

Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication

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Pooled data on human papillomavirus (HPV) type distribution in invasive cervical cancer (ICC) can help to predict the potential impact of HPV type-specific vaccines and screening tests, and to understand the carcinogenicity of HPV types. We performed a meta-analysis of HPV type-specific prevalence data published from 1990 to 2010, including a total of 243 studies and 30,848 ICC. The proportion of ICC associated with HPV16 and/or 18 (HPV16/18) was between 70 and 76% in all world regions except Asia. In Western/Central Asia, 82% of ICC was HPV16/18-associated compared to only 68% in Eastern Asia. The 12 most common HPV types identified, in order of decreasing prevalence, were HPV16 (57%), 18 (16%), 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56. The prevalence of other types, phylogenetically related to those above, ranged from <0.1% for HPV85 to 0.6% for HPV68. Overall HPV prevalence increased significantly from 85.9% in studies published from 1990 to 1999 to 92.9% in studies published from 2006 to 2010. Prevalence increases were large for multiple infections (from 4.0 to 15.7%) and for HPV16 (from 51.8 to 60.0%, including HPV16 alone or in multiple infections). Smaller but significant increases in prevalence were also seen for HPV39, 53 and 58. A large amount of recently published data has improved the understanding of the contribution of a broad range of HPV types to ICC in different world regions. However, estimating the fraction of ICC attributable to different types is increasingly complicated by the detection of multiple HPV infections in ICC.

Since the establishment of human papillomavirus (HPV) as the central cause of invasive cervical cancer (ICC),¹ data on HPV type distribution in ICC have proven useful to predict the potential impact of HPV16 and 18 vaccines, as well as to determine priorities for inclusion of carcinogenic HPV types in future HPV vaccines and HPV-based screening tests.^{2,3}

A previous meta-analysis including 14,595 cases confirmed that a majority of ICC in all world regions is HPV16 and/or 18 (HPV16/18)-related.³ These two types, together with the less frequently detected HPV31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, were recently classified as carcinogenic to humans,³ and they cluster together in a few species (alpha-species 5, 6, 7, 9 and 11) that are phylogenetically related (*i.e.*, they belong to the same evolutionary branch or clade of the mucosotropic

HPV alpha-genus).^{4,5} This clade has therefore been referred to as the high-risk clade.⁵ Many of the rarest types of the high-risk clade have not been studied systematically. However, the number of studies available and the sensitivity of polymerase chain reaction (PCR)-based HPV testing protocols have increased over time, as has the ability to detect a broad range of HPV types and multiple infections.

The aims of our study were thus to update previous meta-analyses with the large amount of new data on HPV type-specific prevalence in ICC published in 2006–2010 and to evaluate whether HPV type-specific prevalence has changed during 20 years of evolving HPV DNA testing protocols.

Material and Methods

The detailed methods used for this meta-analysis of HPV type-specific prevalence in ICC have been reported previously.^{2,3} In brief, Medline was used to search for citations published from January 1990 to February 2010 using combinations of the following MeSH terms: “cervical cancer”, “cervical intraepithelial neoplasia”, “HPV”, “human”, “female” and “polymerase chain reaction”. Additional relevant references cited in retrieved articles were also evaluated. Included studies had to meet the following criteria: (i) use of PCR-based technology to detect HPV DNA and (ii) reporting of type-specific prevalence for at least one HPV type. For the purpose of our study, ICC refers to squamous cell carcinoma (SCC), adeno/adenosquamous carcinoma (ADC) and cervical

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cancer of unspecified histology, but does not include carcinoma *in situ*. For each included study, the following key information was extracted: country of sample, sample size, distribution of cancer cases by histological type (SCC, ADC or unspecified), HPV DNA source (fresh/fixed biopsies or exfoliated cervical cells), PCR primers used to detect HPV-positive samples, and type-specific and overall prevalence of HPV DNA. Studies were classified into seven broad geographical regions (Africa, Eastern Asia, Western/Central Asia, Europe, North America, South/Central America and Oceania). Crude type-specific prevalence is presented for all HPV types in the high-risk clade (*i.e.*, HPV16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82 and 85)⁵ other than HPV97, which has been too recently discovered to be included in our study. HPV6 and 11 from alpha-species 10 (the most well-studied non-carcinogenic types) were also included for comparison purposes. Each HPV type was evaluated independently of all others. Type-specific prevalence was estimated only among those studies testing for the HPV type in question, and thus denominators of prevalence estimates vary by type. Unless otherwise specified, therefore, type-specific prevalence includes that in either single or multiple HPV infections. However, the prevalence of single and multiple infections could be evaluated separately in a subset of studies from which such data were available. Owing to the predominance of SCC worldwide, ICC of unspecified histology was combined with SCC for comparison of HPV type-specific prevalence by SCC and ADC.

