BRIEF REPORT

Human Polyomavirus 7–Associated Pruritic Rash and Viremia in Transplant Recipients

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Human polyomavirus 7 (HPyV7) is one of 11 HPyVs recently discovered through genomic sequencing technologies. Two lung transplant recipients receiving immunosuppressive therapy developed pruritic, brown plaques on the trunk and extremities showing a distinctive epidermal hyperplasia with virus-laden keratinocytes containing densely packed 36–45-nm icosahedral capsids. Rolling circle amplification and gradient centrifugation testing were positive for encapsidated HPyV7 DNA in skin and peripheral blood specimens from both patients, and HPyV7 early and capsid proteins were abundantly expressed in affected tissues. We describe for the first time that HPyV7 is associated with novel pathogenicity in some immunosuppressed individuals.

Keywords. polyomavirus; HPyV7; skin viral infection; transplantation-associated disease; immunosuppression.

Polyomaviridae encompasses a family of small (approximately 45 nm) nonenveloped icosahedral viruses with approximately 5-kb double-stranded, circular DNA genomes encoding the early nonstructural proteins, small tumor antigen (sTAg) and large tumor antigen (TAg), and the late virion proteins (VPs) VP1, VP2, and VP3 [1, 2]. Until recently, only 2 human polyomaviruses (HPyVs), JC polyomavirus and BK polyomavirus, were known. Both HPyVs cause disease among immunosuppressed populations but are not associated with skin disorders. Molecular biologic technologies have uncovered hundreds of novel viral sequences since 2007, including 11 additional HPyVs that are normal components of human flora. Of these newly discovered polyomaviruses, only 2 are known to cause human skin disease. Merkel cell polyomavirus (MCV) causes most cases of Merkel cell carcinoma after the virus integrates into the host cell genome and undergoes a specific pattern of mutation [3, 4]. Viral particles are not formed in Merkel cell carcinoma and cancer cell proliferation is driven by expression of viral oncogenes [5]. Trichodysplasia spinulosa polyomavirus (TSV) causes a rare facial skin disorder of transplant recipients with prominent hyperplastic epidermal inner root sheath structures and abundant TSV virion production [6].

HPyV7 was discovered as an incidental infection by rolling circle amplification (RCA) of healthy human skin swabs in 2010 [7]. Serologic studies suggest that HPyV7, like most other HPyVs, is a common and persistent infection, with a prevalence of 64% in adults [8, 9]. So far, no disease has been ascribed to HPyV7 infection.

CASE REPORTS

Patient 1

A 73-year-old man was admitted to the hospital with nausea, odynophagia, a diffuse rash, and acute kidney injury. He had received a single lung transplant for idiopathic pulmonary fibrosis 4 years prior to admission. After induction of immunosuppression with alemtuzumab, he initiated a continuing immunosuppression regimen of prednisone, azathioprine, and tacrolimus. Everolimus was subsequently added. Four months prior to admission, he developed a brownish pink, velvety plaque on his right flank, initially diagnosed as a drug eruption. Pruritus was severe and interfered with sleep. Treatment with topical corticosteroids and antihistamines and discontinuation of filgrastim, trimethoprim-sulfamethoxazole, and everolimus therapy provided no relief, and the rash continued to progress to the buttocks and trunk. He developed nausea and a severe burning sensation with swallowing, and laboratory analyses revealed acute kidney injury. On admission, skin examination revealed multiple brownish pink, scaly, thin papules and plaques involving the gluteal cleft and back, with progression up to the neck and chest but sparing acral areas and the face (Figure 1*A*). Analysis of a skin biopsy specimen showed a distinctive epidermal

