MAJOR ARTICLE

Natural History of Polyomaviruses in Men: The HPV Infection in Men (HIM) Study

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Background. Several new polyomaviruses have been discovered in the last decade, including *Merkel cell polyomavirus* (MCPyV). Little is known about the natural history of the more recently discovered polyomaviruses. We estimated the incidence, prevalence, and persistence of 9 polyomaviruses (MCPyV, *BK polyomavirus*, *KI polyomavirus*, *JC polyomavirus*, *WU polyomavirus*, *Human polyomavirus 6* [HPyV6], HPyV7, HPyV9, and *Trichodysplasia spinulosa–associated polyomavirus*) and examined factors associated with MCPyV infection in a prospective cohort of 209 men initially enrolled in the HPV Infection in Men (HIM) study.

Methods. Participants enrolled at the US site of the HIM study were recruited into a substudy of cutaneous viral infections and followed for a median of 12.6 months. Eyebrow hair and normal skin swab specimens were obtained at each study visit, and the viral DNA load was measured using multiplex polymerase chain reaction.

Results. MCPyV infection showed the highest prevalence (65.1% of normal skin swab specimens and 30.6% of eyebrow hair specimens), incidence (81.7 cases per 1000 person-months among normal skin swab specimens, and 24.1 cases per 1000 person-months among eyebrow hair specimens), and persistence (85.8% of normal skin swab specimens and 58.9% of eyebrow hair specimens) among all polyomaviruses examined. Age of >44 years (odds ratio [OR], 2.11; 95% confidence interval [CI], 1.03–4.33) and Hispanic race (OR, 2.64; 95% CI, 1.01–6.88) were associated with an increased prevalence of MCPyV infection in eyebrow hair and normal skin swab specimens, respectively.

Conclusion. MCPyV infection is highly prevalent in adults, with age and race being predisposing factors.

Keywords. polyomavirus; natural history; eyebrow hair; skin swabs.

Polyomaviruses are small, nonenveloped viruses with double-stranded DNA [1, 2]. *JC polyomavirus* (JCV) and *BK polyomavirus* (BKV) were the first polyomaviruses to be discovered, in 1971 [3, 4]. JCV and BKV are associated with a wide range of diseases, including nephropathy, hemorrhagic cystitis, and progressive multifocal leukoencephalopathy [4, 5]. According to the International Agency for Research on Cancer (IARC), JCV and BKV are each classified as a "possible carcinogen," based on evidence from experimental and human studies [6, 7]. A few decades after the discovery of JCV and BKV, *KI polyomavirus* (KIV) [8], *Merkel cell polyomavirus* (MCPyV) [9], and *WU polyomavirus* (WUV) [10] were identified. MCPyV was first identified in 2008, when Feng et al detected clonal integration of MCPyV DNA in Merkel cell carcinoma (MCC) tumor genome [9]. MCPyV has been detected in >80% of MCC cases [11, 12] and is recognized by the IARC as a "probable carcinogen" [13]. Alteration of expression of oncogenes has been observed in MCPyV-positive tumors [14]. Polyomaviruses have been detected in several cancers, including squamous cell carcinoma of the cervix [2], cutaneous squamous cell carcinoma [15, 16], basal cell carcinoma [16, 17], and melanoma [17]. Discovery of additional polyomaviruses continues with the recent addition of *Trichodysplasia spinulosa–associated polyomavirus* (TSPyV) [18] and *Human polyomavirus 6* (HPyV6) and HPyV7 [19]. While a recent case report has suggested cutaneous pathology in association with HPyV7 infection in immunocompromised individuals [20], the pathological outcome of HPyV6 infection is unknown.

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Antibodies to viral antigens are often used as markers of polyomavirus infection. For example, we previously reported a statistically significant association between MCPyV seropositivity and MCPyV DNA–positive squamous cell carcinoma [15]. In a large case-control study, Robles et al observed that higher seroreactivity to BKV and MCPyV was associated with an increased risk of bladder cancer [21]. These positive associations have been reported despite the ubiquitous presence of polyomaviruses in the general population, with >80% and >50% of healthy, immunocompetent adults exhibiting seropositivity to BKV and JCV, respectively [22]. Polyomaviruses have also been detected in saliva and oral tissues from individuals with oral lesions [23], as well as in nonneoplastic tissues, such as skin and bone marrow [24]. Antiviral antibodies provide a marker of past viral infection and are therefore useful biomarkers in epidemiological studies examining prevalence of polyomaviruses. However, biomarkers of recent infection, such as eyebrow hair and skin swab specimens, are required for estimating incidence and persistence [25]. It is important to understand the natural history of these potentially pathogenic viral infections to identify individuals at high risk for infection and virus-associated disease. However, very few studies have evaluated the natural history of recently discovered polyomaviruses, such as KIV [26], WUV [26, 27], HPyV6 [28, 29], HPyV7 [28, 29], and MCPyV [24, 30].

We recently reported on the natural history of cutaneous human papillomavirus infection [25]. Using the same underlying study population, here we estimated the incidence, prevalence, and persistence of 9 polyomaviruses (MCPyV, BKV, KIV, JCV, WUV, HPyV6, HPyV7, HPyV9, and TSPyV) in normal skin and eyebrow hairs and investigated risk factors associated with these infections in a cohort of 209 healthy men.

