WEB MATERIALS

"Seroepidemiology of human polyomaviruses in a United States population"
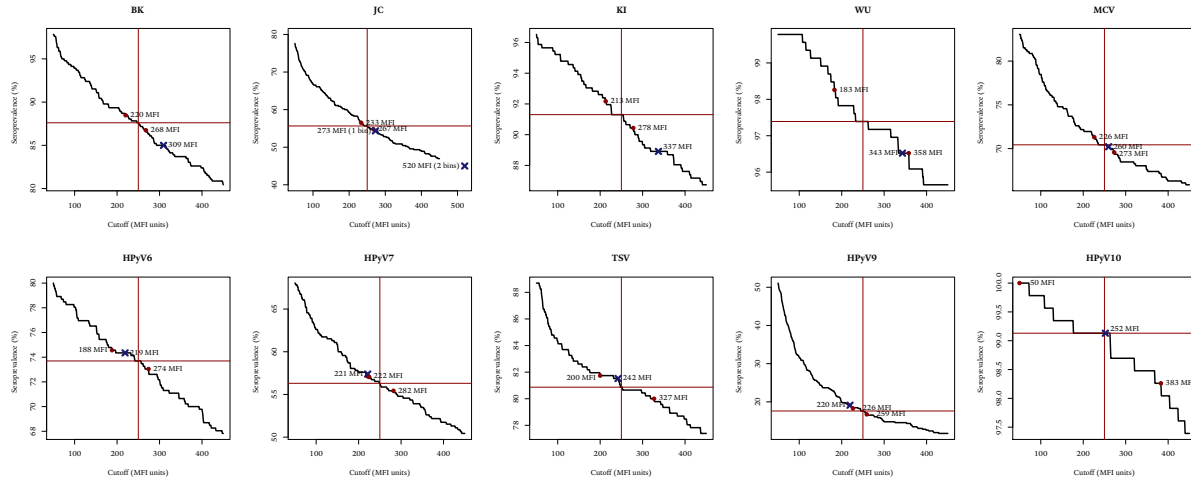
Included in the Web Material:

Web Table 1. Distribution of Human Polyomavirus (PyV) Seropositivity among 460 Study Participants by Sociodemographic Factors, New Hampshire, USA (1993-1995).

Web Table 2. Distribution of Human Polyomavirus (PyV) Seropositivity among 194 Study Participants by Sociodemographic Factors, New Hampshire, USA (1993-1995).

Web Figure 1. Robustness of human polyomavirus (PyV) seroprevalence estimates as determined by VP1 seroreactivity among 460 study participants, New Hampshire, USA (1993-1995). The cut points were varied from 50 to 450 median fluorescence intensity (MFI) units (x axis), and the resulting seroprevalences were calculated using the new cutoffs (y axis). The red cross hairs show the seroprevalences for each PyV VP1 using the recommended cutoff=250 MFI units. The two red dots show the range of MFI units that would result in seroprevalences ±1% of the seroprevalences calculated using the recommended cutoff. The blue crosses denote the cut points calculated using a frequency distribution analysis as described in van der Meijden *et al*, 2013 (34), with two JC cutoffs calculated using 1 bin versus 2 bins to illustrate the large difference in seroprevalence estimates depending upon binned samples included.

Web Figure 2. Phylogenetic tree relating human polyomaviruses (PyV) from human isolates (left). This simplified tree was constructed from the complete genome sequences aligned using MUSCLE 3.8.31 with default parameters. Branch length is in substitutions per site. The RefSeq accession numbers for the viruses are listed in the text. Spearman rank correlation coefficients, ρ, between the median fluorescence intensity (MFI) values against each PyV VP1 antigen among 460 study participants from New Hampshire, USA (1993-1995) are shown (right), where * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Web Figure 3. Spearman rank correlation coefficients, ρ, between immune cell proportions and median fluorescence intensity (MFI) values for each human polyomavirus (PyV) VP1 among 194 study participants, New Hampshire, USA (1993-1995). The red square emphasizes the correlations between immune cell proportions and MFI values for each PyV.