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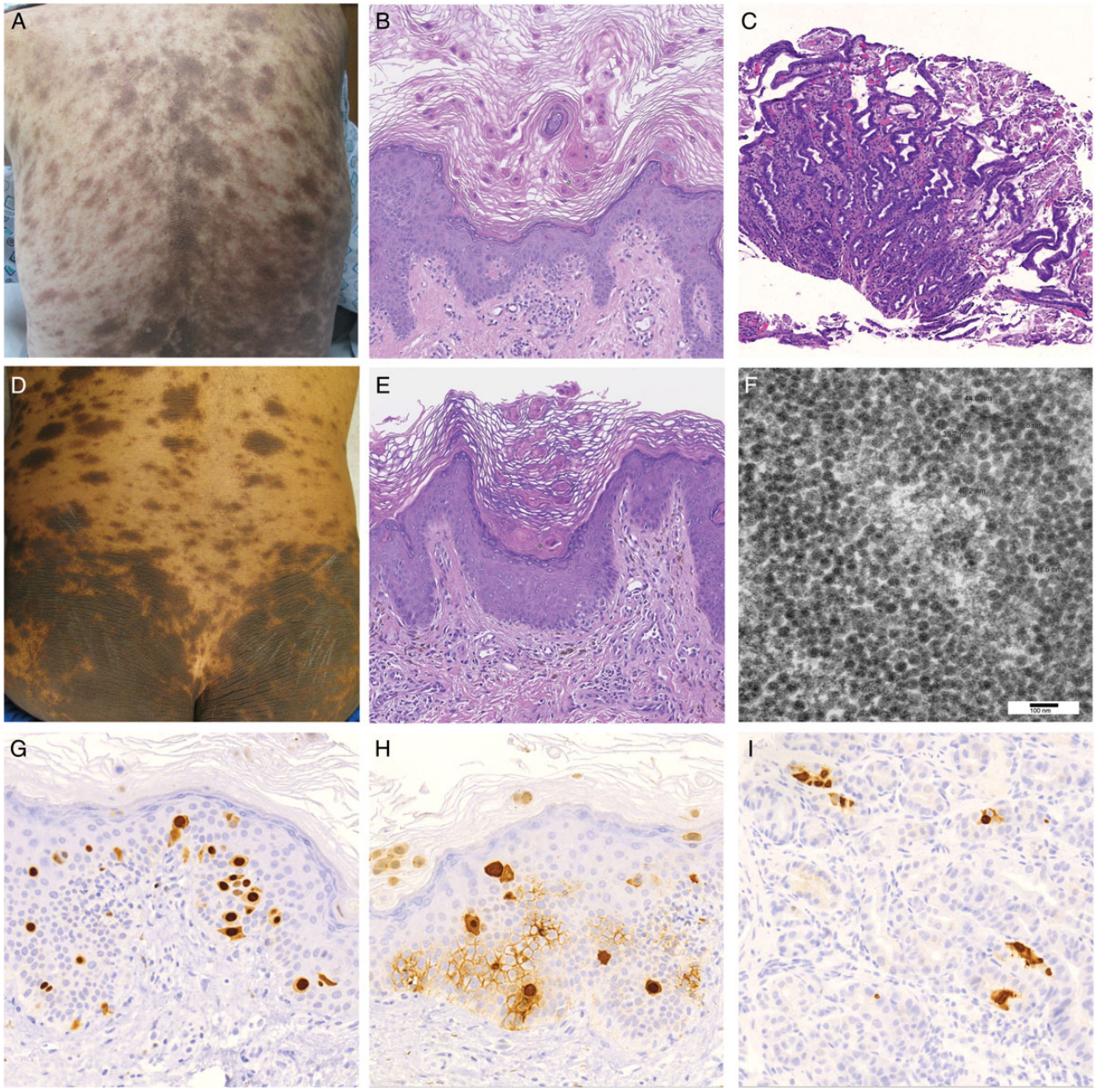