METHODS

Study Population

The present analysis was conducted on a subcohort of men enrolled in the US site of the HPV Infection in Men (HIM) study, a large, multinational prospective cohort study of the natural history of human papillomavirus (HPV) infection in men [31, 32]. The HIM study methods have been described in detail previously [31, 32]. Briefly, between July 2005 and September 2009, students, faculty, and staff from the University of South Florida, as well as members of the general population, were recruited through mass advertisement for participation in the Tampa site of the HIM study. Inclusion criteria were as follows: (1) male and aged 18–70 years, (2) residence in Florida, (3) no prior diagnosis of penile or anal cancers, (4) no prior diagnosis of genital and/or anal warts, (5) no participation in an HPV vaccine study, (6) no prior diagnosis of HIV/AIDS, (7) no current penile discharge or burning during urination, (8) no current receipt of treatment for a sexually transmitted disease, (9) no imprisonment or homelessness during the past 6 months, and (10) no participation in a drug or alcohol treatment program over the last 6 months at enrollment. In the parent HIM study, the participants were followed every 6 months up to four years. As described previously [25], between November 2008 and June 2010, a subcohort of 1082 participants residing in Tampa, Florida, who were initially enrolled in the parent HIM study was invited to participate in a substudy of natural history of cutaneous viruses. A total of 967 men enrolled in the parent HIM study had at least 1 eyebrow hair specimen or normal skin swab specimen at their baseline visit. Of these 967 men, 965 (99.8%) had 3 samples (1 eyebrow hair specimen and 1 normal skin swab specimen each, from sun-exposed and unexposed skin) obtained at their baseline visit, while 89.4% and 66.7% men had all 3 samples obtained at 2 and 3 visits, respectively. To maximize observation time and facilitate estimation of incidence and persistence, the substudy was restricted to 211 men who had all 3 samples for at least 4 visits. Of these, 209 men had viable biospecimens and were included in the final analysis. Written informed consent was obtained from all participants in the parent HIM study. The study protocol was approved by the review boards of recruiting sites as described previously [32].

Data Collection

Questionnaires

At their enrollment visit, the HIM study participants completed a comprehensive self-administered questionnaire that included questions related to demographic characteristics (age, race, education level, and marital status), socioeconomic status, medical history, smoking status, alcohol consumption, and sexual history. Additional questions on risk factors for skin cancer (such as response to season's first sun exposure and history of blistering sunburn) were added to the cutaneous viral infection substudy [25].

Eyebrow Hairs and Swabs of Normal Skin

Normal skin swab specimens were collected from the top of the forearm (sun exposed) and underneath the upper arm (sun unexposed) for each study participant. A 5 × 5-cm area of normal skin was prewetted (0.9% NaCl) and swabbed back and forth 5 times, using a cotton-tipped Dacron swab (Digene, Gaithersburg, Maryland). All swabs were placed in a separate vial and preserved in 500 μL of Digene Standard Transport Medium for HPV DNA testing (only swabs from sun-exposed skin were tested for polyomavirus DNA in the present substudy, owing to limited funding). Between 3 and 4 hairs were plucked from each eyebrow, using disposable tweezers as described previously [33, 34]. All tissues were stored at −70°C until testing for cutaneous HPV DNA.

DNA Extraction From Skin Swab and Eyebrow Hair Specimens

Eyebrow hair specimens and swab specimens of normal, sun-exposed skin were shipped on dry ice to the Infection and

Cancer Biology Group at the IARC in Lyon, France. DNA extraction was performed using the Qiagen BioRobot EZ1 with the EZ1 DNA tissue kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Briefly, the Dacron swabs were carefully cut into an Eppendorf tube, using scissors, and incubated overnight in proteinase K and buffer G2 (Qiagen, Hilden, Germany) at 56°C. An EZ1 DNA Forensic protocol was used to extract the DNA from eyebrow hair specimens according to the manufacturer's instructions. To monitor the possible occurrence of cross-contamination between the different specimens during DNA extraction, tubes containing buffer only were also included (1 tube with buffer every 10 specimens).

Viral DNA Detection Using Multiplex Polymerase Chain Reaction (PCR)/Luminex Assay

Samples collected during the study were banked until the end of study follow-up visits and were then screened using a multiplex PCR-based assay that identified the presence of 9 polyomaviruses (JCV, BKV, MCV, WUV, KIV, HPyV6, HPyV7, TSPyV, and HPyV9). Nine pairs of specific primers were designed to amplify a fragment of approximately 200 bp in the N-terminal part of the large T antigen gene (sequences available upon request). The accession numbers of the GenBank sequences that we used as references, with the corresponding polyomavirus given in parentheses, were NC_009539 (WUV), EF520287 (KIV), NC_001699 (JCV), NC_001538 (BKV), EU375804 (MCV), NC_014406 (HPyV6), NC_014407 (HPyV7), GU989205 (TSPyV), and HQ696595 (HPyV9). Oligonucleotides were synthesized by MWG Biotech (Ebersberg, Germany). Two primers for the amplification of β-globin were also added in the assay, to provide a positive control for the quality of the template DNA [35].