Statistical analysis

HPV prevalence in SCC and ADC was compared by logistic regression, adjusted, when appropriate, for region (Africa, Eastern Asia, Western/Central Asia, Europe, North America, South/Central America, Oceania), year of publication (1990–1999, 2000–2005, 2006–2010), specimen type (fresh/fixed biopsies, exfoliated cervical cells) and PCR primer used (GP5+/6+, MY09/11, PGMY09/11, SPF10, combination of the above, other). The statistical significance of trends in HPV prevalence by year of publication was assessed by considering the categorical variable as a continuous variable in the logistic model. Because of multiple statistical testing, only *p* values equal to or smaller than 0.01 were considered significant. Trends in single *versus* multiple infections with any HPV type, HPV16 and HPV18 were assessed in a subset of studies in which all 12 carcinogenic HPV types were tested for, and information on whether an individual type was found in single or multiple infections could be retrieved.

Results

Two hundred forty-three studies met our inclusion criteria, including a total of 30,848 ICC (Table 1). This constituted a gain of 16,253 ICC over a previous meta-analysis.³ The proportional gain was greatest for Asia, particularly for Western/Central Asia (from 386 to 2,051 ICC) where five countries were newly represented. Thirty-eight percent of all ICC came

from Eastern Asia, 29% from Europe, 10% from South/Central America, 8% from North America, 7% from Western/Central Asia, 7% from Africa and 2% from Oceania (Table 1). The proportion of cases diagnosed as SCC, ADC and unspecified histology varied by region, with the proportion of ICC classified as ADC ranging from 3.8% in Western/Central Asia up to 23.7% in North America (Table 1).

The prevalence of 26 selected HPV types are shown overall and by histological type in Table 2. Overall, prevalence of any HPV type, HPV16 and HPV18 were 89.9%, 56.6% and 16.0%, respectively. Among the studies reporting the relevant information, overall prevalence of single and multiple infections were 79.0% and 11.2%, respectively. The 12 most common HPV types identified were, in order of decreasing prevalence, HPV16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56. Prevalence of other types belonging to the high-risk clade ranged from less than 0.1% for HPV85 to 0.6% for HPV68. HPV6 and 11 were detected in 0.4% and 0.5% of ICC, respectively.

Overall HPV positivity was significantly higher among 26,667 cases of SCC/unspecified histology (90.9%) than in 3,525 ADC (82.0%; Table 2). HPV18 was over-represented in ADC (36.8%) compared to SCC (13.2%). Conversely, HPV16 was significantly under-represented in ADC (36.3%) compared to SCC (59.3%), as were many other carcinogenic types belonging to the same species as HPV16 (*i.e.*, HPV31, 33, 52 and 58). Two very rarely detected types (HPV6 and 69) were significantly more frequent in SCC and ADC, respectively. The prevalence of multiple infections did not differ significantly by histological type.

Overall HPV prevalence ranged from 88.3% in studies from Oceania to 94.2% in those from Africa (Fig. 1). The ten most common HPV types in ICC are shown by region in Figure 1. HPV16 was the most common type in all regions (ranging from 52% in Africa to 67% in Western/Central Asia), and HPV18 the second most common type (ranging from 14% in Eastern Asia to 20% in Oceania). The third to eighth most common HPV types were almost always HPV31, 33, 35, 45, 52 and 58, although their relative importance slightly differed by region. Of notice, HPV58 was the third most common type in Eastern Asia. In addition, HPV51 was in the seventh place in Africa, HPV59 in the eighth place in Eastern Asia, HPV39 in the eighth place in North America and HPV73, 39 and 53 in the fourth, fifth and eighth place, respectively (although sample sizes were small), in Oceania.

Overall and type-specific HPV prevalence in ICC and ADC are shown in Table 3 stratified by year of publication. The prevalence of any HPV type in ICC increased significantly from 85.9% in studies published in 1990–1999 to 92.9% in studies published in 2006–2010. Over the same time period, the prevalence of multiple infections increased from 4.0 to 15.7%, whereas the prevalence of single infections slightly decreased from 82.1 to 76.8%. The prevalence of HPV16, including both single and multiple infections, increased significantly from 51.8% in 1990–1999 to 60.0% in 2006–2010. After adjustment for histological type, region,

Table 1. Distribution of studies and invasive cervical cancer (ICC), by region and histological type