Web Figure 1. Robustness of human polyomavirus (PyV) seroprevalence estimates as determined by VP1 seroreactivity among 460 study participants, New Hampshire, USA (1993-1995). The cut points were varied from 50 to 450 median fluorescence intensity (MFI) units (x axis), and the resulting seroprevalences were calculated using the new cutoffs (y axis). The red cross hairs show the seroprevalences for each PyV VP1 using the recommended cutoff=250 MFI units. The two red dots show the range of MFI units that would result in seroprevalences ±1% of the seroprevalences calculated using the recommended cutoff. The blue crosses denote the cut points calculated using a frequency distribution analysis as described in van der Meijden *et al*, 2013 (34), with two JC cutoffs calculated using 1 bin versus 2 bins to illustrate the large difference in seroprevalence estimates depending upon binned samples included.

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Web Table 1. Distribution of Human Polyomavirus (PyV) Seropositivity among 460 Study Participants by Sociodemographic Factors, New Hampshire, USA (1993-1995). [a]

Variable	Total [d] No.	%	No. PyV types seropositive, mean (SD) [e]	Seropositive [b,c] BK No.	BK %	JC No.	JC %	KI No.	KI %	WU No.	WU %	MCV No.	MCV %	HPyV6 No.	HPyV6 %	HPyV7 No.	HPyV7 %	TSV No.	TSV %	HPyV9 No.	HPyV9 %	HPyV10 No.	HPyV10 %
Overall	460	100	7.3 (1.4)	403	87.6	256	55.6†	420	91.3	448	97.4	324	70.4†††	339	73.7	259	56.3††	372	80.9	81	17.6	456	99.1
Gender																							
Male	280	100	7.5 (1.5)***	246	87.8	168	60.0*	256	91.4	271	96.8	204	72.8	212	75.7	164	58.6	239	85.4***	54	19.3	277	98.9
Female	180	100	7.0 (1.3)	157	87.2	88	48.9	164	91.1	177	98.3	120	66.7	127	70.6	95	52.8	133	73.9	27	15.0	179	99.4
Education																							
Elementary to high or technical school	227	100	7.4 (1.4)	201	88.5	126	55.5	211	93.0	221	97.4	163	71.8	169	74.4	131	57.7	193	85.0	41	18.1	226	99.6
Any college	144	100	7.1 (1.4)	123	85.4	78	54.2	128	88.9	140	97.2	99	68.8	102	70.8	77	53.5	110	76.4	18	12.5	142	98.6
Graduate or professional school	89	100	7.4 (1.6)	79	88.8	52	58.4	81	91.0	87	97.8	62	69.7	68	76.4	51	57.3	69	77.5	22	24.7	88	98.9
Smoking status																							
Never	146	100	7.3 (1.3)	130	89.0	81	55.5	133	91.1	145	99.3	111	76.0*[g]	103	70.5	79	54.1	119	81.5	15	10.3*[g]	144	98.6
Former	230	100	7.4 (1.5)	205	89.1	128	55.6	210	91.3	222	96.5	159	69.1	178	77.4	138	60.0	191	83.0	49	21.3	228	99.1
Current	84	100	7.0 (1.6)	68	81.1	47	56.0	77	91.7	81	96.4	54	64.3	58	69.0	42	50.0	62	73.8	17	20.2	84	100
Glucocorticoid use [f]																							
Yes	39	100	7.4 (1.3)	35	89.7	22	56.4	36	92.3	37	94.9	23	59.0	30	76.9	30	76.9***[g]	32	82.0	5	12.8	39	100
No	415	100	7.3 (1.4)	363	87.5	231	55.7	278	67.0	406	97.8	296	71.3	305	73.5	226	54.4	334	80.5	74	17.8	411	99.0

[a] Numbers may not sum to the overall total due to missing data.
[b] Percentages indicate the proportion of healthy adults who are PyV seropositive versus PyV seronegative in each given strata.
[c] PyV infection was determined using seropositivity for the VP1 protein.
[d] All participants were seropositive for at least one of the ten PyVs assayed.
[e] Standard deviation referred to as "SD".
[f] Glucocorticoid use was defined as having used oral steroid or corticosteroid medications (for example, cortisone or prednisone) for one month or longer.
[g] Age group and gender-adjusted P-value, as determined by logistic regression.
† $P < 0.05$, †† $P < 0.01$, ††† $P < 0.005$ to test P for trend across age groups 25-44 (n=46), 45-54 (n=70), 55-64 (n=106), 65-69 (n=131), and 70-75 (n=107) years.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$ to test difference in proportions between groups, as determined by X^2 or Fisher's exact tests for categorical variables, and by Kruskal-Wallis or Wilcoxon rank sum tests for continuous variables.

