Human papillomavirus types in 115,789 HPV-positive women: A meta-analysis from cervical infection to cancer

Peng Guan1,2, Rebecca Howell-Jones1,3, Ni Li1,4, Laia Bruni5, Silvia de Sanjosé5, Silvia Franceschi1 and Gary M. Clifford1

1 International Agency for Research on Cancer, Lyon, France
2 China Medical University, Shenyang, China
3 Health Protection Agency, London, United Kingdom
4 Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China
5 Institut Catalan d’Oncologia, Barcelona, Spain

Genotyping may improve risk stratification of high-risk (HR) human papillomavirus (HPV)-positive women in cervical screening programs; however, prospective data comparing the natural history and carcinogenic potential of individual HR types remain limited. A meta-analysis of cross-sectional HR HPV-type distribution in 115,789 HPV-positive women was performed, including 33,154 normal cytology, 6,810 atypical squamous cells of undetermined significance (ASCUS), 13,480 low-grade squamous intraepithelial lesions (LSIL) and 6,616 high-grade SIL (HSIL) diagnosed cytologically, 8,106 cervical intraepithelial neoplasia grade 1 (CIN1), 4,068 CIN2 and 10,753 CIN3 diagnosed histologically and 36,374 invasive cervical cancers (ICCs) from 423 PCR-based studies worldwide. No strong differences in HPV-type distribution were apparent between normal cytology, ASCUS, LSIL or CIN1. However, HPV16 positivity increased steeply from normal/ASCUS/LSIL/CIN1 (20–28%), through CIN2/HSIL (40/47%) to CIN3/ICC (58/63%). HPV16, 18 and 45 accounted for a greater or equal proportion of HPV infections in ICC compared to normal cytology (ICC:normal ratios = 3.07, 1.87 and 1.10, respectively) and to CIN3 (ICC:CIN3 ratios = 1.08, 2.11 and 1.47, respectively). Other HR types accounted for important proportions of HPV-positive CIN2 and CIN3, but their contribution dropped in ICC, with ICC:normal ratios ranging from 0.94 for HPV33 down to 0.16 for HPV51. ICC:normal ratios were particularly high for HPV45 in Africa (1.85) and South/Central America (1.79) and for HPV58 in Eastern Asia (1.36). ASCUS and LSIL appear proxies of HPV infection rather than cancer precursors, and even CIN3 is not entirely representative of the types causing ICC. HPV16 in particular, but also HPV18 and 45, warrant special attention in HPV-based screening programs.

Thirteen human papillomavirus (HPV) genotypes have been judged to be carcinogenic or probably carcinogenic,1 hereafter referred to as high-risk (HR) types, and are the cause of virtually all invasive cervical cancers (ICCs) worldwide.2 This has led to the development of screening tests that detect HR types as a group, an approach which is more sensitive than cytology in primary screening and offers a longer negative predictive value for cervical intraepithelial neoplasia grade 3 (CIN3) and cervical cancers.3–8 Nevertheless, it is recognized that individual HR types differ enormously in their relative carcinogenic potential.3 Thus, new screening tests are emerging to provide some degree of genotyping with the aim to improve risk stratification among HPV-positive subjects and to allow adapted follow-up protocols. However, the use of distinguishing individual HPV types beyond HPV16 remains ill-defined due to the limited prospective evidence on cervical cancer risk for individual HR types, which, in addition, may vary by geographical region.

Key words: human papillomavirus, cervical cancer, precancerous cervical lesions, genotype, epidemiology, meta-analysis

Abbreviations: ADC: adeno/adenosquamous cell carcinoma; ASCUS: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; HR: high risk; HSIL: high-grade squamous intraepithelial lesions; ICC: invasive cervical cancers; LSIL: low-grade squamous intraepithelial lesions; SCC: squamous cell carcinoma

Drs. Clifford, Franceschi, Guan, Howell-Jones and Li have no conflict of interest to declare. Drs. Bruni and de Sanjosé were occasional recipients of travel grants from Sanofi and GSK, and Dr. de Sanjosé also from Qiagen. Their unit has unrestricted grants from GSK, Merck and Sanofi.

Grant sponsor: Bill and Melinda Gates Foundation; Grant number: 35537; Grant sponsors: International Agency for Research on Cancer; Fondation Innovations en Infectiologie (FINOVI); Department of Health, United Kingdom.