Region	Studies (N)	Countries represented	Cases (N)	ICC cases by histological type					
				SCC		ADC		UNSPEC	
				N	%	N	%	N	%
Africa	18	Algeria, Benin, Ethiopia, Guinea, Kenya, ¹ Mali, Morocco, Mozambique, Senegal, South Africa, Tanzania, Uganda, Zimbabwe	2,011	1,440	71.6	86	4.3	485	24.1
Eastern Asia	78	China, Indonesia, Japan, Malaysia, Mongolia, ¹ Philippines, South Korea, Taiwan, Thailand	11,651	7,572	65.0	972	8.3	3,107	26.7
Western/Central Asia	23	India, Iran, Jordan, ¹ Nepal, ¹ Pakistan, ¹ Syria, ¹ Turkey ¹	2,051	1,218	59.4	77	3.8	756	36.9
Europe	79	Austria, Belarus, ¹ Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Greenland, Hungary, Iceland, ¹ Ireland, Italy, Latvia, Lithuania, Luxembourg, ¹ Norway, Poland, Portugal, Russia, Slovenia, ¹ Spain, Sweden, the Netherlands, UK	9,015	5,182	57.5	1,358	15.1	2,475	27.5
North America	19	Canada, USA	2,485	1,627	65.5	588	23.7	270	10.9
South/Central America	28	Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Honduras, Jamaica, Mexico, Nicaragua, ¹ Panama, Paraguay, Peru, Surinam ¹	3,010	1,976	65.6	374	12.4	660	21.9
Oceania	6	Australia	625	169	27.0	83	13.3	373	59.7
Total	243 ²		30,848	19,184	62.2	3,538	11.5	8,126	26.3

¹Country not represented in previous meta-analyses. ²Total is less than the sum of the individual regions due to some multi-centre studies. Abbreviations: ADC: adeno/adenosquamous carcinoma; SCC: squamous cell carcinoma; UNSPEC: unspecified histological type.

specimen type and PCR primer, statistically significant increases in prevalence were also seen for HPV58, 39 and 53.

The absolute increase in overall HPV prevalence in ADC (from 77.8 to 86.0%) was similar to that for all ICC, as was the significant increase in multiple infections (from 3.9 to 15.9%). The prevalence of HPV16 increased (from 28.8% in 1990–1999 to 41.6% in 2006–2010), whereas HPV18 prevalence did not significantly change.

The distribution of ICC by year of publication is shown across strata of region, specimen type and PCR primer in Table 4, as well as the corresponding trends in HPV16 prevalence. With respect to distribution by region, there was an increase in the proportion of cases from Asia over time. The increase in HPV16 prevalence was observed in most regions and it was statistically significant in Eastern Asia. HPV16 prevalence was consistently higher among ICC tested from fresh/fixed biopsies than exfoliated cervical cells, but the increase in HPV16 prevalence over time was consistent in both types of specimen. The two most frequently used PCR primer sets over the entire study period were GP5+/6+ and MY09/11. The proportion of cases tested with MY09/11 decreased over time, with a parallel increase in those tested

by PGMY09/11, SPF10 or a combination of the four primers listed in Table 4. A significant upward trend in HPV16 prevalence over time was found only for MY09/11 (Table 4).

A subset of 66 studies, including 12,106 HPV-positive ICC and 1,156 ADC, tested for all 12 carcinogenic types and had the information to distinguish whether an individual HPV type was found in single or multiple infections (Table 5). Overall estimates of HPV prevalence were slightly higher in this subset of studies than the respective figures among the overall meta-analysis, but prevalence of HPV16 and HPV18 were similar. The increases in the prevalence of any HPV type (from 91.4 to 94.0%), multiple HPV infections (from 3.0 to 16.4%) and HPV16 (from 51.6 to 59.1%) by the year of publication were consistent with the overall meta-analysis. In ICC, the prevalence of HPV16 (49.7% in 1990–1999 and 49.0% in 2006–2010) and HPV18 (14.6 and 10.4%) in single infections stayed constant, while the corresponding prevalence in multiple infections increased substantially (from 2.0 to 10.1%, and from 1.6 to 5.9%, respectively). These shifts from single to multiple infections for any HPV-, HPV16- and HPV18-positive ICC were all significant. In ADC, similar shifts from single to multiple infections were seen by year of

Table 2. Selected human papillomavirus (HPV) types in invasive cervical cancer (ICC), overall and by histological type

