Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study

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Summary

Background Knowledge about the distribution of human papillomavirus (HPV) genotypes in invasive cervical cancer is crucial to guide the introduction of prophylactic vaccines. We aimed to provide novel and comprehensive data about the worldwide genotype distribution in patients with invasive cervical cancer.

Methods Paraffin-embedded samples of histologically confirmed cases of invasive cervical cancer were collected from 38 countries in Europe, North America, central South America, Africa, Asia, and Oceania. Inclusion criteria were a pathological confirmation of a primary invasive cervical cancer of epithelial origin in the tissue sample selected for analysis of HPV DNA, and information about the year of diagnosis. HPV detection was done by use of PCR with SPF-10 broad-spectrum primers followed by DNA enzyme immunoassay and genotyping with a reverse hybridisation line probe assay. Sequence analysis was done to characterise HPV-positive samples with unknown HPV types. Data analyses included algorithms of multiple infections to estimate type-specific relative contributions.

Findings 22 661 paraffin-embedded samples were obtained from 14 249 women. 10 575 cases of invasive cervical cancer were included in the study, and 8977 (85%) of these were positive for HPV DNA. The most common HPV types were 16, 18, 31, 33, 35, 45, 52, and 58 with a combined worldwide relative contribution of 8196 of 8977 (91%, 95% CI 90–92). HPV types 16 and 18 were detected in 6357 of 8977 of cases (71%, 70–72) of invasive cervical cancer. HPV types 16, 18, and 45 were detected in 443 of 470 cases (94%, 92–96) of cervical adenocarcinomas. Unknown HPV types that were identified with sequence analysis were 26, 30, 61, 67, 69, 82, and 91 in 103 (1%) of 8977 cases of invasive cervical cancer. Women with invasive cervical cancers related to HPV types 16, 18, or 45 presented at a younger mean age than did those with other HPV types (50·0 years [49·6–50·4], 48·2 years [47·3–49·2], 46·8 years [46·6–48·1], and 55·5 years [54·9–56·1], respectively).

Interpretation To our knowledge, this study is the largest assessment of HPV genotypes to date. HPV types 16, 18, 31, 33, 35, 45, 52, and 58 should be given priority when the cross-protective effects of current vaccines are assessed, and for formulation of recommendations for the use of second-generation polyvalent HPV vaccines. Our results also suggest that type-specific high-risk HPV-DNA-based screening tests and protocols should focus on HPV types 16, 18, and 45.

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Introduction

Invasive cervical cancer is the second most common cancer among women worldwide. 585 278 incident cases and 327 899 cases of attributable deaths are predicted for 2010.[1] More than 80% of cases arise in developing countries; invasive cervical cancer accounts for 15% of cancers in women and ranks first or second among cancers in women in 13 of 23 regions in the world.[1,2] Infection with one of the few oncogenic human papillomavirus (HPV) types is a necessary cause of invasive cervical cancer.[3,4] More than 118 different HPV types have been isolated and sequenced, and about 40 of these are known to infect the genital tract and 12 are classified as carcinogens.[5,6]

Distribution of HPV types in invasive cervical cancer has been reported for several countries.[2,7–11] However, no

reliable information exists about the type-specific contribution of HPV in invasive cervical cancer in many, mostly developing, countries. Country or regional data for the prevalence of HPV types in cervical cancer have been used to estimate the contribution of the various HPV types in invasive cervical cancer. Although not much is known about HPV types that are classified phylogenetically as strongly linked to carcinogenesis, some epidemiological data are available for HPV α5 (26, 51, 69, 82), α6 (30, 53, 56, 66), and α7 (39, 59, 68, 70), excluding types 18 and 45 for which we have consolidated information.[12]

The present study is an international project in which we aim to provide information about HPV genotype distribution in samples of invasive cervical cancer from several countries with different incidences of this disease; investigate the contribution of rare HPV types and the type attribution in specimens with several HPV types; identify HPV types associated with specific histological types of cervical cancer; investigate the role of exclusively low-risk HPV types; and generate insight into the secular trends of HPV-type distribution in invasive cervical cancer. The prespecified hypothesis was that the distribution of the five most common HPV types would be consistent geographically for the main histological categories of invasive cervical cancer.

Methods

Study design

A retrospective cross-sectional study was designed and coordinated by the Institut Català d'Oncologia, Barcelona, Spain, and DDL Diagnostic Laboratory, Voorburg, Netherlands, to estimate the prevalence of HPV DNA types in women with invasive cervical cancer during 1949–2009. Paraffin-embedded specimens from cases (aged 16–97 years) with cervical cancer were obtained from hospital pathology archives in 38 countries—Europe (Bosnia-Herzegovina, Croatia, Czech Republic, France, Greece, Italy, Netherlands, Poland, Portugal, and Spain); North America (USA); central South America (Argentina, Brazil, Chile, Colombia, Guatemala, Honduras, Mexico, Paraguay, Peru, and Venezuela); Africa (Algeria, Mozambique, Nigeria, and Uganda); Asia (Bangladesh, China, India, Israel, Japan, South Korea, Kuwait, Lebanon, Philippines, Taiwan, Thailand, and Turkey); and Oceania (Australia).