To evaluate the sensitivity of our assay, a multiplex PCR was performed using serial dilutions of DNA (from 1000 to 0 copies of viral genome) from polyomavirus types as a template. PCR products were obtained even when only 10 copies of the viral genome were used as template ([36] and data not shown). Following PCR amplification, 10 μL of each reaction was analyzed by multiplex genotyping using a Luminex-based assay [37]. Typing of the specific polyomaviruses was performed by hybridization of the PCR products to type-specific Luminex-bead–coupled polyomavirus probes (sequences available upon request) [15, 38]. The specificities of the polyomavirus probes were determined by coupling them to defined bead sets that were subsequently hybridized with the PCR products of all polyomaviruses. All of the probes were highly specific, and no cross-hybridization was found (data not shown). The positivity of the assay was given by the intensity of the fluorescent signal detected by the Luminex apparatus and was expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. The cutoff was calculated for each polyomavirus-specific probe by adding 5 MFIs to 1.1 × the median background value, as described by Schmitt et al [37]. All MFIs above the cutoff were considered positive. All of the tubes containing buffer only tested negative for polyomavirus DNA.

Statistical Analyses

Baseline characteristics were summarized using descriptive statistics, as appropriate. Prevalence was defined as the proportion of individuals who were DNA positive for a specific polyomavirus out of all participants with viable samples at baseline. Incidence was defined as the number of cases of new polyomavirus infection per 1000 person-months. The individuals contributing to the incidence calculation for a given polyomavirus were DNA negative for that virus at baseline. Samples that were found to be β-globin negative at baseline (n = 17) were not included in the estimation of prevalence, but these samples were included in the denominator in the estimation of incidence rate if the sample at the second visit was DNA negative for the specific polyomavirus infection. Persistence was defined as presence of polyomavirus infection at ≥2 consecutive visits. For a given polyomavirus persistence, only participants who were positive for the polyomavirus and had at least 1 subsequent follow-up visit were included. The κ coefficient was used to determine the concordance of viral infections across eyebrow hair and normal skin swab specimens for each polyomavirus [39]. Age-adjusted logistic regression analyses were conducted to derive odds ratios (ORs) and 95% confidence intervals (CIs) estimating the association between risk factors and incidence, prevalence, and persistence of MCPyV and HPyV6 infections. Because of the small numbers of subjects who were positive for other polyomaviruses, risk factors for these infections could not be examined. All analyses were conducted using r v2.13. Adjustment for multiple comparisons was not conducted.

RESULTS

Demographic characteristics of the study population have been previously described [25]. Briefly, a majority of the study population was white (74.2%), nonsmoking (84.2%), and aged 18–30 years (51.7%). Among the polyomaviruses examined, MCPyV was the most prevalent (65.1% of normal skin swab specimens) at baseline, followed by HPyV6 (12% of normal skin swab specimens; Table 1). The prevalence of MCPyV infection at baseline was higher when DNA was measured in normal skin swab specimens (65.1%), compared with eyebrow hair specimens (30.6%). There was a moderate concordance between viral DNA measured in normal skin swab specimens and that measured in eyebrow hair specimens (κ = 0.38 for MCPyV, 0.44 for HPyV6, and 0.43 for HPyV7; data not shown).

A high prevalence of MCPyV infection was mirrored by high incidence and persistence rates among all polyomaviruses examined (Table 1). BKV, KIV, WUV, and JCV were not detected

Table 1. Baseline Prevalence, Incidence, and Persistence of Polyomaviruses Among Men Residing in Tampa, Florida

	Baseline Prevalence, Samples Positive, No. (%)		Incidence,[a] Cases Per 1000 Person-months		Persistence,[b] %	
Species	Eyebrow Hair (n = 209)	Normal Skin Swab (n = 192)	Eyebrow Hair	Normal Skin Swab	Eyebrow Hair	Normal Skin Swab
MCPyV	64 (30.6)	125 (65.1)	24.1	81.7	58.9	85.8
BKV	6 (2.9)	0 (0)	1.1	0.4	. . .	. . .
KIV	0 (0)	0 (0)	0.3	0.4	. . .	. . .
JCV	0 (0)	0 (0)	1.0	0.0	. . .	. . .
WUV	0 (0)	0 (0)	0.0	0.4	. . .	. . .
HPyV6	10 (4.8)	23 (12)	4.3	15.6	53.3	73.0
HPyV7	5 (2.4)	4 (2.1)	1.1	2.6	42.9	37.5
HPyV9	0 (0)	1 (0.5)	0.0	0.4	. . .	. . .
TSPyV	0 (0)	0 (0)	0.0	0.0	. . .	. . .

Abbreviations: BKV, *BK polyomavirus*; HPyV, human polyomavirus; JCV, *JC polyomavirus*; KIV, *KI polyomavirus*; MCPyV, *Merkel cell polyomavirus*; TSPyV, *Trichodysplasia spinulosa–associated polyomavirus*; WUV, *WU polyomavirus*.

[a] Person must be negative for a given polyomavirus at baseline or, if the baseline sample was β-globin negative, at their second visit, to contribute person-time to the calculation of incidence. First event of new viral infection was considered in calculation of incidence.