DOI: 10.1002/ijc.27485

History: Received 6 Dec 2011; Accepted 27 Jan 2012; Online 9 Feb 2012

Correspondence to: Gary M. Clifford, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon, Cedex 08, France, Tel.: +33-47-273-8425, Fax: +33-47-273-8345, E-mail: clifford@iarc.fr

Int. J. Cancer: 131, 2349–2359 (2012) © 2012 UICC
With the aim to improve our understanding of the complete carcinogenic process for individual HR types from infection to cervical cancer, we have performed a systematic literature review and meta-analysis of type distribution in more than 100,000 HPV-positive women across the full spectrum of cytopathological and histopathological cervical diagnoses from normal cytology to ICC. This builds on previous meta-analyses of type-specific HPV prevalence by specific cervical disease grade.2,12–16

Material and Methods

Methods have been previously reported for meta-analyses focusing on specific cervical lesion grades.2,12–16 No meta-analysis including atypical squamous cells of undetermined significance (ASCUS) has previously been published. In brief, Medline was used to search for publications from January 1990 to November 2011 using combinations of the MeSH terms: “cervical cancer,” “cervical intraepithelial neoplasia,” “HPV,” “human,” “female” and “polymerase chain reaction.” References cited in retrieved articles were also evaluated. Eligible studies met the following criteria: (i) use of broad-spectrum consensus PCR assays based on the primers MY09/11, PGMY09/11, GP5+/6+, SPF10, SPF1/GP6+ or L1C1/L1C2, and (ii) reporting of overall and type-specific HPV prevalence by strata of cytopathological and/or histopathological cervical diagnoses (see definitions below).

For each included study, the following data were extracted by cervical diagnosis: country, source of HPV DNA (cells versus biopsies/tissue), PCR primers, sample size and overall and type-specific prevalence of HPV DNA. Subjects were classified into seven geographical regions (Africa, Eastern Asia, Western/Central Asia, Europe, North America, South/Central America and Oceania). If study methods suggested that additional relevant information was available (e.g., additional HPV types and/or more detailed stratification by cervical diagnosis), data requests were made to authors. For a subset of studies reporting such data, the overall prevalence of multiple infections (which may also include low-risk HPV types) was also extracted.

Cases were classified into eight grades of cervical diagnosis: those diagnosed by cytology as (i) normal; (ii) ASCUS; (iii) low-grade squamous intraepithelial lesion (LSIL) or (iv) high-grade squamous intraepithelial lesion (HSIL); those diagnosed by histology as (v) CIN1; (vi) CIN2 or (vii) CIN3 (including squamous carcinoma in situ) and those diagnosed as (viii) ICC, which refers to squamous cell carcinoma (SCC), adeno/adenosquamous carcinoma (ADC) or cervical cancer of other/unspecified histology.

To retain appropriate sample size in each category, cervical diagnoses were further collapsed into four categories for comparisons by geographical region: (i) normal, including normal cytology only; (ii) low-grade, encompassing ASCUS, LSIL and CIN1; (iii) high-grade, including HSIL, CIN2 and CIN3 and (iv) ICC.

Overall HPV DNA prevalence is reported as a percentage of all women tested by consensus PCR. Type-specific HPV positivity is presented as the proportion of HPV-positive cases in which the particular HPV type was detected, and for the 13 HR HPV types judged to be carcinogenic or probably carcinogenic (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) in order of their frequency of detection in ICC.2 This is somewhat different from previous meta-analyses2,12–16 that have focused on type-specific prevalence among all women tested for a given HPV type. Each HPV type was evaluated independently. Type-specific positivity was estimated only among those studies that both genotyped and reported the prevalence of the HPV type in question, and thus, denominators vary by type (Tables A1 and A2). Type-specific positivity includes that contributed by multiple HPV infections. Standard errors of type-specific positivity estimates were calculated assuming the nonindependence of cases within the same study using cluster-correlated robust variance estimates.17

Type-specific positivity was compared between HPV-positive normal cytology and CIN3 with that in HPV-positive ICC by ratios. ICC:CIN3 ratios were also assessed separately by the source of HPV DNA in CIN3 (i.e., exfoliated cells versus biopsies/tissue).