Samples from consecutive cases of invasive cervical cancer with information about age at diagnosis, year of diagnosis, and original histological diagnosis were obtained from the participating centres. We did not have information about the quality control for storage of specimens in the 38 participating countries. After the blocks were processed, inclusion criteria were a pathological confirmation of a primary invasive cervical cancer of epithelial origin in the tissue sample selected for analysis of HPV DNA, and information about the year of diagnosis. Reasons for exclusion were absence of confirmation of invasive cervical cancer in the first and fourth sections (n=2169); blocks that were moldy, or had a yellow colour resulting from Bouin's fixative (n=884); missing information about year of diagnosis (n=121); squamous or glandular differentiation in which confirmation of the cervical origin was not possible on the basis of histology and therefore the tumour was thought to be metastatic (n=89); cervical origin not confirmed (n=53); and no epithelial histogenesis (n=7). 351 cases were internal controls and excluded from the final analysis.

All protocols were approved by the local and Institut Català d'Oncologia ethics committees, and the entire study progress was overseen by an international steering committee.

Paraffin blocks were processed under strict conditions to avoid contamination. Four paraffin sections were systematically obtained from each block (sandwich method). First and fourth sections were used for histopathological assessment after haematoxylin and eosin staining, and the second and third sections were used for detection of and genotyping HPV DNA. The sections were processed and the disease was diagnosed at the reference pathology laboratory at the Institut Català d'Oncologia. Diagnosis included confirmation of invasive cervical cancer; and assessment of the histological type (eg, squamous cell carcinoma, adenocarcinoma, adenosquamous cell carcinoma, non-invasive cervical preneoplastic lesions); presence of normal mucosa or preneoplastic lesions adjacent to invasive cervical cancer (cervical intraepithelial neoplasia grade 1, 2, or 3; adenocarcinoma in situ); presence and quantification of tumour necrosis, and the proportion of tumour in the whole tissue section; and adequacy of the sample for HPV testing. Diagnosis of adenocarcinoma had to be confirmed by a panel of three pathologists. A blank paraffin section was cut and processed in between specimens to control for any carryover contamination in addition to the routine controls.

For each specimen, a paraffin tissue section was digested with 250 µL of freshly prepared proteinase K solution to extract the DNA. SPF-10 PCR was done in a final reaction volume of 50 µL containing 10 µL of extracted DNA that was diluted ten times. The amplified PCR products were tested by use of hybridisation with a cocktail of conservative probes that recognised at least 54 mucosal HPV genotypes in a microtitre plate format for the detection of HPV DNA with a DNA enzyme immunoassay. A microtitre plate reader was used to measure optical densities at 450 nm and the samples were classified as negative, positive, or borderline for HPV DNA. Borderline samples were rechecked by use of DNA enzyme immunoassay. After PCR, 10 µL of the amplimers that were positive for HPV DNA in the DNA immunoassay were used in the reverse hybridisation line probe assay ($LiPA_{25}$)[13] (version 1, Laboratory Biomedical Products, Rijswijk, Netherlands). $LiPA_{25}$ can be used to detect 25 high-risk and low-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52,

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	Cases of invasive cervical cancer analysed for HPV	HPV-positive cases*	Single HPV types†	Multiple HPV types†	Undetermined HPV types†
Geographical region					
Europe	2364	2058 (87%)	1914 (93%)	134 (7%)	10 (<1%)
North America	176	160 (91%)	154 (96%)	6 (4%)	0
Central South America	4171	3404 (82%)	3190 (94%)	199 (6%)	15 (<1%)
Africa	691	544 (79%)	430 (79%)	101 (19%)	13 (2%)
Asia	2994	2641 (88%)	2501 (95%)	128 (5%)	12 (<1%)
Oceania	179	170 (95%)	149 (88%)	19 (11%)	2 (1%)
Histological type					
Squamous cell carcinoma	9486	8252 (87%)	7678 (93%)	530 (6%)	44 (<1%)
Adenocarcinoma	760	470 (62%)	426 (91%)	39 (8%)	5 (1%)
Adenosquamous cell carcinoma	191	155 (81%)	142 (92%)	10 (6%)	3 (2%)
Other diagnosis‡	138	100 (72%)	92 (92%)	8 (8%)	0
Total	10 575	8977 (85%)	8338 (93%)	587 (7%)	52 (<1%)

Data are number or number (%). *Denominators are number of cases analysed for HPV. †Denominators are number of HPV-positive cases. ‡Includes undifferentiated, neuroendocrine, not otherwise specified, basal adenoid, and cystic adenoid carcinomas.

Table 1: **Human papillomavirus (HPV) types in cases of invasive cervical cancer by geographical region and histological categories**

53, 54, 56, 58, 59, 66, 68, 70, and 74). All detectable types belonged to nine species of the α-papillomavirus genus.[12] The sequence variation within the SPF-10 interprimer region allows the recognition of these different HPV genotypes, but not types 68 and 73 because their interprimer regions are identical and cannot be distinguished with this test. Positive hybridisation on the strips is seen as a purple band that results from the precipitation of a colour substrate on the probe site.