[b] Defined as the percentage of men who tested positive for the specified polyomavirus at 2 consecutive visits among those who tested positive at the first of ≥2 visits.

at baseline in eyebrow hair and/or normal skin swab specimens, and few incident cases were observed upon follow-up. TSPyV infection was not detected at baseline or during follow-up in normal skin and eyebrow hairs. Persistent infections were only observed for MCPyV, HPyV6, and HPyV7 in this population.

Given the relatively high prevalence, incidence, and persistence of MCPyV in this population, we further examined factors associated with MCPyV infection. Table 2 presents estimates of association between baseline demographic and lifestyle characteristics and MCPyV infection prevalence in eyebrow hairs and normal skin. Men aged >44 years were significantly more likely (OR, 2.11; 95% CI, 1.03–4.33) to have prevalent MCPyV infection in eyebrow hairs, compared with men aged 18–30 years. No association was observed between age and prevalence of MCPyV infection in normal skin. Hispanic ethnicity was associated with a significantly higher prevalence of MCPyV infection in normal skin (OR, 2.64; 95% CI, 1.01–6.88), while no association was observed with MCPyV infection in eyebrow hairs. None of the other factors examined were associated with MCPyV infection prevalence.

Unlike its positive association with prevalent MCPyV infection, age was not associated with incident or persistent MCPyV infection (Table 3; ORs not shown). None of the other demographic or lifestyle factors were associated with incident or persistent MCPyV infection (Table 3; ORs not shown).

Age was significantly associated with HPyV6 infection prevalence in both normal skin and eyebrow hairs and with persistent HPyV6 infection in normal skin (Table 4). Lifetime number of blistering sunburns and frequency of alcohol consumption were significantly associated with the incidence (in normal skin; $P < .05$) and persistence (in eyebrow hairs; $P < .05$) of HPyV6 infection, respectively (Table 4).

DISCUSSION

We examined the prevalence, incidence, and persistence of 9 polyomaviruses in a subcohort of 209 men enrolled in the HIM study at the US site. Among the 9 viruses examined, MCPyV had the highest prevalence in normal skin swab specimens (65.1%) and eyebrow hair specimens (30.6%). We also observed high incidence and persistence of MCPyV in our population, indicating that a majority of adult men harbor MCPyV infection. Previously, Nicol et al observed a higher prevalence of MCPyV infection (79%–96.2%) in adults, based on detection of antibodies to MCPyV [40]. The MCPyV seroprevalence is most likely higher than the prevalence of MCPyV DNA observed in normal skin swab specimens and eyebrow hair specimens in the current study because antibody analyses measure both current and past infections, whereas DNA-based biomarkers measure current infection. Compared with our study, Mertz et al observed a much lower prevalence of MCPyV DNA (21.3%) in normal skin specimens from healthy adults [16]. It should be noted that although we used normal skin swab specimens, Mertz et al measured MCPyV DNA in normal skin samples obtained from surgical margins of scars, from cysts, and from tumor-free margins of skin cancer, breast cancer, or benign tumors [16]. Furthermore, apart from their small sample size (n = 47), a third of skin samples in their study had actinic damage [16]. These differences in population and methods may explain variation in MCPyV prevalence across studies. While we observed a much lower prevalence of HPyV6 (12% in normal

Table 2. Association Between Baseline Characteristics and Prevalence of *Merkel cell polyomavirus* Infection Among Men Residing in Tampa, Florida

