV, WUPyV, HPyV6, HPyV7, and HPyV9—have not yet been performed in TS lesions.

### Table 2. Positive Response to Treatment in Reported Cases of TS and Presumed TS\(^a\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Source</th>
<th>Response Rate, No./Total No. (%)</th>
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<tbody>
<tr>
<td>Oral valganciclovir hydrochloride</td>
<td>Holzer and Hughey, (^4) 2009; Benoit et al., (^10) 2010; Elaba et al., (^12) 2010; Schiewer-Briel et al., (^11) 2010</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td>Topical cidofovir</td>
<td>van der Meijden et al., (^1) 2010; Wanat et al., (^12) 2012; Osswald et al., (^7) 2007; Sperling et al., (^2) 2004; Benoit et al., (^10) 2010</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>Topical tazarotene with shaving of lesions</td>
<td>Campbell et al., (^8) 2006</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Topical compounded acyclovir, 2-deoxy-(\alpha)-glucose, and epigallocatechin (green tea extract)</td>
<td>Blake et al., (^9) 2011</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Change in immunosuppressant regimen</td>
<td>van der Meijden et al., (^1) 2010; Holzer and Hughey, (^4) 2009; Heapthy et al., (^6) 2004; Chastain and Millikan, (^2) 2000; Izakovic et al., (^4) 1995; Benoit et al., (^10) 2010; Schiewer-Briel et al., (^11) 2010; Wyatt et al., (^14) 2005; Sadler et al., (^16) 2007</td>
<td>5/10 (50)</td>
</tr>
</tbody>
</table>

Abbreviation: TS, trichodysplasia spinulosa.

\(^a\)Excludes cases with no reported follow-up (cidofovir)\(^14\) or early discontinuation owing to adverse effects (tazarotene).\(^4\)

### Table 3. Summary of Human Polyomaviruses Identified to Date

<table>
<thead>
<tr>
<th>Human Polyomavirus</th>
<th>Source</th>
<th>Anatomic Sites of Identification</th>
<th>Disease Associations</th>
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<tbody>
<tr>
<td>BK polyomavirus</td>
<td>Gardner et al., (^22) 1971; Moens et al., (^33) 2011; Drachenberg et al., (^23) 2003</td>
<td>Skin, blood, central nervous system, kidney, urine, lymphoid tissues</td>
<td>Polyomavirus allograft nephropathy, hemorrhagic cystitis</td>
</tr>
<tr>
<td>JC polyomavirus</td>
<td>Padgett et al., (^24) 1971; Moens et al., (^33) 2011; Drachenberg et al., (^23) 2003</td>
<td>Skin, blood, central nervous system, cerebrospinal fluid, kidney, urine, lymphoid tissues</td>
<td>Progressive multifocal leukoencephalopathy, polyomavirus allograft nephropathy (rare)</td>
</tr>
<tr>
<td>KI polyomavirus</td>
<td>Allander et al., (^26) 2007; Mourez et al., (^35) 2009; Debiaggi et al., (^36) 2010</td>
<td>Blood, brain, central nervous system, lungs, tonsils</td>
<td>Uncertain (pneumonia?)</td>
</tr>
<tr>
<td>WU polyomavirus</td>
<td>Gaynor et al., (^25) 2007; Mourez et al., (^35) 2009; Debiaggi et al., (^36) 2010</td>
<td>Blood, brain, central nervous system, lungs, tonsils</td>
<td>Uncertain (pneumonia?)</td>
</tr>
<tr>
<td>Merkel cell polyomavirus</td>
<td>Schowalter et al., (^28) 2010; Fong et al., (^27) 2008; Moens et al., (^34) 2011</td>
<td>Skin, blood, nasopharynx, lungs, tonsils/lymphoid, hepatobiliary and gastrointestinal tracts</td>
<td>Merkel cell carcinoma</td>
</tr>
<tr>
<td>Human polyomavirus 6</td>
<td>Schowalter et al., (^23) 2010</td>
<td>Skin</td>
<td>Unknown</td>
</tr>
<tr>
<td>Human polyomavirus 7</td>
<td>Schowalter et al., (^23) 2010</td>
<td>Skin</td>
<td>Unknown</td>
</tr>
<tr>
<td>TS-associated polyomavirus</td>
<td>van der Meijden et al., (^1) 2010; Matthews et al., (^35) 2011</td>
<td>Skin, kidney (allograft), urine (presumptive)</td>
<td>TS</td>
</tr>
<tr>
<td>Human polyomavirus 9, including IPPyV strain (^36)</td>
<td>Scuda et al., (^29) 2011; Sauvage et al., (^30) 2011 (IPPyV strain); Moens et al., (^33) 2011</td>
<td>Skin, blood, urine</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations: IPPyV, Institut Pasteur polyomavirus; TS, trichodysplasia spinulosa.
Our findings of TSPyV DNA in the renal allograft biopsy specimen and subsequent evidence of a productive polyomavirus infection in the urinary tract of our patient that failed to react with antibody to SV40 strongly suggest that TSPyV may be tropic to the urinary tract in addition to skin. Another possibility is that the SV40 immunostain lacked sufficient sensitivity to detect reactivation of latent BKPyV or JCPyV infection in this patient’s urine cytologic material; however, the paired urine cytospin positive control specimen reacted strongly.