HPV type ¹	Alpha-species	Histological type									SCC vs. ADC p value ³
		Overall			SCC ²			ADC			
		N	%	95% CI	N	%	95% CI	N	%	95% CI	
Any		30,357	89.9	88.2, 91.3	26,667	90.9	89.3, 92.3	3,525	82.0	78.4, 85.1	<0.001
Single		23,934	79.0	76.6, 81.1	19,860	79.4	76.8, 81.8	2,542	73.4	69.2, 77.1	<0.001
Multiple		23,934	11.2	9.1, 13.8	19,860	11.6	9.2, 14.5	2,542	9.3	6.8, 12.6	0.255
HPV16	9	30,743	56.6	54.3, 58.9	27,155	59.3	56.8, 61.7	3,538	36.3	33.0, 39.7	<0.001
HPV18	7	30,405	16.0	14.6, 17.4	26,826	13.2	12.0, 14.5	3,529	36.8	34.0, 39.7	<0.001
HPV58	9	23,487	4.7	2.9, 7.5	21,161	5.1	3.2, 8.0	2,276	1.5	0.5, 4.3	<0.001
HPV33	9	26,187	4.6	3.8, 5.6	23,157	4.9	4.0, 5.9	2,743	2.2	1.3, 3.6	<0.001
HPV45	7	20,659	4.5	3.8, 5.5	18,279	4.4	3.6, 5.4	2,330	5.2	4.0, 6.7	0.069
HPV31	9	24,652	3.8	3.3, 4.4	21,967	4.0	3.4, 4.6	2,398	2.3	1.4, 3.8	0.007
HPV52	9	22,555	3.4	2.5, 4.6	20,112	3.6	2.7, 4.9	2,275	1.2	0.7, 2.2	<0.001
HPV35	9	21,334	1.7	1.3, 2.3	18,879	1.9	1.4, 2.4	2,168	0.6	0.3, 1.4	0.027
HPV59	7	19,415	1.3	1.0, 1.6	17,156	1.4	1.1, 1.7	2,091	0.8	0.4, 1.3	0.140
HPV39	7	18,806	1.3	0.9, 1.8	16,571	1.3	0.9, 1.9	1,859	0.8	0.4, 1.8	0.055
HPV51	5	18,623	1.0	0.7, 1.5	16,265	1.0	0.7, 1.6	1,863	0.6	0.1, 2.9	0.604
HPV56	6	18,681	0.8	0.6, 1.0	16,560	0.8	0.7, 1.1	2,071	0.2	0.1, 0.6	0.005
HPV68	7	16,168	0.6	0.4, 0.9	14,208	0.5	0.3, 0.9	1,732	0.5	0.1, 1.5	0.619
HPV11	10	19,669	0.5	0.2, 1.3	17,841	0.6	0.3, 1.4	1,782	0.1	0.0, 0.4	0.064
HPV53	6	14,875	0.5	0.4, 0.7	13,229	0.6	0.4, 0.8	1,388	0.2	0.0, 1.1	0.255
HPV73	11	12,588	0.5	0.3, 0.8	11,510	0.5	0.3, 0.9	976	0.0	–	–
HPV6	10	20,081	0.4	0.3, 0.7	18,045	0.5	0.3, 0.7	1,990	0.1	0.0, 0.4	<0.001
HPV66	6	17,641	0.4	0.3, 0.6	15,856	0.4	0.3, 0.6	1,646	0.1	0.0, 0.4	0.081
HPV70	7	14,912	0.3	0.2, 0.7	12,914	0.3	0.2, 0.7	1,285	0.2	0.0, 1.2	0.338
HPV67	9	8,468	0.3	0.2, 0.5	7,170	0.3	0.2, 0.5	605	0.2	0.0, 0.6	0.142
HPV82	5	13,959	0.2	0.1, 0.4	11,920	0.3	0.2, 0.4	1,010	0.1	0.0, 0.7	0.489
HPV69	5	4,792	0.2	0.1, 0.6	3,747	0.2	0.1, 0.5	237	0.8	0.2, 3.5	0.002
HPV26	5	13,707	0.2	0.1, 0.5	11,673	0.1	0.1, 0.2	1,005	0.0	–	–
HPV34	11	8,045	0.1	0.0, 0.2	6,259	0.0	0.0, 0.2	750	0.0	–	–
HPV30	6	2,851	0.0	0.0, 0.3	2,612	0.0	0.0, 0.3	239	0.0	–	–
HPV85	7	1,172	0.0	–	1,155	0.0	–	17	0.0	–	–

¹All types in the high-risk clade (alpha-species 5, 6, 7, 9, 11)⁵ plus HPV 6 and 11 (alpha-species 10). ²Also includes ICC of unspecified histology.

³Adjusted for region, publication period, specimen type and PCR primer. Abbreviations: ADC: adeno/adenosquamous carcinoma; CI: confidence interval; N: number of cases tested for the given HPV type; SCC: squamous cell carcinoma.

publication and trends over time were significant for any HPV type (Table 5).

Discussion

By updating with the large amount of data published after 2006, our study includes twice as many cases as previous meta-analyses of HPV types in ICC.^{2,3} The accrual of new information has been especially large for studies from Asia, and for the rarest types in the high-risk clade.⁴ Furthermore, for the first time, our study allowed a description of time

trends in the prevalence of HPV types and multiple infections in ICC over 20 years of publications on the topic.