Characteristic	Eyebrow Hair (n = 209)			Normal Skin Swab (n = 192)		
	Samples Tested, No.	Samples Positive, No. (%)	Age-Adjusted OR (95% CI)[a]	Samples Tested, No.	Samples Positive, No. (%)	Age-Adjusted OR (95% CI)[a]
Age, y[b]						
18–30	108	22 (20.4)	1.00	95	61 (64.2)	1.00
31–44	44	22 (50)	3.91 (1.84–8.31)	43	29 (67.4)	1.15 (.54–2.48)
>44	57	20 (35.1)	2.11 (1.03–4.33)	54	35 (64.8)	1.03 (.51–2.06)
Self-identified race						
White	155	48 (31)	1.00	141	92 (65.2)	1.00
Other	53	16 (30.2)	0.98 (.48–1.98)	50	33 (66)	1.03 (.52–2.04)
Spanish/Hispanic/Latino ethnicity						
No	177	51 (28.8)	1.00	161	100 (62.1)	1.00
Yes	32	13 (40.6)	2.08 (.91–4.75)	31	25 (80.6)	2.64 (1.01–6.88)
Marital status						
Single, never married, or divorced/separated	148	45 (30.4)	1.00	136	87 (64)	1.00
Married or cohabiting, living together	61	19 (31.1)	0.7 (.34–1.41)	56	38 (67.9)	1.18 (.59–2.35)
Highest level of education						
High school or below	38	15 (39.5)	1.00	38	27 (71.1)	1.00
Vocational school/some college	110	29 (26.4)	0.73 (.32–1.66)	98	61 (62.2)	0.68 (.3–1.55)
Graduated college/ graduate school	61	20 (32.8)	0.64 (.26–1.55)	56	37 (66.1)	0.8 (.32–1.97)
Skin reaction to season's first sun exposure						
No change in skin color	37	8 (21.6)	1.00	37	23 (62.2)	1.00
Tan with no sunburn	53	19 (35.8)	2.6 (.95–7.17)	48	35 (72.9)	1.65 (.66–4.18)
Mild sunburn that becomes a tan	80	26 (32.5)	2.02 (.78–5.22)	73	47 (64.4)	1.1 (.49–2.51)
Sunburn	37	10 (27)	1.37 (.46–4.12)	32	19 (59.4)	0.89 (.34–2.35)
Ever had a blistering sunburn						
No	103	33 (32)	1.00	97	68 (70.1)	1.00
Yes	104	30 (28.8)	0.82 (.44–1.52)	93	56 (60.2)	0.65 (.35–1.18)
Lifetime no. of blistering sunburns						
0	103	33 (32)	1.00	97	68 (70.1)	1.00
1	38	11 (28.9)	0.93 (.4–2.17)	34	22 (64.7)	0.78 (.34–1.79)
2	28	11 (39.3)	1.45 (.59–3.57)	25	13 (52)	0.46 (.19–1.13)
>2	38	8 (21.1)	0.45 (.18–1.12)	34	21 (61.8)	0.69 (.3–1.61)
Alcohol use in past month						
No	35	10 (28.6)	1.00	33	22 (66.7)	1.00
Yes	174	54 (31)	1.07 (.47–2.45)	159	103 (64.8)	0.91 (.41–2.02)
No. of days of alcohol use in past month						
0	35	10 (28.6)	1.00	33	22 (66.7)	1.00
1–8	81	25 (30.9)	1.14 (.46–2.84)	73	46 (63)	0.83 (.35–1.97)
≥9	60	19 (31.7)	1.1 (.43–2.84)	54	32 (59.3)	0.7 (.28–1.74)
Current smoker						
No	176	48 (27.3)	1.00	159	103 (64.8)	1.00
Yes	33	16 (48.5)	1.86 (.84–4.13)	33	22 (66.7)	1.06 (.47–2.39)
Ever smoker						
No	123	29 (23.6)	1.00	108	69 (63.9)	1.00
Yes	84	34 (40.5)	1.63 (.84–3.13)	82	54 (65.9)	1.05 (.55–2.01)

Table 2 continued.

Characteristic	Eyebrow Hair (n = 209)			Normal Skin Swab (n = 192)		
	Samples Tested, No.	Samples Positive, No. (%)	Age-Adjusted OR (95% CI)[a]	Samples Tested, No.	Samples Positive, No. (%)	Age-Adjusted OR (95% CI)[a]
Smoking status						
Never	123	29 (23.6)	1.00	108	69 (63.9)	1.00
Former	51	18 (35.3)	1.36 (.64–2.91)	49	32 (65.3)	1.04 (.49–2.18)
Current	33	16 (48.5)	2.12 (.91–4.98)	33	22 (66.7)	1.08 (.46–2.57)
Ever received an STD diagnosis						
No	180	54 (30)	1.00	163	107 (65.6)	1.00
Yes	29	10 (34.5)	0.88 (.36–2.13)	29	18 (62.1)	0.83 (.35–1.95)
Lifetime no. of female vaginal sex partners						
0–1	42	9 (21.4)	1.00	36	21 (58.3)	1.00
2–9	61	15 (24.6)	1.08 (.41–2.82)	54	36 (66.7)	1.46 (.6–3.52)
≥10	96	37 (38.5)	1.53 (.59–3.99)	92	64 (69.6)	1.74 (.7–4.34)
Female vaginal sex partners in past 6 months						
0	53	18 (34)	1.00	52	35 (67.3)	1.00
1	105	28 (26.7)	0.71 (.34–1.48)	94	56 (59.6)	0.71 (.35–1.46)
≥2	49	17 (34.7)	1.19 (.49–2.9)	44	32 (72.7)	1.31 (.53–3.27)
Lifetime no. of male anal-sex partners						
0	158	46 (29.1)	1.00	143	93 (65)	1.00
≥1	17	6 (35.3)	1.15 (.39–3.4)	16	11 (68.8)	1.16 (.38–3.53)

Abbreviations: CI, confidence interval; OR, odds ratio; STD, sexually transmitted disease.

[a] By logistic regression.

[b] By unadjusted logistic regression.

skin swab specimens) and HPyV7 (2.1% in normal skin swab specimens), compared with MCPyV, Nicol et al observed high seroprevalence of HPyV6 (61.8%–98.2%) and HPyV7 (36%–85.7%) among individuals aged 15 to ≥80 years [40]. Again, the higher seroprevalence may reflect both current and past exposure to the virus.

The prevalence of other polyomaviruses examined was very low in our population. Recently, a prevalence of 13.9% and 1.4% was reported for WUV and KIV, respectively, among Japanese children [41]. It is likely that these viruses are predominantly acquired in childhood and possibly cleared with increasing age. If this is true, it might explain the lack of prevalent WUV or KIV infection in our adult population. Furthermore, these viruses were initially discovered in respiratory secretions [8, 10]. The low prevalence in normal skin may reflect tissue tropism of these polyomaviruses.

