

# **Association between Human Papillomavirus Particle Production and the Severity of Recurrent Respiratory Papillomatosis**

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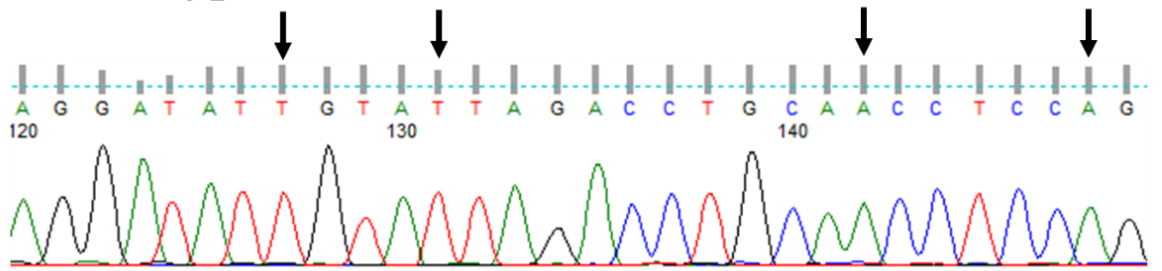
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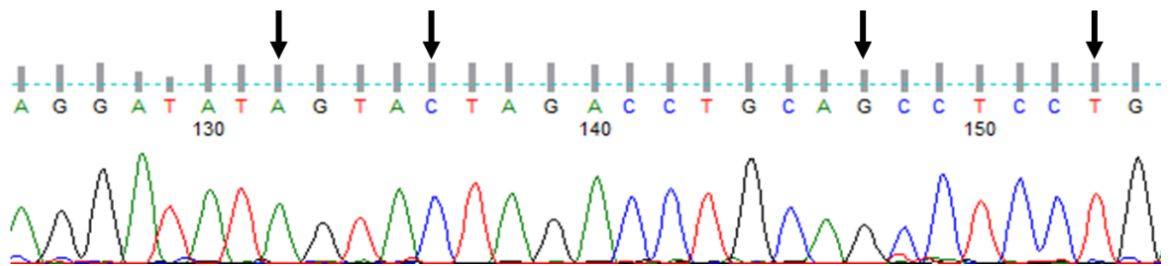
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Supplemental Figure S1. HPV sequencing analysis

Case No. 9 HPV type 6



Case No. 11 HPV type 11



HPV type 6 and HPV type 11 sequencing results. Arrows indicate different nucleotide sequences.  
HPV: human papillomavirus

## Supplemental Figure S2. Immunoreactive score

Intensity Positive tumor cells	Weak Staining = 1 point	Moderate Staining = 2 points	Strong Staining = 3 points
< 10% = 1 point	IRS = 1	IRS = 2	IRS = 3
10 - 50% = 2 points	IRS = 2	IRS = 4	IRS = 6
51 - 80% = 3 points	IRS = 3	IRS = 6	IRS = 9
> 80% = 4 points	IRS = 4	IRS = 8	IRS = 12

The immunoreactive score (IRS) is used to evaluate p16 IHC staining  
IHC: immunohistochemistry

**Supplemental Table S1 Detailed data on patient characteristics**

Case No.	Operation No. from this study	Age	Sex	Smoking	Alcohol	HPV	Total operation No.	DS	Operation interval (days)	p16 IRS	L1 IHC	MIB-1 index	Viral load (Copies/ng DNA)	E2/E6	MT
1	1-1	43	M	-	+	6	3	9	N.A.	2	-	54.62%	$1.00 \times 10^6$	0.79	-
	1-2							3	329	0	-	36.22%	$1.15 \times 10^6$	0.52	-
2	2-1	30	M	+	+	6	5	8	-	6	-	31.42%	$3.94 \times 10^6$	1.11	-
	2-2							7	664	6	-	22.77%	$2.42 \times 10^6$	0.71	-
	2-3							7	302	6	+	39.59%	$6.48 \times 10^5$	0.77	-
	2-4							7	1190	4	-	20.28%	N.A.	N.A.	-
	2-5							5	1078	2	-	36.38%	$2.79 \times 10^6$	0.57	-
3	3-1	42	M	+	+	6	2	4	-	0	-	17.95%	$2.72 \times 10^6$	0.64	-
	3-2							5	896	2	-	28.68%	$2.63 \times 10^6$	0.38	-
4	4-1	57	M	+	+	6	5	8	N.A.	2	-	39.66%	$4.05 \times 10^6$	0.78	-
	4-2							5	688	6	-	47.81%	$2.27 \times 10^6$	0.86	-
5	5-1	47	M	+	+	11	10	12	N.A.	1	+	34.16%	$2.79 \times 10^6$	0.51	-
	5-2							10	49	1	+	27.44%	$4.96 \times 10^6$	0.37	-
	5-3							10	34	1	-	31.47%	$6.34 \times 10^6$	0.15	-
	5-4							8	113	2	+	58.57%	$4.52 \times 10^6$	0.34	-
	5-5							6	92	4	-	18.94%	$1.40 \times 10^6$	0.16	-
6	6-1	70	M	+	+	-	3	3	-	1	-	0.60%	-	-	-
	6-2							5	2542	0	-	58.93%	-	-	-
	6-3							5	743	0	-	63.71%	-	-	+
7	7-1	54	F	-	-	-	1	6	-	0	-	54.93%	-	-	+
8	8-1	52	M	-	+	6	8	10	N.A.	4	+	47.51%	$4.01 \times 10^6$	0.60	-
	8-2							7	63	1	-	56.60%	$1.63 \times 10^6$	0.96	-
	8-3							9	108	1	-	N.A.	$1.66 \times 10^6$	0.61	-
	8-4							6	93	1	-	40.19%	$1.79 \times 10^6$	1.53	-
	8-5							4	187	0	-	N.A.	$1.38 \times 10^6$	0.62	-
	8-6							6	211	4	+	56.77%	$2.93 \times 10^6$	0.67	-
9	9-1	46	M	+	+	6	3	5	N.A.	6	-	45.10%	$2.95 \times 10^6$	0.60	-
10	10-1	45	F	+	-	6	1	3	-	4	-	17.95%	$2.81 \times 10^6$	0.66	-