Figure 1. Clinical and pathologic features of human polyomavirus 7 (HPyV7)-related skin manifestations in patient 1 (the index patient; *A, B, C, F, G, H,* and *I*) and patient 2 (*D* and *E*). *A* and *D*, Pruritic velvety plaques on the trunk. *B* and *E*, Viropathic keratinocytes in a peacock plumage pattern (original magnification, 20×; hematoxylin–eosin staining). *C*, The antral biopsy specimen shows a pseudomembrane-like pattern (original magnification, 10×; hematoxylin–eosin staining). *F*, Electron microscopy of affected keratinocytes (original magnification, 71 000×), with numerous 36–45-nm icosahedral virions. *G*, Strong HPyV7 T antigen (2t10) nuclear and cytoplasmic immunostaining in the keratinocytes (original magnification, 40×). *H*, HPyV7 viral capsid staining in the skin (6V32) shows nuclear and cytoplasmic staining in keratinocytes, as well as a lacy pattern representing postlysed virions in the intercellular space (original magnification, 40×). *I*, T antigen (2t10) also shows nuclear and cytoplasmic staining in the antrum (original magnification, 40×).

histopathologic pattern resembling peacock plumage, with plump, eosinophilic keratinocytes containing viral inclusions at different levels of the epidermis. An expanded stratum corneum with clusters of nucleated keratinocytes was present (Figure 1*B*). The dermis contained mild, superficial perivascular lymphocytic infiltrates. On upper gastrointestinal endoscopy, multiple linear superficial ulcerations were noted in the mid-esophagus, which became confluent in the distal esophagus. Large amounts of food were seen in the fundus and body of the stomach, suggestive of gastroparesis. Analyses of esophageal

biopsy specimens showed only mild reactive changes, but investigation of an antral biopsy specimen showed pseudomembrane-like changes and glandular epithelium with viral inclusion bodies (Figure 1*C*).

Patient 2

A 72-year-old man, who received a double lung transplant followed by alemtuzumab-based induction immunosuppression for idiopathic pulmonary fibrosis 5 years previously, presented with pruritic, velvety plaques of 4 months' duration. The patient was receiving an immunosuppression regimen of tacrolimus, mycophenolic acid, and prednisone. His rash consisted of brown, well-defined thin papules and plaques involving the neck, back, chest, axillae, buttocks, and legs (Figure 1*D*). Pruritus was graded as severe and interfered with sleep, but no gastrointestinal symptoms were noted. Diphenhydramine therapy provided minimal improvement, while topical steroid treatment caused the rash to worsen. Histologic examination of skin specimens from affected sites revealed a histopathologic pattern similar that observed for the index patient (patient 1), with an expanded stratum corneum containing keratinocytes with viral inclusions (Figure 1*E*).

Informed consent was obtained from both patients to use tissues for diagnostic and research purposes, and written permission was obtained from both patients for use of clinical photographs.

METHODS

Antibodies and Immunohistochemistry (IHC) Studies

Monoclonal antibodies (mAbs) were raised using previously reported methods [10]. 2t10 recognizes a linear epitope present in the common J-domain present in HPyV7 TAg and sTAg. Control experiments showed that 2t10 was weakly cross-reactive with the TAgs of HPyV6 but not MCV, SV40, or HPyV10. 6V32 was raised against virus-like particles composed of HPyV6 VP1. However, it cross-reacted with HPyV7 VP1 and was used in IHC studies since initial polymerase chain reaction (PCR) studies showed no evidence for HPyV6 infection in patient tissues. Formalin-fixed, paraffin-embedded tissue biopsy sections were immunostained according to previously published protocols [11], using 10 mM citrate buffer (pH 6.0) for antigen retrieval and a 1:100 dilution (approximately 2 μg/mL) of 2t10 or 6V32 for 1.5 hours at 37°C.

PCR and Viral Genome Sequencing

DNA was extracted from skin biopsy specimens and amplified by traditional and RCA PCR with HPyV-specific and consensus primers (Supplementary Table 1). Quantitative real-time PCR analysis was performed using SsoFast EvaGreen Supermix with the CFX96 Real-Time PCR detection system (BioRad). For virus genome sequencing, 10 pairs of HPyV7-specific primers with overlapping coverage of the entire genome were designed using the sequence of the 727a HPyV7 isolate (accession no. HM011569; Supplementary Table 2). Sequences were assembled using the CAP3 assembly computer program [12].