Overall, approximately 73% of ICC were associated with either HPV16 (57%) or 18 (16%), which, as shown previously,³ were by far the most common types in ICC in all regions studied. As new data accrue, the proportion of cases associated with HPV16/18 appears increasingly similar across all regions (70–76%), with the only partial exception of Asia. In Western/Central Asia, 82% of ICC was HPV16/18-associated, compared to only 68% in Eastern Asia. These figures suggest, therefore, a difference in HPV type distribution in

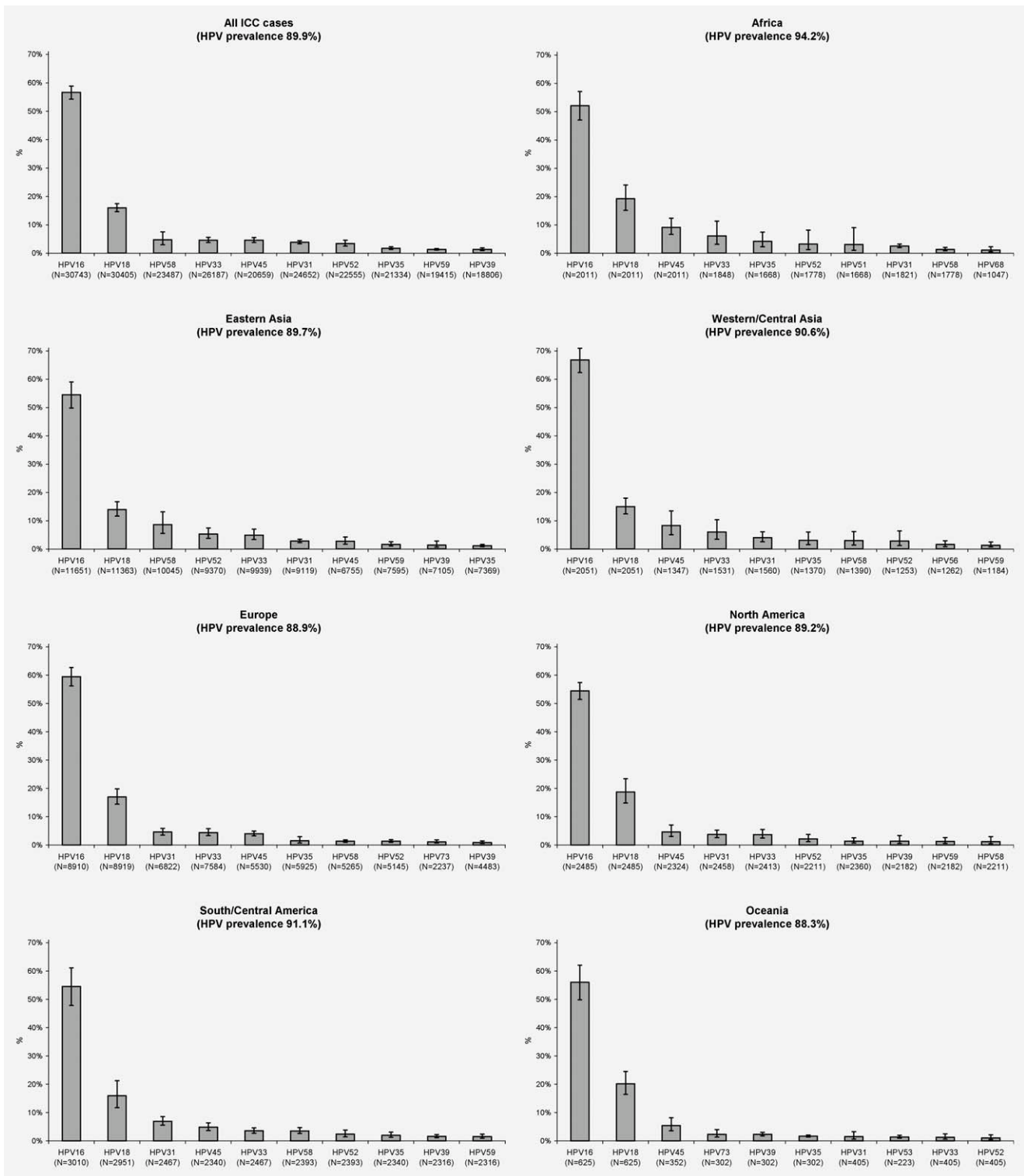


Figure 1. The ten most frequently detected human papillomavirus (HPV) types in invasive cervical cancer (ICC) 1990–2010, by region. Abbreviation: N: number of cases tested for the given HPV type.

ICC across the Asian continent, which is estimated to bear more than half of the world's cervical cancer burden.⁶

The most common types in ICC after HPV16 and HPV18 were confirmed to be consistent in all world regions and over

time, namely a combination of HPV31, 33, 35, 45, 52 and 58, with few exceptions. Our present findings are in agreement with those from our previous meta-analysis,³ as well as with those from the uniformly tested samples of the IARC

Table 3. Selected human papillomavirus (HPV) types in invasive cervical cancer (ICC) and adeno/adenosquamous carcinoma (ADC), by year of publication