Specimens that were positive for HPV DNA by use of the DNA immunoassay, but did not hybridise with any of the 28 probes in $LiPA_{25}$ were analysed further by DNA sequencing. Specimens that were positive for HPV types 68 or 73, or 39, 68, or 73 were also sequenced to confirm the specific type. Because the 65 bp SPF-10 amplimer is too short for direct sequencing analysis of the 22 bp inner primer region, it was reamplified with elongated SPF-10 primers that contained T7 SP6 linker sequences attached to the forward and reverse primers, respectively. Reamplification PCR was done in a final volume of 50 µL, containing 10 µL of SPF-10 amplimer that was diluted ten times, 2 µmol/L magnesium chloride, 1X GeneAmp PCR buffer II (Applied Biosystems, Nieuwerkerk a/d Ijssel, Netherlands), 0·2 µmol/L of each of the four deoxynucleoside triphosphates, 1·5 U AmpliTaq Gold DNA polymerase (Applied Biosystems), and the elongated primer set. The PCR consisted of a 9 min preheating step at 94°C, followed by 30 cycles of amplification, each lasting 30 s at 94°C, 45 s at 52°C, and 45 s at 72°C, and a final elongation step at 72°C for 5 min. Reamplified SPF-10 PCR products were analysed on a 2·2% FlashGel (Lonza, Basel, Switzerland) to check for the presence of the 105 bp reamplification product. The reamplified PCR product was purified by use of ExoSAP-IT (USB, Staufen, Germany) according to the manufacturer's instructions to remove the unused primers. T7 and SP6 primers were used for forward and reverse sequence analysis of the complete SPF-10 PCR region. DNA sequencing was done with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using the ABI3100 Avant Genetic Analyzer (Applied Biosystems). If no HPV type could be attributed after DNA sequencing, the HPV was labelled HPV undetermined.

Statistical analysis

Variables analysed were country, year at diagnosis, age at diagnosis, pathological report that included histopathological diagnosis, presence of normal mucosa (as percentage of total), presence of preneoplastic lesions (grade if any), presence of necrosis (as percentage of total), and HPV genotype.

HPV and type-specific detection percentages were ascertained for the different continents, countries, histologies, age of the patient, and year of diagnosis. Unconditional logistic regression analysis was used to assess HPV detection for a particular variable, and other relevant variables were taken into account. The best fitting model was selected by use of the logarithm-likelihood ratio test. Mean age at diagnosis was estimated by use of a linear regression model, with the effect of region, period, and histology taken into account. Significance for all analyses was set at the two-sided 0·05 level.

The HPV type-specific relative contribution refers to the percentage of positive samples for a specific HPV type in relation to all the HPV-positive samples. HPV type-specific information always includes information about multiple infections. When more than one HPV type was

identified in a sample, two algorithms were used to represent the range of relative contributions for each HPV type. The lower estimate (more conservative) consists of single type infections and the multiple type infections restricted to the combination of the exact types (ie, relative contribution of HPV 16 and HPV 18 will include HPV 16, HPV 18, and both types). In the higher estimate, the multiple infections are added to single types in accordance with a proportional weighting attribution,[14,15] with the distributions in which single HPV types were identified used as references.

Statistical power was sufficient (>80%) to investigate a time trend analysis (change per year and change per 5 years) for the past 60 years for the three most common HPV types—16, 18, and 45. Data were analysed with SPSS (version 13.0) and STATA (version 10.0).

Role of the funding source

The sponsors did not have any role in the study design, and collection, analysis, or interpretation of the data. None of the sponsors had access to the raw data. The corresponding author had full access to all of the data, and had the final responsibility to submit for publication.

Results

After histological assessment of 22 661 blocks of archived paraffin-embedded tissues that were obtained from 14 249 women with cervical cancer, 10 575 cases of invasive cervical cancer were included in the study. Webappendix pp 5–7 lists the cases included in the study by region, country, period of sample collection, mean age at diagnosis, and histological group.