Older age was associated with a higher prevalence of MCPyV infection in eyebrow hairs. As previously suggested, the association of age with the prevalence of MCPyV infection may be due to reduced immunity with increasing age that may lead to reactivation of virus in older individuals [40]. In contrast, in a study conducted by Baez et al, age was not related to MCPyV positivity in oral samples [23]. While we observed a significant association between age and prevalent MCPyV infection in eyebrow hairs, no association was seen with MCPyV infection in normal skin swab specimens.

Apart from age, ethnicity was associated with prevalence of MCPyV infection. Hispanic men were more likely than non-Hispanic men to harbor MCPyV DNA in normal skin and thus may represent a high-risk group for chronic MCPyV infection. Evaluation of lifestyle factors that differ by ethnicity may provide an insight into potential sources of transmission of MCPyV infection that differ by ethnicity. No other risk factors were associated with prevalence, incidence, or persistence of MCPyV infection in this population. While an evaluation of risk factors for prevalence of other polyomaviruses was restricted in our study because of small sample size, age [22] and race [42] have previously been reported to be associated with BKV infection.

The findings of our study should be interpreted with caution. Our analyses were restricted to men, and therefore the findings may not be generalizable to women. However, sex has not been previously associated with MCPyV DNA positivity in oral samples [23]. While we evaluated 9 polyomaviruses, additional polyomaviruses are being discovered [43], with many more likely unidentified to date. The estimates of persistence were based on inclusion of both incident and prevalent polyomavirus infection. Thus, the persistence rates presented here may be overestimated. Conversely, if persistent viral infection at a skin site

Table 3. Associations Between Baseline Characteristics and Incident and Persistent Infection With *Merkel cell polyomavirus* (MCPyV) Among Men Residing in Tampa, Florida

Characteristic	Incident Infection, Samples Positive, No. (%)		Persistent Infection, Samples Positive, No. (%)	
	Eyebrow Hair (n = 145)	Normal Skin Swab (n = 75)	Eyebrow Hair (n = 73)	Normal Skin Swab (n = 169)
Age, y				
18–30	17 (19.8)	31 (79.5)	14 (48.3)	75 (86.2)
31–44	4 (18.2)	9 (60)	13 (59.1)	30 (88.2)
>44	10 (27)	18 (85.7)	16 (72.7)	40 (83.3)
Self-identified Race				
White	22 (20.6)	42 (73.7)	30 (54.5)	102 (84.3)
Other	9 (24.3)	15 (88.2)	13 (72.2)	43 (89.6)
Spanish/Hispanic/Latino ethnicity				
No	28 (22.2)	53 (76.8)	38 (62.3)	121 (85.2)
Yes	3 (15.8)	5 (83.3)	5 (41.7)	24 (88.9)
Marital status				
Single, never married, or divorced/ separated	21 (20.4)	46 (85.2)	28 (54.9)	104 (86)
Married or cohabiting, living together	10 (23.8)	12 (57.1)	15 (68.2)	41 (85.4)
Highest level of education				
High school or below	6 (26.1)	10 (90.9)	8 (50)	31 (93.9)
Vocational school/some college	17 (21)	32 (76.2)	22 (62.9)	76 (84.4)
Graduated college/ graduate school	8 (19.5)	16 (72.7)	13 (59.1)	38 (82.6)
Skin reaction to season's first sun exposure				
No change in skin color	6 (20.7)	10 (71.4)	4 (50)	29 (93.5)
Tan with no sunburn	11 (32.4)	12 (85.7)	14 (60.9)	38 (84.4)
Mild sunburn that becomes a tan	7 (13)	23 (74.2)	16 (53.3)	53 (82.8)
Sunburn	7 (25.9)	12 (80)	8 (72.7)	24 (85.7)
Ever had a blistering sunburn				
No	17 (24.3)	29 (93.5)	21 (53.8)	76 (85.4)
Yes	14 (18.9)	28 (65.1)	21 (63.6)	68 (86.1)
Lifetime no. of blistering sunburns				
0	17 (24.3)	29 (93.5)	21 (53.8)	76 (85.4)
1	3 (11.1)	9 (69.2)	7 (53.8)	25 (83.3)
2	5 (29.4)	10 (71.4)	8 (80)	22 (100)
>2	6 (20)	9 (56.2)	6 (60)	21 (77.8)
Alcohol use in past month				
No	6 (24)	9 (69.2)	8 (80)	25 (89.3)
Yes	25 (20.8)	49 (79)	35 (55.6)	120 (85.1)
No. of days of alcohol use in past month				
0	6 (24)	9 (69.2)	8 (80)	25 (89.3)
1–8	9 (16.1)	25 (80.6)	16 (55.2)	57 (87.7)
≥9	9 (22)	18 (78.3)	13 (59.1)	41 (83.7)
Current smoker				
No	27 (21.1)	50 (78.1)	35 (62.5)	122 (84.7)
Yes	4 (23.5)	8 (72.7)	8 (47.1)	23 (92)
Ever smoker				
No	20 (21.3)	39 (84.8)	22 (64.7)	85 (82.5)
Yes	11 (22)	19 (65.5)	21 (55.3)	58 (90.6)
Smoking status				
Never	20 (21.3)	39 (84.8)	22 (64.7)	85 (82.5)
Former	7 (21.2)	11 (61.1)	13 (61.9)	35 (89.7)
Current	4 (23.5)	8 (72.7)	8 (47.1)	23 (92)
Ever received an STD diagnosis				
No	26 (20.6)	49 (76.6)	37 (59.7)	122 (85.3)
Yes	5 (26.3)	9 (81.8)	6 (54.5)	23 (88.5)
Lifetime no. of female vaginal sex partners				
0–1	6 (18.2)	12 (63.2)	5 (55.6)	25 (78.1)
2–9	10 (21.7)	15 (78.9)	9 (45)	42 (85.7)
≥10	15 (25.4)	26 (83.9)	27 (65.9)	71 (89.9)
Female vaginal sex partners in past 6 mo				
None	7 (20)	14 (82.4)	11 (61.1)	37 (80.4)
1	16 (20.8)	33 (73.3)	18 (58.1)	68 (84)
≥2	8 (25)	11 (84.6)	13 (56.5)	38 (95)
Lifetime no. of male anal-sex partners				
0	26 (23.2)	43 (75.4)	33 (61.1)	109 (87.2)
≥1	2 (18.2)	6 (100)	2 (33.3)	13 (92.9)