HPV type	1990–1999			2000–2005			2006–2010			p for trend ¹
	N	%	95% CI	N	%	95% CI	N	%	95% CI	
All ICC										
Any	7,523	85.9	82.5, 88.8	7,877	87.9	85.7, 89.8	14,957	92.9	90.8, 94.5	0.001
Single	5,963	82.1	78.1, 85.5	5,621	80.4	77.3, 83.2	12,350	76.8	73.0, 80.2	0.010
Multiple	5,963	4.0	3.0, 5.4	5,621	9.0	5.9, 13.5	12,350	15.7	12.8, 19.2	<0.001
HPV16	7,662	51.8	48.5, 55.1	7,871	54.7	49.5, 59.8	15,210	60.0	56.2, 63.7	0.002
HPV18	7,561	16.8	14.6, 19.3	7,906	15.5	12.5, 19.0	14,938	15.8	13.9, 17.8	0.619
HPV58	4,305	2.5	1.6, 3.8	6,361	3.5	2.7, 4.6	12,821	6.1	3.3, 10.9	<0.001
HPV33	6,932	3.2	2.4, 4.2	6,090	5.9	4.4, 8.1	13,165	4.7	3.5, 6.4	0.181
HPV45	4,013	4.9	3.0, 7.9	4,739	3.4	2.4, 4.7	11,907	4.9	3.8, 6.2	0.937
HPV31	5,515	3.4	2.5, 4.7	5,691	4.3	3.3, 5.7	13,446	3.8	3.1, 4.6	0.039
HPV52	4,305	2.0	1.3, 3.0	5,519	2.6	1.8, 3.8	12,731	4.2	2.9, 6.1	0.029
HPV35	4,742	1.1	0.7, 2.0	4,935	2.6	1.5, 4.4	11,657	1.6	1.1, 2.2	0.634
HPV59	3,680	1.1	0.7, 1.6	3,610	1.1	0.7, 1.8	12,125	1.4	1.0, 1.9	0.245
HPV39	4,085	0.7	0.4, 1.2	3,141	0.7	0.4, 1.3	11,580	1.6	1.2, 2.3	0.001
HPV51	3,589	0.5	0.2, 1.3	4,007	0.8	0.5, 1.4	11,027	1.2	0.7, 2.1	0.429
HPV56	3,725	0.7	0.3, 1.6	4,143	0.7	0.4, 1.3	10,813	0.8	0.6, 1.1	0.658
HPV68	3,491	0.4	0.1, 1.2	3,288	0.5	0.2, 1.0	9,389	0.6	0.4, 1.1	0.944
HPV11	5,749	0.2	0.1, 0.5	3,688	0.3	0.1, 0.8	10,232	0.8	0.3, 2.4	0.042
HPV53	2,656	0.0	0.0, 0.3	2,267	0.4	0.2, 1.0	9,952	0.7	0.5, 0.9	<0.001
HPV73	3,071	0.2	0.1, 0.5	2,641	0.8	0.4, 1.3	6,876	0.5	0.2, 1.1	0.165
HPV6	5,824	0.3	0.1, 0.8	4,025	0.7	0.4, 1.4	10,232	0.4	0.2, 0.7	0.842
HPV66	3,637	0.2	0.1, 0.6	3,261	0.5	0.3, 1.0	10,743	0.5	0.3, 0.7	0.011
HPV70	3,413	0.1	0.1, 0.4	2,709	0.2	0.1, 0.6	8,790	0.4	0.2, 1.0	0.340
HPV67	524	0.0	0.0, 0.0	1,868	0.2	0.0, 0.5	6,076	0.3	0.2, 0.5	0.094
ADC only										
Any	1,003	77.8	72.6, 82.2	1,154	80.9	76.9, 84.4	1,368	86.0	78.3, 91.2	0.024
Single	838	73.4	67.4, 78.6	703	77.1	74.2, 79.7	1,001	70.7	61.4, 78.6	0.777
Multiple	838	3.9	2.5, 6.3	703	6.4	3.6, 11.2	1,001	15.9	12.0, 20.7	<0.001
HPV16	1,003	28.8	24.0, 34.1	1,154	36.3	30.6, 42.4	1,381	41.6	37.3, 46.1	0.010
HPV18	994	41.0	36.0, 46.2	1,154	34.6	30.6, 38.8	1,381	35.6	30.8, 40.8	0.999

¹Adjusted for histological type, region, specimen type and PCR primer. Abbreviations: CI: confidence interval; N: number of cases tested for the given HPV type.

Multicentric Cervical Cancer Study of 3,085 ICC,⁷ and *ad interim* data from a large multi-centre study.⁸ The most important regional differences in the ranking of HPV types was represented by HPV58, which is more frequent in Eastern Asia compared to elsewhere. For this reason, the total prevalence of HPV58 in published studies (4.7%) was inflated by the large contribution of cases from Eastern Asia. HPV31, 33, 35, 45, 52 and 58 clearly remain the priority candidates for additions in future generations of prophylactic vaccines. The remaining four HPV types recently classified as carcinogenic to humans,^{5,9} namely HPV39, 59, 51 and 56, were the next most common HPV types in ICC, each found in about 1% of cases.