	Total (n=8977)	Europe (n=2058)	North America (n=160)	Central South America (n=3404)	Africa (n=544)	Asia (n=2641)	Oceania (n=170)
HPV 6	10 (<1%)	3 (<1%)	..	3 (<1%)	..	1 (<1%)	3 (2%)
HPV 11	2 (<1%)	..	..	1 (<1%)	..	1 (<1%)	..
HPV 16	5439 (61%)	1348 (66%)	115 (72%)	2015 (59%)	259 (48%)	1597 (60%)	100 (59%)
HPV 18	918 (10%)	150 (7%)	11 (7%)	309 (9%)	123 (23%)	295 (11%)	34 (20%)
HPV 26	31 (<1%)	3 (<1%)	..	17 (<1%)	..	11 (<1%)	..
HPV 30	31 (<1%)	5 (<1%)	..	14 (<1%)	3 (<1%)	9 (<1%)	..
HPV 31	335 (4%)	69 (3%)	5 (3%)	166 (5%)	10 (2%)	80 (3%)	1 (<1%)
HPV 33	345 (4%)	117 (6%)	5 (3%)	119 (3%)	8 (1%)	92 (3%)	3 (2%)
HPV 34	6 (<1%)	1 (<1%)	1 (<1%)	3 (<1%)	..	1 (<1%)	..
HPV 35	175 (2%)	46 (2%)	..	72 (2%)	27 (5%)	27 (1%)	4 (2%)
HPV 39	143 (2%)	27 (1%)	2 (1%)	76 (2%)	3 (<1%)	31 (1%)	3 (2%)
HPV 39*	3 (<1%)	1 (<1%)	..	1 (<1%)	1 (<1%)	..	..
HPV 42	3 (<1%)	3 (<1%)	..	..	..	..	..
HPV 44	1 (<1%)	1 (<1%)	..	..	..	..	..
HPV 45	528 (6%)	80 (4%)	9 (6%)	230 (7%)	54 (10%)	146 (6%)	9 (5%)
HPV 51	114 (1%)	28 (1%)	2 (1%)	53 (2%)	13 (2%)	19 (<1%)	..
HPV 52	253 (3%)	40 (2%)	5 (3%)	91 (3%)	14 (3%)	101 (4%)	1 (<1%)
HPV 53	24 (<1%)	10 (<1%)	1 (<1%)	9 (<1%)	..	1 (<1%)	3 (2%)
HPV 56	75 (<1%)	32 (2%)	1 (<1%)	20 (<1%)	4 (<1%)	18 (<1%)	..
HPV 58	203 (2%)	27 (1%)	3 (2%)	67 (2%)	4 (<1%)	102 (4%)	..
HPV 59	95 (1%)	15 (<1%)	..	42 (1%)	1 (<1%)	36 (1%)	..
HPV 61	1 (<1%)	..	..	1 (<1%)	..	..	..
HPV 66	7 (<1%)	2 (<1%)	..	2 (<1%)	2 (<1%)	1 (<1%)	..
HPV 67	26 (<1%)	3 (<1%)	..	13 (<1%)	..	10 (<1%)	..
HPV 68	59 (<1%)	13 (<1%)	..	20 (<1%)	1 (<1%)	25 (<1%)	..
HPV 68†	31 (<1%)	4 (<1%)	..	17 (<1%)	1 (<1%)	3 (<1%)	6 (4%)
HPV 69	7 (<1%)	..	..	6 (<1%)	1 (<1%)	..	..
HPV 70	9 (<1%)	1 (<1%)	..	3 (<1%)	..	5 (<1%)	..
HPV 73	43 (<1%)	16 (<1%)	..	14 (<1%)	1 (<1%)	12 (<1%)	..
HPV 74‡	2 (<1%)	..	..	..	..	1 (<1%)	..
HPV 82	6 (<1%)	..	..	4 (<1%)	..	2 (<1%)	..
HPV 91	1 (<1%)	1 (<1%)	..	..	..	..	..
HPV undetermined	52 (<1%)	10 (<1%)	0	15 (<1%)	13 (2%)	12 (<1%)	2 (1%)

Data are number (%) and are based on the upper estimate attribution of multiple HPV types. *HPV type 39, 68, or 73. †HPV type 68 or 73. ‡One case in the total attributable to infection with multiple HPV types, and no case with exclusively HPV 74.

Table 2: **Human papillomavirus (HPV) genotypes in cases of invasive cervical cancer that were positive for HPV DNA, by region**

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See **Online** for webappendix

For more on **HPV and cervical cancer** see http://www.who.int/hpvcentre/en/

Most infections were present as single infections, with the lowest proportion of single infections detected in samples from Africa and in adenocarcinomas. Undetermined HPV types were rarely detected, with the highest proportion noted in specimens from Africa (table 1). Histological subtype was the most important characteristic that was correlated with HPV DNA (webappendix pp 8–9). Adenocarcinoma had the lowest positivity for HPV DNA, followed by the other category and adenosquamous cell carcinoma (table 1).

All HPVs identified according to the regions of the world belonged to nine of 15 species (α1, α3, α5, α6, α7, α8, α9, α10, and α11) within the α-papillomavirus genus. The eight most common HPV types detected were 16, 18, 31, 33, 35, 45, 52, and 58 (table 2). Their combined relative contribution was 8196 of 8977 cases (91%, 95% CI 90–92). Some discrepancies were noted in the ranking by regions, with substantial differences noted for HPV 33 (ranked third in Europe), and HPV 58 and HPV 52 (ranked fourth and fifth, respectively, in Asia; table 2; webappendix p 10).

Notably, the use of sequencing analysis to identify HPV type in HPV-positive samples that were not characterised by use of hybridisation showed that 103 (1%) of 8977 cases were positive for HPV types 26, 30, 61, 67, 69, 82, and 91. Low-risk HPV types were occasionally identified as single infections: HPV 6 (n=9), HPV 11 (n=2), HPV 42 (n=3), HPV 44 (n=1), and HPV 74 (n=1). During the study period (60 years), the relative contribution of HPV 16 slightly decreased while that of the other HPV types slightly increased. However, these variations were not significant (data not shown).

HPV types 16, 18, and 45 were the three most common types in each histological group (squamous cell carcinoma, adenocarcinoma, and adenosquamous cell carcinoma; table 3). Together, they accounted for 6223 of 8252 cases (75%, 95% CI 75–76) of squamous cell carcinoma and 443 of 470 cases (94%, 92–96) of adenocarcinoma (table 3). HPV 18 and HPV 45 together were significantly more common in cases of adenocarcinoma than in cases of squamous cell carcinoma (208 [44%] of 470 *vs* 1133 [14%] of 8252; p<0·0001). Figure 1 shows the lower and higher estimates of the relative contribution of the eight most common types of HPV overall and according to histological categories (squamous cell carcinoma and adenocarcinoma). HPV DNA was detected in 155 (81%) of 191 cases of adenosquamous cell carcinoma and 100 (72%) of 138 histologically rare cases of invasive cervical cancer (neuroendocrine tumours and undifferentiated tumours); the predominant HPV types were 16, 18, and 45, with a relative contribution closer to that noted in adenocarcinoma.