Web Table 2. Distribution of Human Polyomavirus (PyV) Seropositivity among 194 Study Participants by Sociodemographic Factors, New Hampshire, USA (1993-1995). [a]

Variable	Total, No. (%) [d]	No. PyV types seropositive, mean (SD) [e]	Seropositive, No. (%) [b,c]									
			BK	JC	KI	WU	MCV	HPyV6	HPyV7	TSV	HPyV9	HPyV10
Overall	194 (100)	7.3 (1.4)	164 (84.5)	105 (54.1)	177 (91.2)	189 (97.4)	130 (67.0)	144 (74.2)	120 (61.8)	163 (84.0)	38 (19.6)	192 (99.0)
Gender												
Male	121 (100)	7.4 (1.6)	101 (83.5)	69 (57.0)	111 (91.7)	118 (97.5)	82 (67.8)	91 (75.2)	79 (65.3)	107 (88.4)	24 (19.8)	120 (99.2)
Female	73 (100)	7.1 (1.2)	63 (86.3)	36 (49.3)	66 (90.4)	71 (97.3)	48 (65.7)	53 (72.6)	41 (56.2)	56 (76.7)	14 (19.2)	72 (98.6)
Age (years)												
<45	23 (100)	6.5 (1.3)	22 (95.6)	8 (34.8)	19 (82.6)	23 (100)	14 (60.9)	17 (73.9)	8 (34.8)	17 (73.9)	2 (8.7)	23 (100)
45-54	28 (100)	7.4 (1.7)	26 (92.8)	15 (53.6)	24 (85.7)	27 (96.4)	19 (67.9)	23 (82.1)	17 (60.7)	23 (82.1)	6 (21.4)	27 (96.4)
55-64	45 (100)	7.4 (1.3)	38 (84.4)	27 (60.0)	43 (85.6)	44 (97.8)	34 (75.6)	31 (68.9)	25 (55.6)	40 (88.9)	8 (17.8)	44 (97.8)
65-69	52 (100)	7.3 (1.5)	42 (80.8)	32 (61.5)	47 (90.4)	50 (96.1)	30 (57.7)	37 (71.2)	38 (73.1)	44 (84.6)	9 (17.3)	52 (100)
70-75	46 (100)	7.5 (1.4)	36 (78.3)	23 (50.0)	44 (95.6)	45 (97.8)	33 (71.7)	36 (78.3)	32 (69.6)	39 (84.8)	13 (28.3)	46 (100)
Education												
Elementary to high or technical school	98 (100)	7.3 (1.4)	83 (84.7)	53 (54.1)	94 (95.9)	95 (96.9)	64 (65.3)	69 (70.4)	57 (58.2)	87 (88.8)	18 (18.4)	97 (99.0)
Any college	59 (100)	7.1 (1.4)	50 (84.7)	28 (47.5)	52 (88.1)	57 (96.6)	40 (67.8)	44 (74.6)	37 (62.7)	46 (78.0)	7 (11.9)	59 (100)
Graduate or professional school	37 (100)	7.7 (1.6)	31 (83.8)	24 (64.9)	31 (83.8)	37 (100)	26 (70.2)	31 (83.8)	26 (70.3)	30 (81.1)	13 (35.1)	36 (97.3)
Smoking status												
Never	64 (100)	7.2 (1.3)	56 (87.5)	32 (50.0)	57 (89.1)	63 (98.4)	49 (76.6)	43 (67.2)	37 (57.8)	57 (89.1)	6 (9.4)	62 (96.9)
Former	97 (100)	7.4 (1.5)	82 (84.5)	53 (54.6)	90 (92.8)	93 (95.9)	64 (66.0)	76 (78.4)	65 (67.0)	77 (79.4)	25 (25.8)	97 (100)
Current	33 (100)	7.2 (1.4)	26 (78.8)	20 (60.6)	30 (90.9)	33 (100)	17 (51.5)	25 (75.8)	18 (54.5)	29 (87.9)	7 (21.2)	33 (100)
Glucocorticoid use [f]												
Yes	22 (100)	7.4 (1.4)	19 (86.3)	12 (54.5)	21 (95.4)	21 (95.4)	13 (59.1)	16 (72.7)	18 (81.8)	18 (81.8)	3 (13.6)	22 (100)
No	168 (100)	7.3 (1.4)	142 (84.5)	92 (54.8)	152 (90.5)	165 (98.2)	114 (67.9)	126 (75.0)	101 (60.1)	141 (83.9)	34 (20.2)	166 (98.8)
Transplant recipient												
Yes	0	NA	0	0	0	0	0	0	0	0	0	0
No	193 (100)	7.3 (1.4)	164 (85.0)	105 (54.4)	176 (91.2)	188 (97.4)	129 (66.8)	143 (74.1)	120 (62.2)	162 (83.9)	38 (19.7)	191 (99.0)