HPV68, which was classified as probably carcinogenic to humans (Group 2A), was detected in 0.6% of ICC.

In a recent working group at IARC, other types in the high-risk clade (HPV26, 30, 34, 53, 66, 67, 69, 70, 73, 82 and 85) were all classified for cautionary reasons as possibly carcinogenic to humans (Group 2B) even in the absence of adequate epidemiological data on their carcinogenic potential.^{5,9} Our study revealed that most of these types were identified in less than 0.5% of ICC and less often than HPV6 and/or HPV11, which are not considered to be causally related to ICC. Furthermore, some of these types, such as HPV53 and HPV66, are relatively common in women with

Table 4. Human papillomavirus (HPV) 16 prevalence in invasive cervical cancer by year of publication, across strata of region, specimen type and PCR primer

	1990–1999		2000–2005		2006–2010		<i>p</i> for trend
	<i>N</i>	% HPV16-positive	<i>N</i>	% HPV16-positive	<i>N</i>	% HPV16-positive	
	7,662		7,871		15,210		
Region							
Africa	457	47.5	880	57.8	674	47.6	0.779
Eastern Asia	2,357	45.7	3,283	51.7	6,011	59.3	0.009
Western/Central Asia	153	64.7	373	60.6	1,525	68.5	0.215
Europe	2,688	56.7	1,647	61.0	4,575	60.5	0.351
North America	670	51.0	637	57.3	1,178	54.8	0.160
South/Central America	1,064	51.4	890	46.3	1,056	64.7	0.026
Oceania	273	59.3	161	54.0	191	52.9	0.069
Specimen type							
Fresh/fixed biopsies	6,339	53.3	5,665	57.5	12,427	60.6	0.013
Exfoliated cervical cells	1,323	44.7	2,206	47.4	2,783	57.1	0.012
PCR primer							
GP5+/6+	1,155	51.2	1,925	55.1	3,026	62.2	0.036
MY09/11	3,749	51.8	1,705	53.8	1,863	65.9	<0.001
PGMY09/11			50	54.0	1,238	55.7	0.662
SPF10	200	64.0	351	39.9	2,589	64.0	0.284
Combination of above	542	61.4	1,271	63.6	2,419	57.7	0.333
Other	2,016	48.4	2,569	52.5	4,075	55.9	0.136

Abbreviation: N: number of cases tested for the given HPV type.

normal cytology⁹ and in women with low-grade cervical lesions,¹⁰ Hence, including these types in screening assays would decrease the specificity and positive predictive value of the assays with virtually no gain in sensitivity and negative predictive value towards ICC and severe precancerous lesions.¹¹

Ninety percent of all ICC in our present study were positive for HPV DNA, and this percentage has increased over time. Given that HPV is accepted as a necessary cause of ICC, being found in more than 99% of ICC tested under the best conditions,¹² this increase is expected to be related to general improvements in HPV DNA testing protocols and not to any real changes in the contribution of HPV to ICC aetiology. It is also worth noting that year of publication is not always representative of the year of cancer diagnosis and sample collection, which may have been much earlier. The statistical significance of these increases remained after adjustment for specimen type and PCR primers used, suggesting that other improvements, not captured in our present meta-analysis approach, were at least partly responsible for the increases in HPV detection in ICC (*e.g.*, the general quality of biological specimen and/or reagents used, including the use of different DNA polymerases).

The progressive reduction in the proportion of HPV-negative ICC seen in our present meta-analysis has been accom-

panied by a substantial increase in the detection of HPV16, as well as smaller increases in some other rarer types. This would be expected if the type distribution in the falsely HPV-negative ICC in earlier published studies was similar to that of HPV-positive cases. Indeed, this was found to be the case when the HPV-negative cases were retested under gold-standard conditions by Walboomers *et al.*¹²

Although the increase in HPV16 prevalence was consistently observed across many strata of region, specimen type, histological type and PCR primers, the phenomenon was particularly strong in ADC. Thus, the contribution of HPV16 and HPV18 to ADC now seems more similar than in the earliest studies that showed a predominance of HPV18.^{2,3} This increase in the proportion of HPV16-positive ADC may be in part related either to increases in the joint detection of ADC and squamous intraepithelial lesions (in large part HPV16-positive) in screening programmes or to an increase in the fraction contributed by adenosquamous carcinoma, which appears to be related to HPV16 more often than adenocarcinoma.¹³ It is interesting that differences in prevalence by histology are not limited to HPV16 and HPV18, but extend to other HPV types that are phylogenetically related to these two types.¹⁴

Our study, however, also sends a warning: the attribution of ICC to different HPV types is increasingly complicated by

Table 5. Prevalence of all, single and multiple infections by year of publication, for any human papillomavirus (HPV) type, HPV16 and HPV18¹