Age was much lower for women with invasive cervical cancers that were positive for HPV 45 or HPV 18 than for women with cancers that were positive for HPV 16 or any other HPV type as a single infection. The mean ages of women with invasive cervical cancer related to HPV types 16, 18, and 45 were 50·0 years (95% CI 49·6–50·4), 48·2 years (47·3–49·2), and 46·8 years (46·6–48·1), respectively, and were much lower than the average age of women with this cancer related to any other HPV type (55·5 years [54·9–56·1]; figure 2).

	Total (n=8977)	Squamous cell carcinoma (n=8252)	Adenocarcinoma (n=470)	Adenosquamous cell carcinoma (n=155)	Other* (n=100)
HPV 6	10 (<1%)	9 (<1%)	1 (<1%)	..	..
HPV 11	2 (<1%)	2 (<1%)	..	..	..
HPV 16	5439 (61%)	5090 (62%)	235 (50%)	61 (39%)	51 (51%)
HPV 18	918 (10%)	687 (8%)	152 (32%)	49 (32%)	30 (30%)
HPV 26	31 (<1%)	31 (<1%)	..	..	..
HPV 30	31 (<1%)	30 (<1%)	1 (<1%)	..	..
HPV 31	335 (4%)	328 (4%)	3 (<1%)	3 (2%)	1 (1%)
HPV 33	345 (4%)	338 (4%)	2 (<1%)	4 (3%)	1 (1%)
HPV 34	6 (<1%)	6 (<1%)	..	..	..
HPV 35	175 (2%)	169 (2%)	2 (<1%)	2 (1%)	2 (2%)
HPV 39	143 (2%)	134 (2%)	1 (<1%)	4 (3%)	3 (3%)
HPV 39†	3 (<1%)	3 (<1%)	..	..	..
HPV 42	3 (<1%)	3 (<1%)	..	..	..
HPV 44	1 (<1%)	1 (<1%)	..	..	..
HPV 45	528 (6%)	446 (5%)	56 (12%)	18 (12%)	9 (9%)
HPV 51	114 (1%)	108 (1%)	3 (<1%)	1 (<1%)	1 (1%)
HPV 52	253 (3%)	252 (3%)	..	1 (<1%)	..
HPV 53	24 (<1%)	23 (<1%)	1 (<1%)	..	..
HPV 56	75 (<1%)	72 (<1%)	2 (<1%)	..	1 (1%)
HPV 58	203 (2%)	199 (2%)	..	3 (2%)	1 (1%)
HPV 59	95 (1%)	90 (1%)	3 (<1%)	2 (1%)	..
HPV 61	1 (<1%)	1 (<1%)	..	..	..
HPV 66	7 (<1%)	6 (<1%)	..	1 (<1%)	..
HPV 67	26 (<1%)	26 (<1%)	..	..	..
HPV 68	59 (<1%)	57 (<1%)	1 (<1%)	1 (<1%)	..
HPV 68‡	31 (<1%)	28 (<1%)	2 (<1%)	1 (<1%)	..
HPV 69	7 (<1%)	7 (<1%)	..	..	..
HPV 70	9 (<1%)	9 (<1%)	..	..	..
HPV 73	43 (<1%)	43 (<1%)	..	..	..
HPV 74	2 (<1%)	2 (<1%)	..	..	..
HPV 82	6 (<1%)	6 (<1%)	..	..	..
HPV 91	1 (<1%)	1 (<1%)	..	..	..
HPV undetermined	52 (<1%)	44 (<1%)	5 (1%)	3 (2%)	0

Data are number (%). *Includes undifferentiated, neuroendocrine, not otherwise specified, basal adenoid, and cystic adenoid carcinomas. †HPV type 39, 68, or 73. ‡HPV type 68 or73.

Table 3: **Human papillomavirus (HPV) genotypes in cases of invasive cervical cancer that were positive for HPV DNA, by histological diagnosis**

Discussion

We provide reference data for the distribution of HPV genotypes among women with invasive cervical cancer in 38 countries, and estimates of their relative contributions in five continents. The data confirm the universal contribution of the eight most common HPV types (16, 18, 31, 33, 35, 45, 52, and 58) to invasive cervical

cancer and the predominant role of types 16, 18, and 45 in cervical adenocarcinoma. We also provide data for the HPV types that are phylogenetically classified as oncogenic, such as HPV types 26, 30, 67, 69, and 82, but seldom described in epidemiological studies (panel).[12] Little is known about the exact mechanism of HPV-associated carcinogenesis of these rare types because of insufficient epidemiological evidence. The biological properties of the rare high-risk HPV types have only been investigated in a few studies, which included mostly lesions that were cervical intraepithelial neoplasms and a few cases of invasive cervical cancer.[16]

Our findings confirm that the detection of a single low-risk HPV type, mainly HPV 6, in invasive cervical cancer is a rare event (16 [<1%] of 8977). Other biological markers of viral activity in these specimens are also being studied to further document whether a low-risk type can indeed induce cervical cancer in rare circumstances. Invasive cervical cancers, irrespective of histological type, related to HPV types 16, 18, and 45 are diagnosed an average of 4 years earlier than are those caused by other high-risk HPV types.