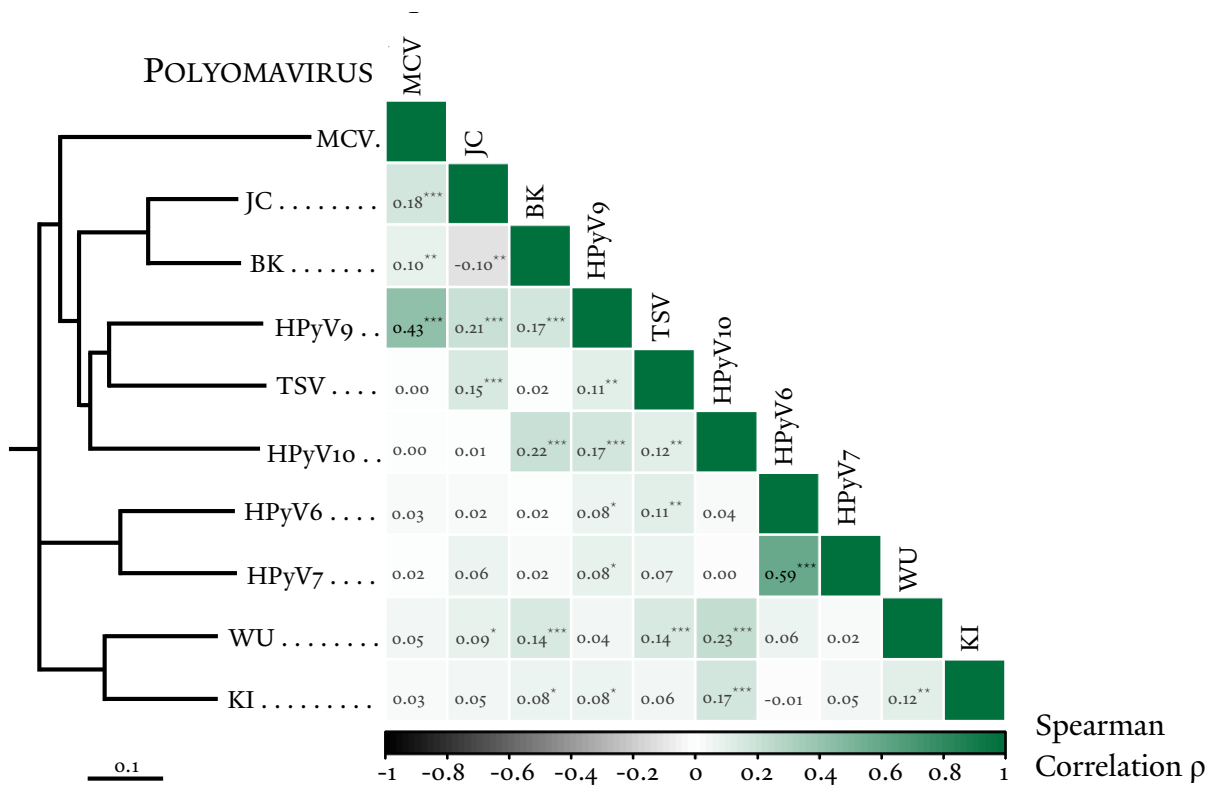[a] Numbers may not sum to the overall total due to missing data.