		Overall		1990–1999		2000–2005		2006–2010		<i>p</i> for trend (single vs. multiple)
		<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	
All ICC		12,106		1,673		2,938		7,495		
Any HPV-positive	All	11,307	93.4	1,529	91.4	2,733	93.0	7,045	94.0	} <0.001
	Single	9,766	80.7	1,479	88.4	2,470	84.1	5,817	77.6	
	Multiple	1,541	12.7	50	3.0	263	9.0	1,228	16.4	
HPV16-positive	All	6,929	57.2	864	51.6	1,635	55.7	4,430	59.1	} <0.001
	Single	5,984	49.4	831	49.7	1,481	50.4	3,672	49.0	
	Multiple	945	7.8	33	2.0	154	5.2	758	10.1	
HPV18-positive	All	2,077	17.2	272	16.3	587	20.0	1,218	16.3	} <0.001
	Single	1,466	12.1	245	14.6	445	15.1	776	10.4	
	Multiple	611	5.0	27	1.6	142	4.8	442	5.9	
ADC only		1,156		152		405		599		
Any HPV-positive	All	1,036	89.6	129	84.9	360	88.9	547	91.3	} <0.001
	Single	917	79.3	129	84.9	330	81.5	458	76.5	
	Multiple	119	10.3	0	0.0	30	7.4	89	14.9	
HPV16-positive	All	462	40.0	42	27.6	172	42.5	248	41.4	} 0.010
	Single	395	34.2	42	27.6	150	37.0	203	33.9	
	Multiple	61	5.3	0	0.0	22	5.4	39	6.5	
HPV18-positive	All	467	40.4	70	46.1	155	38.3	242	40.4	} 0.357
	Single	374	32.4	70	46.1	104	25.7	200	33.4	
	Multiple	89	7.7	0	0.0	51	12.6	38	6.3	

¹Restricted to the subset of studies that tested for all 12 carcinogenic HPV types and allowed to distinguish whether an individual HPV type was found in single or multiple infections. Abbreviations: ADC: adeno/adenosquamous carcinoma; ICC: invasive cervical cancer.

the growing prevalence of multiple HPV types, which reached 15.7% in the most recently published studies. This increase is expected to be due, in large part, to the same factors that have driven the overall increase in HPV prevalence (*i.e.*, increased sensitivity). In addition, the increase in the types included in genotyping protocols of HPV-positive cases has certainly affected the detection of multiple infections.

A full assessment of multiple HPV infections is not possible in the entirety of our present meta-analysis because of the absence of individual-level data on the type-specific breakdown of multiple infections. Nevertheless, the extent to which multiple infections affect the attribution of ICC to HPV16 and HPV18 could be evaluated in a subset of studies including more than 12,000 ICC. This subset of studies showed that the prevalence of HPV16 in multiple infections has increased more than it has in single infections. HPV16 is expected to be causally related to ICC in a large proportion of these multiple infections, especially as an unknown number of multiple infections may involve non-carcinogenic types, but the estimate of the fraction truly attributable to HPV16 is uncertain. It can vary from the most conservative estimate of 49% (*i.e.*, HPV prevalence in single infections only) up to 63% (the

prevalence of HPV16 among HPV-positive ICC only, irrespective of whether HPV16 was found in single or multiple infections).¹⁵

The increasing prevalence of multiple infections also complicates the estimation of the proportion of cancers that can be attributable to groups of HPV types. For example, we estimated the fraction of ICC attributable to HPV16/18 from our entire meta-analysis to be 73% by simple addition of the type-specific prevalence for HPV16 to that for HPV18. However, the number of HPV16/18 multiple infections cannot be extracted from the majority of published data, and HPV16 and HPV18 are thus sometimes counted twice. The problem of overlapping multiple infections obviously becomes more severe as more types are included in the measure of the attributable fraction. For example, based upon studies published in 1990–1999, where multiple infections accounted for only 4%, the proportion of ICC attributable to the eight most common HPV types could be conservatively estimated at 86%. However, 14.1% of ICC remained HPV-negative in these early studies, suggesting that the fraction attributable to these eight types might actually be much higher. In more recently published studies, the HPV-negative fraction has halved (7.1%), but

the newer estimation of the fraction of ICC attributable to the combination of these types (101.0%) is certainly inflated by the multiple counting of HPV types in multiple infections.

In conclusion, the rise of the fraction of multiple infections in ICC, which is understood to arise from a single cell, poses serious interpretation problems. Unless functional involvement of HPV types in multiple infections is demonstrated, the attribution of the various HPV types present in multiple infections remains uncertain. However, demonstrating which HPV type is functionally relevant to a tumour, by using techniques such as microdissection and/or detection of

E6 or E7 transcripts, remains difficult.¹⁶ It is therefore increasingly challenging to distinguish between clinically relevant and irrelevant HPV infections and thereby to estimate the benefits of tests and vaccines including more or less broad ranges of HPV types.¹⁷

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