Optiprep

A total of 250 μL of plasma was layered onto an iodixanol Optiprep (Sigma) gradient and centrifuged at 234 000 × *g* to purify encapsidated virions, as previously described [13]. A portion of all fractions collected was treated with Benzonase Nuclease (Sigma) to digest nonencapsidated nucleic acids.

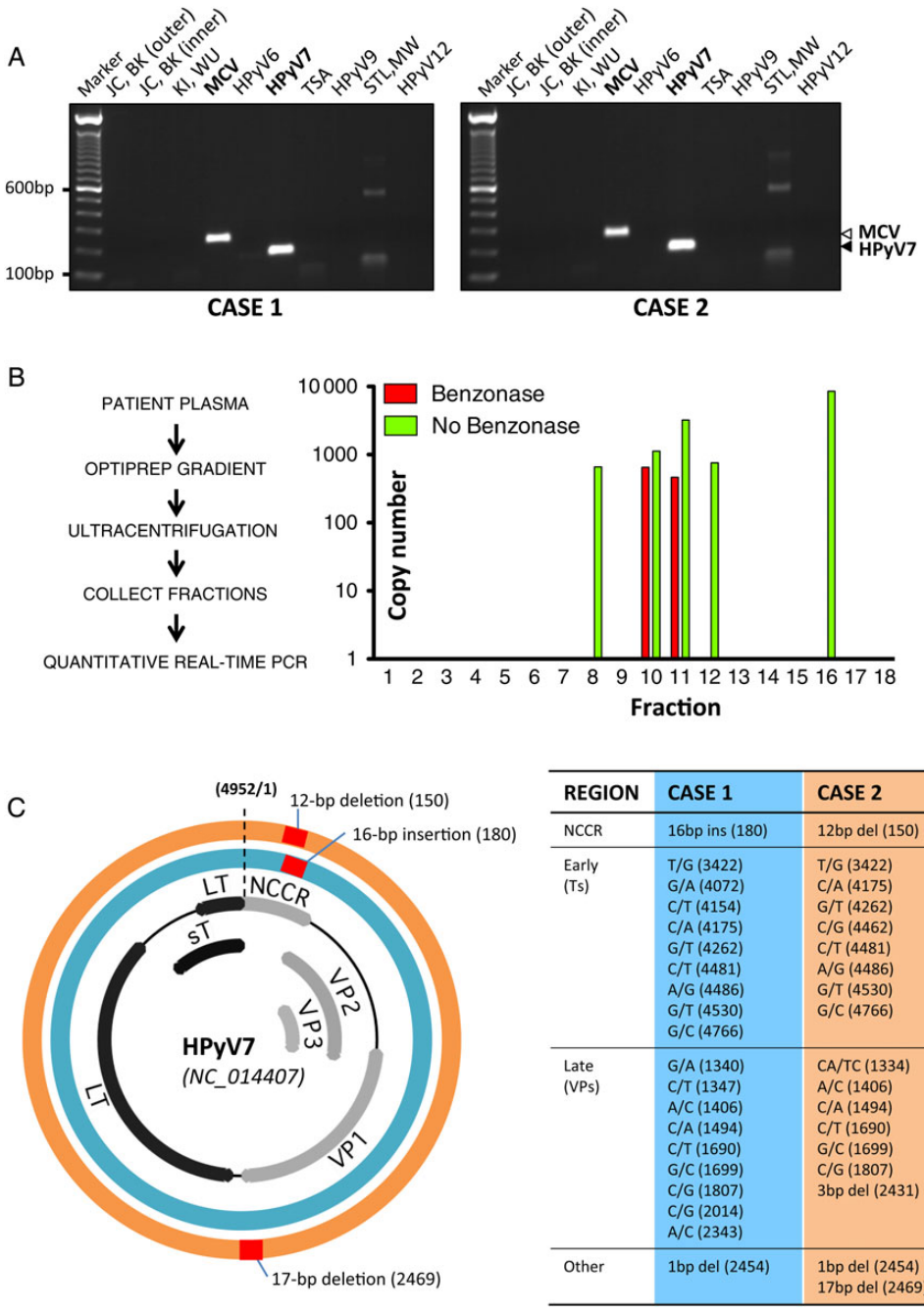
RESULTS

Microbiologic and Histologic Findings

Initial analyses of tissues yielded negative results for standard bacterial, fungal, mycobacterial, and viral cultures and acid-fast bacilli staining. Results of immunostaining for human papillomavirus, cytomegalovirus, and herpes simplex and varicella-zoster virus proteins and in situ hybridization for Epstein-Barr virus–encoded small RNAs were also negative. Immunostaining using an SV40 L TAg antibody (PAb416) [14] cross-reactive for JC and BK polyomavirus TAg showed strong reactivity in the involved skin keratinocyte nuclei of both patients, but JC and BK polyomavirus DNA was not detected by in situ hybridizations. Epithelial cells in esophageal and gastric biopsy specimens from patient 1 were positive for PAb416 as well. Electron microscopy of skin biopsy specimens from both patients revealed numerous keratinocytes laden with approximately 39–46-nm icosahedral virions resembling polyomaviruses (Figure 1*F*).

IHC Analysis for HPyV7 TAg and VP1

Based on initial PCR and sequencing results (presented below), HPyV7 protein expression was assessed by IHC studies. TAg immunopositivity (mAb 2t10) was prominent in the nuclei of scattered keratinocytes in the epidermis (Figure 1*G*) and weaker in nucleated keratinocytes in the stratum corneum of skin biopsy specimens from both patients. Staining for the HPyV7 VP1 capsid protein (mAb 6V32) revealed strong cytoplasmic, variable nuclear, and some intercellular immunopositivity (Figure 1*H*). The antral biopsy specimen in the index case showed HPyV7 TAg immunostaining in mucosal cells (Figure 1*I*); however, HPyV7 VP1 capsid protein immunostaining was present