Results

Four hundred and twenty-three studies met eligibility criteria, including a total of 369,186 eligible women. Their distribution by grade of cervical disease and geographical region is given in Table 1. Women with normal cytology contributed the greatest number of samples (n = 266,611; 72%), followed by ICC (n = 40,679; 11%). Women with ASCUS (4%), LSIL (5%), HSIL (2%), CIN1 (3%), CIN2 (1%) and CIN3 (3%) each contributed smaller fractions. Within each cervical diagnosis grade, studies conducted in Europe contributed the largest sample, but Eastern Asia, North America and South/Central America were also well represented. Western/Central Asia and Africa were poorly represented among all CIN grades. No women with ASCUS diagnoses were available from Oceania.

A total of 115,789 included women were HPV-positive. Overall HPV prevalence increased with increasing severity of cervical disease from 12% in normal cytology to 89% in ICC (Table 1). Figure 1 shows overall HPV prevalence by cervical disease grade and region. Among women with normal cytology, HPV prevalence varied substantially by region, ranging from 8–9% in Western/Central Asia and Europe to more than 20% in Africa, North America, South/Central America and Oceania. Differences in HPV positivity by region were large for most cytopathological and histological categories. Overall HPV prevalence was only consistent (about 90%) for ICC across all regions (Table 1 and Fig. 1).
The proportion of HPV-positive women in which HPV16, 18 and 45 were detected is shown by cervical disease grade in Figure 2. HPV16 was the most frequently detected HR type in every grade. HPV16 positivity varied little across normal cytology (20.4% to 3.6%), ASCUS (22.9% to 2.9%) and LSIL (25.1% to 2.8%), but increased substantially in HSIL (47.5% to 5.5%). HPV16 positivity in CIN1 (27.6% to 4.3%) was similar to that for LSIL, but increased through CIN2 (39.8% to 5.0%) and CIN3 (58.2% to 4.1%) to reach 62.6% in ICC.

Figure 3 shows the positivity, as a proportion of HPV-positive samples, for the next five most frequent HR types in ICC, namely HPV31, 33, 35, 52 and 58, by cervical disease grade. ICC:normal ratios ranged from 0.94 for HPV33 down to 0.44 for HPV52. In addition, each of these types was more frequent in HSIL and CIN2 in comparison to normal cytology (3.5% to 0.7%, respectively). Figure 4. shows the positivity, as a proportion of HPV-positive samples, for the five most frequent HR types in ICC, namely HPV31, 33, 35, 52 and 58, by cervical disease grade. ICC:normal ratios were also the only HPV types not to be less frequently detected in ICC than in CIN3 (3.4% to 0.7%, respectively).

Table 1. Distribution of studies, women and human papillomavirus (HPV) DNA prevalence by cervical disease grade and region

| Region                  | Total (studies = 423) | Normal cytology (studies = 147) | ASCUS (studies = 66) | LSIL (studies = 99) | HSIL (studies = 87) | CIN1 (studies = 81) | CIN2 (studies = 66) | CIN3 (studies = 81) | ICC (studies = 229) |
|-------------------------|-----------------------|---------------------------------|----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                         | Tested, HPV, N        | Tested, HPV, n (%)              | Tested, HPV, N (%)   | Tested, HPV, N (%)  | Tested, HPV, N (%)  | Tested, HPV, N (%)  | Tested, HPV, N (%)  | Tested, HPV, N (%)  | Tested, HPV, N (%)  |
| Africa (studies = 35)   | 14,256                | 5,391                           | 10,174               | 2,221 (22)          | 567                 | 198 (35)           | 493                 | 299 (61)           | 245                 |
| Eastern Asia (studies = 97) | 69,909               | 22,466                          | 50,863               | 6,313 (12)          | 988                 | 98 (50)            | 944                 | 704 (25)           | 1,093 (21)         |
| Western/Central Asia (studies = 30) | 14,834              | 3,369                           | 11,499               | 977 (8)             | 300                 | 120 (40)           | 127                 | 102 (80)           | 84 (40)            |
| Europe (studies = 144)  | 197,263               | 47,116                          | 154,782              | 14,636 (9)          | 8,137               | 4,013 (49)         | 10,908              | 7,971 (73)         | 3,345 (84)         |
| North America (studies = 54) | 29,390               | 15,684                          | 14,500               | 3,057 (21)          | 2,440               | 1,757 (72)         | 3,185               | 2,653 (83)         | 1,668 (6)          |
| South/Central America (studies = 69) | 35,895              | 16,277                          | 22,622               | 5,201 (24)          | 941                 | 424 (45)           | 1,909               | 1,550 (81)         | 734                 |
| Oceania (studies = 9)   | 6,145                 | 2,845                           | 2,271                | 749 (33)            | 0                   | –                  | 239                 | 198 (83)           | 47 (86)            |
| Overall (studies = 423) | 369,186               | 115,789                         | 266,611              | 33,154 (12)         | 12,983              | 6,810 (52)         | 17,805              | 13,480 (76)        | 7,743               |

1For ICC, individual regions do not sum to the total due to a multinational study for which it was not possible to separate Eastern from Western/Central Asia (N = 2,994).