Age-adjusted logistic regression analyses did not show any significant association between baseline characteristics and incident or persistent MCPyV infection.

Abbreviation: STD, sexually transmitted disease.

that was not examined allowed infection at another skin site from where a sample was collected, the incidence of infection may have been overestimated. However, this seems unlikely since normal skin swab samples were collected from the same area on the top of the forearm at each visit, albeit with some minor variation in the exact site.

The study has several strengths. We present the first report of the natural history of several polyomaviruses, using biomarkers of infection in 2 different tissues. Viral DNA is a direct measure of polyomavirus infection, as opposed to serum antiviral antibodies, which reflect host immune response related to current or past infections. Furthermore, we evaluated the presence of viral DNA in

Table 4. Association Between Baseline Characteristics and Prevalence, Incidence, and Persistence of *Human polyomavirus 6* Infection Among Men Residing in Tampa, Florida

Characteristic	Prevalent Infection, Samples Positive, No. (%)		Incident Infection, Samples Positive, No. (%)		Persistent Infection, Samples Positive, No. (%)	
	Eyebrow Hair (n = 209)	Normal Skin Swab (n = 192)	Eyebrow Hair (n = 199)	Normal Skin Swab (n = 186)	Eyebrow Hair (n = 15)	Normal Skin Swab (n = 37)
Age, y						
18–30	1 (0.9)[a]	5 (5.3)[b]	5 (4.7)	23 (22.3)	0 (0)	5 (50.0)[b]
31–44	5 (11.4)[a]	8 (18.6)[b]	3 (7.7)	5 (13.9)	3 (50.0)	10 (100)[b]
>44	4 (7)[a]	10 (18.5)[b]	4 (7.5)	9 (19.1)	5 (71.4)	12 (70.6)[b]
Self-identified race						
White	5 (3.2)	18 (12.8)	12 (8.0)[b]	30 (21.9)	5 (50.0)	22 (73.3)
Other	5 (9.4)	5 (10.0)	0 (0)[b]	7 (14.6)	3 (60.0)	5 (71.4)
Spanish/Hispanic/Latino ethnicity						
No	8 (4.5)	20 (12.4)	11 (6.5)	34 (21.7)	7 (53.8)	25 (73.5)
Yes	2 (6.2)	3 (9.7)	1 (3.3)	3 (10.3)	1 (50.0)	2 (66.7)
Marital status						
Single, never married, or divorced/separated	8 (5.4)	13 (9.6)	3 (2.1)[a]	28 (20.7)	6 (66.7)	14 (66.7)
Married or cohabiting, living together	2 (3.3)	10 (17.9)	9 (15.3)[a]	9 (17.6)	2 (33.3)	13 (81.2)
Highest level of education						
High school or below	4 (10.5)	5 (13.2)	1 (2.9)	7 (21.2)	2 (50.0)	4 (50.0)
Vocational school/some college	3 (2.7)	10 (10.2)	5 (4.7)	21 (21.0)	3 (75.0)	13 (81.2)
Graduated college/ graduate school	3 (4.9)	8 (14.3)	6 (10.3)	9 (17.0)	3 (42.9)	10 (76.9)
Skin reaction to season's first sun exposure						
No change in skin color	3 (8.1)	5 (13.5)	2 (5.9)	7 (21.9)	2 (66.7)	6 (85.7)
Tan with no sunburn	2 (3.8)	5 (10.4)	1 (2.0)	10 (20.8)	0 (0)	5 (71.4)
Mild sunburn that becomes a tan	2 (2.5)	6 (8.2)	7 (9.0)	15 (20.3)	2 (33.3)	8 (57.1)
Sunburn	3 (8.1)	7 (21.9)	2 (5.9)	5 (16.7)	4 (100)	8 (88.9)
Ever had a blistering sunburn						
No	7 (6.8)	8 (8.2)	5 (5.2)	20 (21.1)	5 (55.6)	11 (78.6)
Yes	3 (2.9)	15 (16.1)	7 (6.9)	17 (19.1)	3 (50.0)	16 (69.6)
Lifetime no. of blistering sunburns						
0	7 (6.8)	8 (8.2)	5 (5.2)	20 (21.1)[b]	5 (55.6)	11 (78.6)
1	0 (0)	5 (14.7)	3 (7.9)	2 (6.1)[b]	0 (0)	5 (83.3)
2	1 (3.6)	5 (20)	1 (3.7)	9 (39.1)[b]	0 (0)	5 (55.6)
>2	2 (5.3)	5 (14.7)	3 (8.3)	6 (18.2)[b]	3 (75.0)	6 (75.0)
Alcohol use in past month						
No	1 (2.9)	4 (12.1)	4 (11.8)	5 (16.1)	0 (0)	4 (80.0)
Yes	9 (5.2)	19 (11.9)	8 (4.8)	32 (20.6)	8 (61.5)	23 (71.9)
No. of days of alcohol use in past month						
0	1 (2.9)	4 (12.1)	4 (11.8)	5 (16.1)	0 (0)[b]	4 (80.0)
1–8	5 (6.2)	9 (12.3)	4 (5.3)	15 (20.8)	6 (85.7)[b]	10 (71.4)
≥9	4 (6.7)	8 (14.8)	3 (5.4)	12 (23.1)	2 (33.3)[b]	7 (63.6)
Current smoker						
No	8 (4.5)	19 (11.9)	12 (7.1)	35 (22.3)	7 (53.8)	23 (74.2)
Yes	2 (6.1)	4 (12.1)	0 (0)	2 (6.9)	1 (50.0)	4 (66.7)
Ever smoker						
No	5 (4.1)	13 (12.0)	7 (5.9)	21 (19.1)	4 (44.4)	15 (78.9)
Yes	5 (6)	10 (12.2)	5 (6.3)	15 (20.3)	4 (66.7)	12 (66.7)