HPV 16 and HPV 18 were the two most common types in all regions with a relative contribution of 71%, with the highest relative contributions in North America (79%) and Oceania (79%; table 2). For adenocarcinoma, the global relative contribution of HPV types 16 and 18 was 82% (table 3). Together, HPV types 16, 18, and 45 greatly increased the relative contribution in adenocarcinoma to 94%, showing the restricted genotype contribution in the pathogenesis of adenocarcinoma. The relative contribution of the eight most common HPV types in all world regions was 91% (table 2).

The low average age of women with invasive cervical cancer attributed to HPV 16 or HPV 18 has been noted in other studies.[8,10,17,18] Our findings show that cancers related to HPV 45 and HPV 18 arise at a much younger age (<50 years) than do other types and that this occurrence is universal. Vinokurova and colleagues[17] have reported that HPV types 16, 18, and 45 are more likely to be integrated into the human genome than are the other HPV types, and the tumours might present early. This finding is also consistent with the results of prospective studies that women with HPV 16 and HPV 18 infections at study entry had a much higher risk of developing high-grade cervical lesions within a short follow-up than did women infected with other HPV types.[19–21] HPV 45 is rare in women with normal cytology or low-grade lesions (0·4% and 3·7%, respectively) compared with HPV 16 (2·5% and 20%, respectively); however, it is consistently the third most common HPV type in invasive cervical cancer globally and in most of the regions.[22] The early presentation of cases of invasive cervical cancer that are positive for HPV 45 might be indicative of a short time for progression to invasive cancer, with or without transition through the preinvasive stages, perhaps related to a high early integration rate.[23] The under-representation of HPV 18 and HPV 45 noted in preneoplastic lesions in the follow-up data of the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study lend support to the early integration of these HPV types into the human genome.[24]

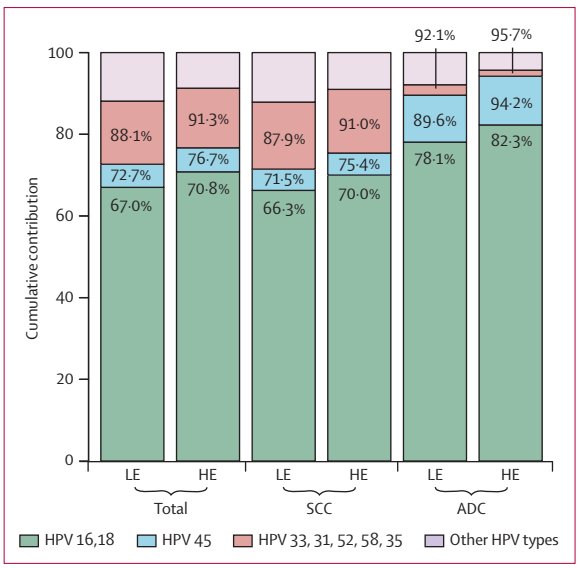

Figure 1: **Cumulative relative contribution of the eight most common human papillomavirus (HPV) types as single-type and multiple-type infections by histological category of invasive cervical cancer**
LE=lower estimate of type-specific contribution. HE=higher estimate of the type-specific contribution. SCC=squamous cell carcinoma. ADC=adenocarcinoma.

Whether massive screening had an effect on the HPV genotype distribution by age among women with invasive cervical cancer in developed countries is difficult to assess. The younger age of women presenting with invasive cervical cancers that are positive for HPV types 16, 18, and 45 was consistent across study regions with substantially different uptake of screening. This age variation is relevant for rationalisation of the newly proposed screening policies and patient management protocols by use of specific genotype information.[10,24,25] In our study, twice (4% [17 of 407]) as many women younger than 30 years had invasive cervical cancers that were positive for HPV 45 than did those with HPV types other than 16, 18, or 45 (2%, [28 of 1605]). Wheeler and colleagues[10] suggested that cervical screening could be delayed in HPV-vaccinated cohorts in the USA because of the late presentation of cases with HPV types that were not HPV 16 or HPV 18. Our findings of the early presentation of cases of invasive cervical cancer that were positive for HPV 45 suggest that this genotype should also be considered in type-specific screening protocols and that women who are positive should be offered increased surveillance that includes the endocervical zone, which is the site of most of the adenocarcinomas.

The carcinogenic mechanism of several HPV types is not known. Some types are rarely identified in human cancers—such as HPV 6 or HPV 11—but are common in