Recent epidemiologic data indicate a high seroprevalence of TSPyV in at-risk and general populations that increases with age. Further basic science studies are needed to determine the exact pathomechanisms of TSPyV, particularly concerning the universal predilection for facial skin and relative sparing of the scalp. Given some similarities between epidermotropic polyomaviruses and papillomaviruses, regional skin specificity may represent yet another similarity. Determination of a possible tumorigenic role of TSPyV in other skin lesions also warrants study.

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References


At about this time, the Celts, an ancient people from Western Europe, especially France, Germany, and England, modified their sexual practice from male to female, and this change was first observed by Dr. Antonio Tagarelli, who gave it the name “male francisco.”

In Germany, also in 1496, Frisian Dirk Van Ulsen, better known as Theodoricus Ulsenius, published 50 aphorisms titled “De epidemia quam Itali morbus gallicus.” In 1498, Bartholomaeus Steber modified it to “mala francosae,” a deference decree on the harlotry called the disease “mal francicus.”

In Italy, also in 1496, Paolo Lagonia, PhD, and Anna Piro, MD, used it, and Karl Sudhoff said that it was derived from the Hebrew language. In 1498, Bartholomaeus Steber modified it to “mala francosae.”

In France, in 1534, a new term, “mal francosae,” was used by Antonino Cammelli, a farchical poet, to define his galiachaeagrudite. He and his son attributed their own syphilis to the fact that they had modified their sexual practice from homosexuality to heterosexuality, because at that time it was common thinking that women spread syphilis. In the 16th century, syphilis was also attributed to the French by the synonyms mal franciscus, morbus francicus, morbus francisci, male francisco, male francosae, male franzosischen, and bozen (pox) franzosae.

Before the end of the 15th century, the gallic adjective was used by Antonio Cammelli, a farchical poet, to define his galiachaeagrudite. He and his son attributed their own syphilis to the fact that they had modified their sexual practice from homosexuality to heterosexuality, because at that time it was common thinking that women spread syphilis. In the 16th century, syphilis was also attributed to the French by the synonyms malum francicum, morbus francicum, morbus francisci, and malo franco. Finally, a new term, lue, which was derived from Latin lues (meaning disease, plague, endemy, and contagion), was followed by the Celts, thus recognizing the Celts, an ancient people from Western Europe, especially France, Spain, Germany, and the British Isles. The term lue celtica was first used by Francesco Roncalli Parolini in 1720. Lue defined syphilis until the first half of the 20th century.

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