only in a small fraction of cells, in contrast to prominent positivity for both viral antigens in skin biopsy specimens. Isotype negative control immunostaining of these tissues had negative results. There were negative results of HPyV7 TAg (mAb 2t10) immunostaining for tissues from 24 normal, reactive, inflammatory, and neoplastic skin cases; 77 inflammatory, hyperplastic, and neoplastic esophageal cases; 30 neoplastic gastroesophageal cases; and 241 normal, inflammatory, hyperplastic, and neoplastic gastric cases (Supplementary Table 3).



REGION	CASE 1	CASE 2
NCCR	16bp ins (180)	12bp del (150)
Early (Ts)	T/G (3422) G/A (4072) C/T (4154) C/A (4175) G/T (4262) C/T (4481) A/G (4486) G/T (4530) G/C (4766)	T/G (3422) C/A (4175) G/T (4262) C/G (4462) C/T (4481) A/G (4486) G/T (4530) G/C (4766)
Late (VPs)	G/A (1340) C/T (1347) A/C (1406) C/A (1494) C/T (1690) G/C (1699) C/G (1807) C/G (2014) A/C (2343)	CA/TC (1334) A/C (1406) C/A (1494) C/T (1690) G/C (1699) C/G (1807) 3bp del (2431)
Other	1bp del (2454)	1bp del (2454) 17bp del (2469)

Figure 2. Molecular characterization of human polyomavirus 7 (HPyV7) in patient tissues. *A*, Specific primers for 12 HPyVs were tested on rolling circle amplification products from 2 patients. Amplification of HPyV7 (closed arrowhead) and Merkel cell polyomavirus (MCV; open arrowhead) are shown. *B*, Plasma from case 1 was fractionated and examined for HPyV7 copy numbers by quantitative polymerase chain reaction (PCR) with (red bars) or without (green bars) Benzonase treatment. From 18 fractions, encapsidated HPyV7 virions are detectable in fractions 10 and 11. *C*, The HPyV7 genomes from patient 1 (blue) and patient 2 (orange) are compared to HPyV7 reference NC_014407, revealing multiple insertions and deletions. The table lists all recoding polymorphisms in the early T antigen and late capsid regions, compared with NC_014407. Abbreviations: LT, large tumor antigen; NCCR, noncoding control region; sT, small tumor antigen; T, tumor antigen; VP, virion protein.