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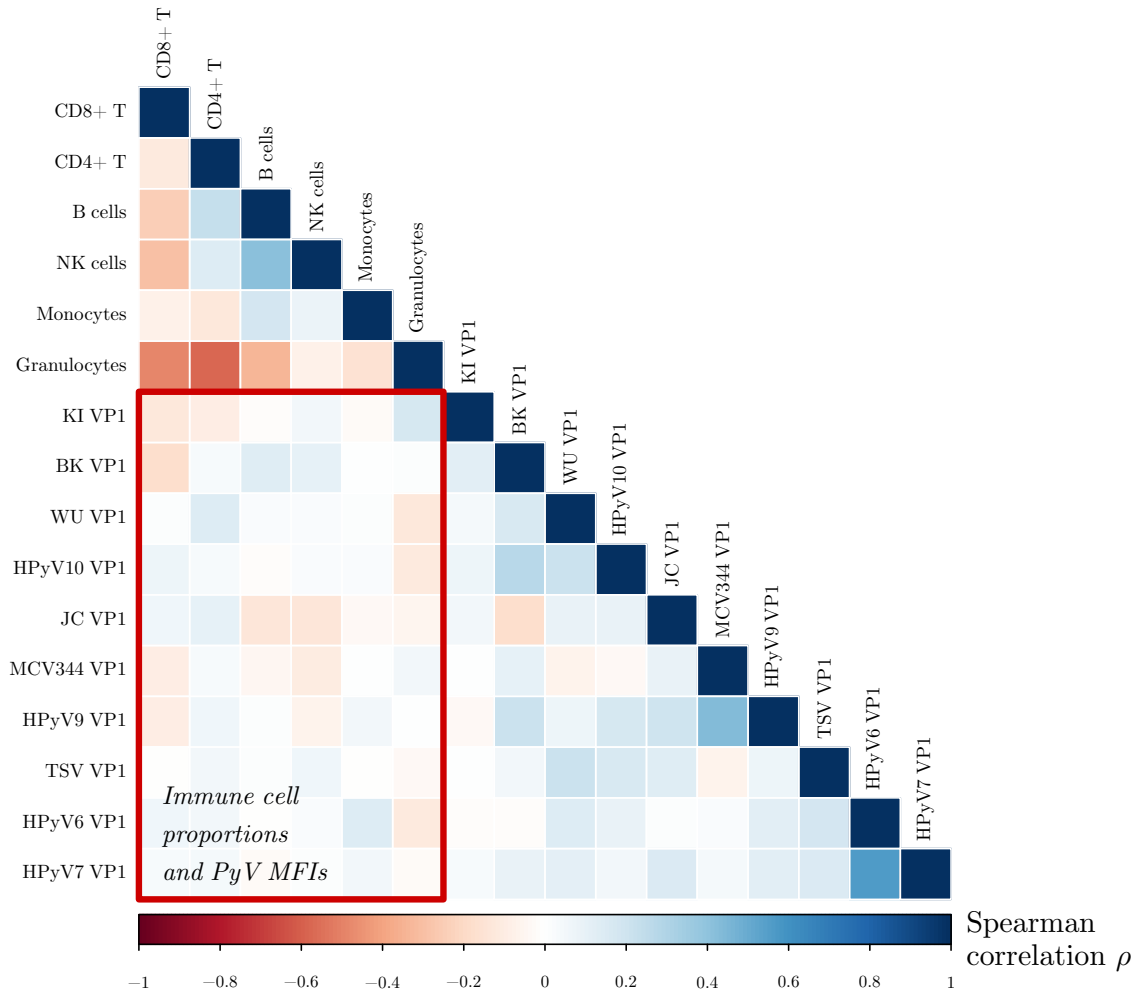[c] PyV infection was determined using seropositivity for the VP1 protein.

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POLYOMAVIRUS

	MCV	JC	BK	HPyV9	TSV	HPyV10	HPyV6	HPyV7	WU	KI
MCV										
JC	0.18^{***}									
BK	0.10^{**}	-0.10^{**}								
HPyV9	0.43^{***}	0.21^{***}	0.17^{***}							
TSV	0.00	0.15^{***}	0.02	0.11^{**}						
HPyV10	0.00	0.01	0.22^{***}	0.17^{***}	0.12^{**}					
HPyV6	0.03	0.02	0.02	0.08^{*}	0.11^{**}	0.04				
HPyV7	0.02	0.06	0.02	0.08^{*}	0.07	0.00	0.59^{***}			
WU	0.05	0.09^{*}	0.14^{***}	0.04	0.14^{***}	0.23^{***}	0.06	0.02		
KI	0.03	0.05	0.08^{*}	0.08^{*}	0.06	0.17^{***}	-0.01	0.05	0.12^{**}	

Spearman Correlation ρ

-1 -0.8 -0.6 -0.4 -0.2 0 0.2 0.4 0.6 0.8 1

0.1

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