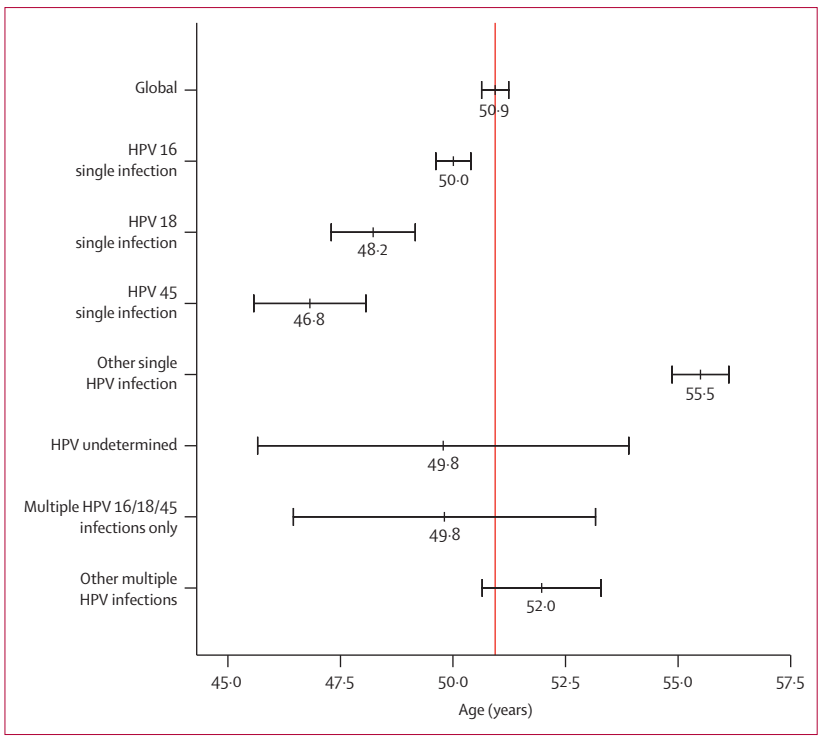

Figure 2: **Mean age (95% CIs) at diagnosis of invasive cervical cancer**
Data were adjusted for histological type of cancer, year of diagnosis, and geographical region. HPV=human papillomavirus.

the population. Further efforts are needed to understand in which conditions these HPV types might induce cancer. The low occurrence of other types—such as HPV 26—in tumour tissue might be attributable to intrinsic viral characteristics, lower prevalence of the virus than of other oncogenic types, or lack of adequate detection with existing HPV assays.

Panel: Research in context

Systematic review

Several meta-analyses of the human papillomavirus (HPV) genotype distribution in cervical cancer have been reported in the medical literature. However, these studies have major limitations in the variability of the sample selection and HPV assays. Furthermore, detailed composition of multiple infections are generally not available. In our study, we overcome these issues, by providing a common protocol for sample selection and individual testing using a common assay.

Interpretation

This work provides estimates of the important contribution of HPV types 16, 18, 31, 33, 35, 45, 52, and 58. These types should be considered when new vaccines with a wider efficacy range are developed. The early detection of tumours associated with HPV types 16, 18, and 45 should be considered in screening programmes aimed at clinical management on the basis of a HPV genotype.

Our study has some potential limitations. The cases included were obtained from pathology laboratories, some of which served as reference centres for the region or the country. Bias could arise in terms of overestimation of the prevalence of a particular HPV type if the centre or the local researcher selected cases on the basis of histological types (ie, adenocarcinomas or neuroendocrine tumours) that were attributable to specific HPV types. To reduce the risk of such an effect, we asked for all consecutive cases that were diagnosed in a defined period without any additional selection criteria. The good concordance in distribution of HPV types with previous country-specific reports suggests that the existence of major deviances in population representation is unlikely, particularly in those regions with large numbers of samples such as Asia, Europe, and Latin America (table 2). We identified a substantial proportion of cases that were HPV negative, particularly among women with adenocarcinoma (290 [38%] of 760), by contrast with the expectation of HPV as a recognised universal cause of cervical cancer. If the negative samples were attributable to a specific HPV type, the missing data for HPV type for these samples could then bias the overall distribution of the series. To assess the potential effect of this bias, a random sample of 200 samples that were negative for HPV DNA were assessed for DNA quality by use of different primers for β globin and β actin during PCR to generate different lengths of amplimers. Besides broad-spectrum PCR, we also did type-specific PCR to analyse 14 different high-risk HPV types. 60% of samples that were negative for HPV DNA were also negative for β globin and β actin. No signs of PCR inhibition were noted in the remaining 40% of HPV-negative samples after we spiked them with HPV DNA and did HPV-type specific tests, showing no difference in the HPV type distribution from the global series (data not shown). Additional assessment of the quality of these HPV-negative specimens and their processing, and the HPV testing methods indicated that low viral load, tissue degradation, and misdiagnosis of endometrial carcinomas could account for less than 10% of HPV-negative adenocarcinomas (data not shown). The use of other potential biomarkers for differentiation of endometrial and cervical adenocarcinomas was reviewed, and pilot exercises were done in laboratories with substantial previous experience. However, in the absence of validated assays, we concluded that HPV negativity was largely attributable to technical artifacts, which accords with previous interpretations.[3] Quantification of the effect of negative cases on estimates of the HPV-type distribution is difficult, particularly with old samples and laboratories that lack current technology. Accordingly, we calculated the type distribution as relative contributions by using the number of HPV-positive cases as the denominator. However, we cannot safely ignore the possibility that a small proportion of cases of invasive cervical cancer, perhaps in the group with adenocarcinomas, might arise independently of exposure. Because our study is a

prevalence survey, we cannot directly infer causality that has been extensively documented by use of other study designs and recognised by all major reviews.

The strengths of the study include the international network of collaborating centres, the use of a common protocol for collection of specimens, histological confirmation and classification, and HPV testing that was centralised in two laboratories with common protocols and parameters for quality control. Furthermore, highly sensitive assays were used for HPV detection when paraffin-embedded specimens were analysed. Robust statistical methods were used to adjust the prevalence rates of the variables that were previously reported to strongly affect the results. This international effort lends support to the results of previous studies and reinforces the rationale for prevention of cervical cancer through the use of existing vaccines. Our results show which HPV types should be given priority when the cross-protective effects of current vaccines are assessed, and are useful for formulation of recommendations about the use of second-generation polyvalent HPV vaccines. These findings suggest that type-specific high-risk HPV-DNA-based screening tests and protocols should focus on HPV types 16, 18, and 45.