11	11-1	42	M	+	-	-	2	5	180	2	-	6.08%	-	-	-
12	12-1	43	M	+	+	6	2	5	732	4	+	N.A.	$2.89 \times 10^7$	0.82	-
13	13-1	49	M	+	-	6	1	3	-	4	-	N.A.	-	-	-
14	14-1	69	M	+	+	11	1	3	-	8	-	26.61%	$2.29 \times 10^5$	0.23	-
15	15-1	66	F	-	-	11	1	3	-	6	-	29.07%	$1.78 \times 10^6$	0.07	-
16	16-1	75	M	+	+	11	1	3	-	2	-	14.61%	$2.61 \times 10^5$	0.27	-
17	17-1	70	M	+	+	-	1	3	-	6	-	33.53%	-	-	-
18	18-1	50	M	+	+	11	1	5	-	8	-	3.65%	$1.08 \times 10^7$	0.40	-
19	19-1	86	M	+	+	-	2	8	-	1	-	29.31%	-	-	-
	19-2							4	35	1	-	12.28%	-	-	-
20	20-1	47	M	+	+	11	1	4	-	6	+	35.17%	N.A.	N.A.	-
21	21-1	44	M	-	+	-	1	4	-	4	-	17.25%	-	-	-
22	22-1	45	M	+	+	11	16	6	-	4	+	28.71%	$3.65 \times 10^6$	0.28	-
	22-2							10	182	4	+	56.46%	$6.33 \times 10^6$	0.45	-
	22-3							5	74	8	+	43.10%	$4.57 \times 10^6$	0.44	-
	22-4							10	290	6	+	54.30%	$1.17 \times 10^6$	0.34	-
	22-5							3	81	4	+	55.24%	$2.29 \times 10^6$	0.31	-
	22-6							14	222	1	-	28.72%	$9.87 \times 10^6$	0.27	-
	22-7							10	173	1	-	N.A.	$3.58 \times 10^5$	0.30	-
	22-8							6	200	4	-	54.88%	$8.80 \times 10^5$	0.36	-
	22-9							8	138	4	+	60.96%	$4.74 \times 10^6$	0.24	-
	22-10							8	198	4	+	55.11%	$3.07 \times 10^6$	0.33	-
	22-11							10	91	1	+	51.42%	$1.17 \times 10^6$	0.33	-
	22-12							5	105	4	+	31.55%	$1.92 \times 10^6$	0.30	-
	22-13							8	336	4	+	59.73%	$1.69 \times 10^7$	0.25	-
	22-14							5	119	6	-	N.A.	$9.97 \times 10^5$	0.28	-
	22-15							4	490	9	-	N.A.	$7.47 \times 10^5$	0.22	-
	22-16							4	213	1	-	45.48%	$6.54 \times 10^6$	0.29	-
23	23-1	58	M	+	+	6	4	10	-	6	-	34.47%	$2.76 \times 10^6$	0.60	-
	23-2							3	1022	4	-	59.45%	$2.27 \times 10^6$	0.56	-
	23-3							3	613	4	+	39.43%	$6.78 \times 10^6$	0.52	-
	23-4							4	76	6	+	36.16%	$3.89 \times 10^6$	0.61	-

24	24-1	59	M	+	-	-	1	8	-	2	-	25.16%	-	-	-
25	25-1	37	M	+	+	-	1	4	-	2	-	N.A.	-	-	-
26	26-1	42	M	+	+	6	2	8	N.A.	4	+	61.98%	$3.05 \times 10^6$	0.83	-
27	27-1	62	M	+	+	-	1	4	-	6	-	45.63%	-	-	+
28	28-1	31	M	+	-	6	3	4	-	6	-	43.35%	$2.17 \times 10^6$	0.46	-
	28-2							3	241	6	-	45.24%	$3.46 \times 10^6$	0.51	-
	28-3							4	1557	6	-	41.23%	$2.29 \times 10^6$	0.53	-
29	29-1	77	M	+	+	-	1	4	-	0	-	11.58%	-	-	-

M: male, F: female, DS: Derkay score, IRS: immunoreactive score, MT: malignant transformation, N.A.: not available (due to missing data or insufficient samples)

**Supplemental Table S2. Real time PCR primers set**

Real-time PCR primers	Sequence (5'-3')	bp
HPV6 E2-F	TGAGTATAGTATGGAACCGTGGA	100
HPV6 E2-R	CTTCTACAGTTTTGCCCCGT	
HPV6 E6-F	GCTACCTGTGTCACAAACCG	100
HPV6 E6-R	CAGCGACCCTTCCACGTA	
HPV11 E2-F	GACCGTCCACTAACAACACC	102
HPV11 E2-R	TCCTTCTTTGGTGCTTGTTGT	
HPV11 E6-F	CAAGCCGTTGTGTGAAATAGAA	100
HPV11 E6-R	CCAGCAGTGTAAGCAACGAC	
ACTB-F	TGCCCTCATTTCCTCTCAG	101
ACTB-R	CGTACAGGTCTTTGCGGATG	

PCR: polymerase chain reaction