Identification of HPyV7 Genome in Patient Tissues

Specific quantitative PCR amplification was performed for 12 HPyVs, for human papillomaviruses 16 and 18, and for consensus polyomavirus or papillomavirus genomes. Extracted DNA from skin biopsy specimens revealed HPyV7 loads of 3227 copies/cell for patient 1 and 728 copies/cell for patient 2. Peripheral blood mononuclear cells (PBMCs) from both patients were weakly positive for the HPyV7 genome, at

approximately 10^{-3} copies/cell. MCV genome was also detected in skin biopsy specimens from both patients but at very low levels (approximately 10^{-4} copies/cell). No other viral sequences assayed were detected. In comparison, a convenience sample of skin specimens from 8 patients with Kaposi sarcoma and 2 healthy controls were amplified by PCR. Only 1 sample, from a patient with Kaposi sarcoma, was HPyV7-genome positive (HPyV7 load, approximately 0.4 copies/cell). Patient 1's gastric antral biopsy specimen also contained HPyV7 sequences amplified by PCR.

RCA of DNA from skin biopsy specimens from each patient was performed to detect intact episomal genomes, and HPyV7 and MCV DNA was detected (Figure 2*A*). To confirm encapsidation of HPyV7 DNA, plasma from patient 1 was loaded onto an Optiprep (Sigma) velocity/density ultracentrifuge gradient. Serial fractions were collected and subjected to Benzonase or no treatment and then tested by quantitative PCR for HPyV7 DNA (Figure 2*B*). Benzonase treatment eliminated amplifiable HPyV7 DNA from fractions 8, 12, and 16, consistent with the presence of naked viral DNA. Benzonase-resistant HPyV7 DNA remained detectable only in fractions 10 and 11, with a buoyant density of 1.19–1.20 g/mL as previously described for encapsidated HPyV7 DNA [7]. Complete HPyV7 genomes were isolated and sequenced from both patients (GenBank KJ733012–KJ733013); virus from patient 1 was 99% (4905/4968) identical to the prototype strain sequence (GenBank NC014407), differing mainly by a 16-bp duplication in the noncoding region between bp 180 and 181. Patient 2's lesion revealed a mixed population of HPyV7 genomes with various small (2–17 bp) deletions and nucleotide substitutions (Figure 2*C*).

DISCUSSION

Prevalent HPyV7 DNA can be detected at low levels in skin specimens from 11% of asymptomatic individuals and can be shed from the skin surface as complete virions [7]. Experimentally distinguishing coincidental HPyV7 infection from hyperinfection, as described here, can be facilitated by specific IHC studies, RCA, or quantitative PCR. The high HPyV7 viral genome copy number, presence of densely packed virions, and widespread TAg and VP1 protein expression in the affected skin tissues make coincidental infection unlikely for these 2 patients. Notably, HPyV7 DNA was also readily detected in blood specimens from both patients and from gastric mucosal tissue specimens from patient 1, indicating that this syndrome can be associated with a previously undescribed HPyV7 viral dissemination. The high level of viremia suggests that HPyV7 may circulate under conditions of immunosuppression, although it predominantly targets skin.