Contributors

SDS, WGVQ, LA, BLL, CJLMM, XC, NM, and FXB participated in the study design, data collection and analysis, interpretation of the results, and writing the report. JEK participated in the study design, and analysis and interpretation of the results, and writing the report. DTG, ST, NG, OC, and MA participated in the analysis, interpretation of the results, and writing the report. AF, LEB, HRS, CSV, PADR, MAL, ALB, CCY, SAT, EK, EI, MO, RP, MS, MG, AU, AJ, GAHS, LEL, AB, CM, EJD, JV, AN, SCBC, YLQ, EL, SMG, TS, AF, DH, LM, AP, IS, EO, WFAJ, EC, TCW, AP, CLL, MT, TA, VGB, CC, JO, MA, AMN, and JB were involved in data collection, and interpretation of the results. GIS was involved in the study design, data collection, interpretation of the results, and final approval of the report. All authors provided approval of the final draft of the report.
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Advisory board: Thomas Iftner (Tübingen University, Tübingen, Germany), Chris J L M Meijer, Peter J F Snijders (Vrije Universiteit Medical Centre, Amsterdam, Netherlands), Massimo Tommasino (International Agency for Research on Cancer, Lyon, France), Janet Kornegay (Roche Diagnostics, San Francisco, CA, USA), Patti Gravitt (John Hopkins, Baltimore, MD, USA), Wim G V Quint (DDL Diagnostic Laboratory, Voorburg, Netherlands), Michael Pawlita (German Cancer Research Centre Deutsches Krebsforschungszentrum, Heidelberg, Germany).

Conflicts of interest

YLQ has received support for travel to meetings for the study or other purposes (personal) from MSD and GlaxoSmithKline. GAHS has received an independent grant from MSD for epidemiological study related to genital warts in Colombia; and payments from MSD for travel accommodation at the International HPV conference in Canada, 2011. NM has received payment for board membership (personal) from Merck, and for lectures (personal) from Merck and Sanofi Pasteur. AMN has received payment for board membership (personal) and consultancy (personal) from GlaxoSmithKline, and for lectures (personal) from GlaxoSmithKline and MSD. CJLMM has received payment for board membership (institution) from Qiagen until 2009; payment for consultancy from Qiagen (institution); payment for lectures including service on speakers bureaus (institution) from Roche and GlaxoSmithKline; payment for patents (institution) from Qiagen for HPV detection by gp5+/6+; royalties (institution) from Oncomethylome Sciences for a patent on promoter methylation of Technological Support for Strategy, Learning and Change specific marker for cervical intraepithelial neoplasia 2+2005; and travel, accommodations, or meeting expenses (personal) from Qiagen and Roche. TCW has received payment for consultancy (personal) from Merck, GlaxoSmithKline, GenProbe, and Roche Molecular Diagnostics; and for lectures including service on speakers bureaus (personal) from Merck, GlaxoSmithKline, and Roche Molecular Diagnostics. SDS has received payment for consultancy (personal) from Qiagen, and for lectures including service on speakers bureaus (personal) from Qiagen, Sanofi, and GlaxoSmithKline. LA has received support for travel to meetings for the study or other purposes (institutional) from Sanofi Pasteur MSD. NG has received institutional support for occasional travel by GlaxoSmithKline and Merck. FXB has received payment for consultancy (personal) from MSD Internal Steering Committee; payment for expert testimony (personal) from GlaxoSmithKline Food and Drug Administration and EMEA clinical expert; and grants (institution) from GlaxoSmithKline, MSD, and Sanofi Pasteur MSD for epidemiological studies; payment for development of educational presentations (institution) from GlaxoSmithKline; travel, accommodations, and meeting expenses (personal) from GlaxoSmithKline and Sanofi Pasteur MSD. JB has received a grant (institution) from MSD Israel for a research assistant working with the pathology laboratory staff, assisting in the assembly and transport of paraffin blocks; consultancy (personal) payments from MSD Israel; and payment for lectures, including service on speakers bureaus (personal) from GlaxoSmithKline Israel. GIS has received a study grant (institution) from Marató de TV3 Foundation, and grants (institution) from Merck. XC has received a study grant (institution) from Sanofi Pasteur MSD and Merck; consultancy payments from Sanofi Pasteur MSD, and GlaxoSmithKline; grants (institution) from GlaxoSmithKline, Merck, Sanofi Pasteur MSD; and payment for lectures including service on speakers bureaus (personal) from GlaxoSmithKline and Sanofi Pasteur MSD. BL has received payment for lectures including service on speakers bureaus (personal) from Roche and Qiagen. SAT has received payment for lectures including service on speakers bureaus from MSD; and travel, accommodations, or meeting expenses from Merck. SMG has received board membership from GlaxoSmithKline, Commonwealth Serum Laboratories advisory board for cervical cancer vaccine; payment for development of education presentations from GlaxoSmithKline, EXCEL program; grants have been paid to SMG's institute by the Royal Women's Hospital Melbourne. CC has received payment for consultancy from Merck (steering committee about HPV vaccination), expert testimony from Roche, and funding for travel from Sanofi Pasteur, MSD, and GlaxoSmithKline. CLL has received institutional grants from GlaxoSmithKline. The other authors declared no conflicts of interest.

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