Table 4 continued.

Characteristic	Prevalent Infection, Samples Positive, No. (%)		Incident Infection, Samples Positive, No. (%)		Persistent Infection, Samples Positive, No. (%)	
	Eyebrow Hair (n = 209)	Normal Skin Swab (n = 192)	Eyebrow Hair (n = 199)	Normal Skin Swab (n = 186)	Eyebrow Hair (n = 15)	Normal Skin Swab (n = 37)
Smoking status						
Never	5 (4.1)	13 (12.0)	7 (5.9)	21 (19.1)	4 (44.4)	15 (78.9)
Former	3 (5.9)	6 (12.2)	5 (10.4)	13 (28.9)	3 (75.0)	8 (66.7)
Current	2 (6.1)	4 (12.1)	0 (0)	2 (6.9)	1 (50.0)	4 (66.7)
Ever received an STD diagnosis						
No	7 (3.9)	21 (12.9)	12 (6.9)	32 (20.1)	6 (50.0)	24 (70.6)
Yes	3 (10.3)	2 (6.9)	0 (0)	5 (18.5)	2 (66.7)	3 (100)
Lifetime no. of female vaginal sex partners						
0–1	0 (0)	4 (11.1)	3 (7.1)	9 (23.7)	0 (0)	4 (80.0)
2–9	2 (3.3)	5 (9.3)	3 (5.1)	9 (16.1)	1 (50.0)	6 (75.0)
≥10	7 (7.3)	13 (14.1)	5 (5.6)	16 (19.3)	5 (50.0)	15 (75.0)
Female vaginal sex partners in past 6 mo						
None	1 (1.9)	9 (17.3)	5 (9.6)	6 (13.6)	2 (66.7)	9 (81.8)
1	6 (5.7)	10 (10.6)	6 (6.1)	20 (21.1)	5 (55.6)	14 (77.8)
≥2	3 (6.1)	4 (9.1)	1 (2.2)	10 (22.2)	1 (33.3)	4 (57.1)
Lifetime no. of male anal-sex partners						
0	8 (5.1)	15 (10.5)	8 (5.3)	30 (21.0)	5 (45.5)	19 (76.0)
≥1	1 (5.9)	3 (18.8)	1 (6.2)	3 (21.4)	1 (50.0)	2 (50.0)

Abbreviation: STD, sexually transmitted disease.

[a] $P < .01$, by the Fisher exact test.

[b] $P < .05$, by the Fisher exact test.

both normal skin and eyebrow hairs and thus have provided more insight into tissue tropism for these infections. We observed higher rates of polyomavirus infection in skin, compared with eyebrow hairs. Furthermore, we observed moderate concordance between viral DNA measured in normal skin and viral DNA measured in eyebrow hairs. Finally, we evaluated a wide range of demographic and lifestyle factors in association with prevalence, incidence, and persistence of MCPyV infection.

In conclusion, we observed a high prevalence, incidence, and persistence of MCPyV infection in adult men. Age and Hispanic ethnicity were associated with the prevalence of MCPyV infection and may represent unknown factors that predispose to polyomavirus infection. The IARC has classified MCPyV as a "probable carcinogen" [13]. Given the oncogenic potential of polyomaviruses, it is important to further elucidate factors associated with polyomavirus infection.

Notes

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