The rash for both patients was visually distinctive and intensely pruritic. Involved areas for both patients included the lower back, immediately above the gluteal cleft. The quality of the lesions also had an unusual velvety and finely scaled texture. Both cases showed unique dermatopathological features, in which viropathic keratinocytes were present in the basket weave–like orthokeratosis of the stratum corneum and in the epidermis. At present, we do not know whether the florid symptoms and pathology described here represent the most common presentation of HPyV7-related skin disease or are exceptional examples for a continuum of disease. However, the degree of similarity in these 2 cases may predict a particular syndrome. The apparent involvement of the antrum of the index patient suggests that HPyV7 can affect different types of epithelium, but HPyV7 TAg expression without VP1 expression in patient 1's gastric biopsy specimen is consistent with the gastric mucosa being a less permissive host cell environment for HPyV7 than skin epithelium.

In comparison to a reference HPyV7 genome (NC014407), the viral genomes sequenced from the 2 patients share many polymorphisms in their coding regions (Figure 2*C*), indicating that these viruses are more closely related to each other. None of these polymorphisms are nonsense mutations, and thus full-length viral early and late proteins are predicted to be translated. However, many missense mutations were found that may lead to functional changes in encoded proteins or antigens. Viral sequences from both cases show mutations in the noncoding control region—a 16-bp insertion in patient 1 and a 12-bp deletion in patient 2—that may affect viral replication activities. It is possible that these polymorphisms may confer increased clinical virulence in the setting of immunosuppression.

Identification of a new polyomaviral cause for disease suggests potential antiviral therapies, although the sensitivity of HPyV7 to antivirals is currently unknown. Epidemiological and molecular studies are needed to identify other risk factors in addition to immunosuppression and to uncover the pathophysiology of HPyV7 infection. These findings support the use of molecular biologic technologies in the search for infectious causes of idiopathic diseases having a suspected viral etiology.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. DeCaprio JA, Garcea RL. A cornucopia of human polyomaviruses. Nat Rev Microbiol **2013**; 11:264–76.
2. White MK, Gordon J, Khalili K. The rapidly expanding family of human polyomaviruses: recent developments in understanding their life cycle and role in human pathology. PLoS Pathog **2013**; 9:e1003206.
3. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science **2008**; 319:1096–100.
4. Shuda M, Feng H, Kwun HJ, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci U S A **2008**; 105:16272–7.
5. Houben R, Shuda M, Weinkam R, et al. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol **2010**; 84:7064–72.
6. van der Meijden E, Janssens RW, Lauber C, Bouwes Bavinck JN, Gorbalenya AE, Feltkamp MC. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. PLoS Pathog **2010**; 6:e1001024.
7. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell Host Microbe **2010**; 7:509–15.
8. van der Meijden E, Bialasiewicz S, Rockett RJ, Tozer SJ, Sloots TP, Feltkamp MC. Different serologic behavior of MCPyV, TSPyV, HPyV6, HPyV7 and HPyV9 polyomaviruses found on the skin. PLoS One **2013**; 8:e81078.
9. Nicol JT, Robinot R, Carpentier A, et al. Age-specific seroprevalences of merkel cell polyomavirus, human polyomaviruses 6, 7, and 9, and trichodysplasia spinulosa-associated polyomavirus. Clin Vaccine Immunol **2013**; 20:363–8.
10. Pastrana DV, Pumphrey KA, Cuburu N, Schowalter RM, Buck CB. Characterization of monoclonal antibodies specific for the Merkel cell polyomavirus capsid. Virology **2010**; 405:20–5.
11. Shuda M, Arora R, Kwun HJ, et al. Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. Int J Cancer **2009**; 125:1243–9.
12. Huang X, Madan A. CAP3: A DNA sequence assembly program. Genome Res **1999**; 9:868–77.
13. Buck CB, Thompson CD. Production of papillomavirus-based gene transfer vectors. Curr Protocol Cell Biol **2007**; Chapter 26:Unit 26:26.1.1–19.
14. Harlow E, Crawford LV, Pim DC, Williamson NM. Monoclonal antibodies specific for simian virus 40 tumor antigens. J Virol **1981**; 39:861–9.