Abbreviations: ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia grade; ICC: invasive cervical cancer.
Figure 3b shows the positivity, as a proportion of HPV-positive samples, for the five least frequent HR types in ICC, namely HPV39, 51, 56, 59 and 68. Each of these types was less frequent in ICC than normal cytology, with ICC:normal ratios ranging from 0.41 for HPV59 down to 0.16 for HPV51. ICC:CIN3 ratios ranged from 0.58 for HPV59 down to 0.20 for HPV51.

HPV type-specific positivity is shown stratified by region in Figure 4, using four grades of cervical disease. The increase in HPV16 positivity with increasing lesion severity was similar in all regions, with ICC:normal ratios ranging from 2.33 in North America to 4.07 in Africa. However, in each cervical disease grade, HPV16 positivity was highest in Western/Central Asia and lowest in Africa. The increase in HPV18 positivity between normal cytology and ICC was seen across all regions (ICC:normal ratios between 1.73 and 2.56), with the biggest increases being between high-grade disease and ICC. HPV45 showed ICC:normal ratios between 0.78 in Europe up to 1.79 and 1.85 in South/Central America and Africa, respectively. HPV45 positivity was low in every cervical disease grade in Eastern Asia. For HPV33, ICC:normal ratios varied by region, from 0.44 to 1.25. For HPV58, there was a particularly high positivity in Eastern Asia across all cervical disease grades and a relatively elevated ICC:normal ratio (1.36) that was not apparent in other regions (0.12–0.83). The relative decrease of HPV58 from high-grade disease to ICC was, however, consistent in Eastern Asia and elsewhere. For HPV31, ICC:normal ratios ranged between 0.32 and 1.06 by region. HPV52 positivity also varied considerably by region, with highest levels in Eastern Asia, but ICC:normal ratios were consistent (0.17–0.67). For HPV35, ICC:normal ratios varied by region (0.36–1.13), and positivity was highest in Africa, including in ICC (4.9% ± 1.8%).

A subset of studies reported overall prevalence of multiple HPV infections (data not shown), which accounted for 31–41% of HPV infections in normal, ASCUS, LSIL and HSIL (among which HPV was almost entirely tested from cells) versus 12% in ICC (irrespective of whether HPV was tested from cells or biopsies/tissue). For CIN1, CIN2 and CIN3, however, the proportion of multiple infections appeared higher when HPV was tested from cells (52, 51 and 32%, respectively) than from biopsies/tissue (21, 18 and 16%, respectively). Hence, ICC:CIN3 ratios were calculated separately for CIN3 tested from cells and biopsies/tissue (Table 2). This sensitivity analysis additionally excluded studies from Africa, Western/Central Asia and South/Central America as each contributed less than 100 HPV-positive CIN3. ICC: CIN3 ratios for most HR HPV types were materially unchanged, except for those of types in the alpha-7 species (HPV18, 45, 39 and 59), which were higher in the analysis including CIN3 tested from biopsies/tissue than cells (Table 2). ICC:CIN3 ratios from biopsies/tissue were also consistent when additionally restricted to SCC only (e.g., SCC:CIN3 ratios = 2.10 for HPV45 and 0.36 for HPV31).

**Discussion**

Our study is the first to analyze worldwide data on HPV types across the complete spectrum of cervical disease. The objective was to compare the cross-sectional distribution of HR HPV types as a proxy for their relative potential to cause ICC. For the most frequent HR types, we also compared type-distribution patterns across world regions. Although this approach cannot estimate absolute type-specific risks, it is nevertheless useful to identify variations in the importance of different HPV types by severity of lesions. Such information is of practical use for identifying types, or groups of types, that are particularly important in different regions. For example, HPV16 and HPV18 are the most frequent types in most regions, except in Eastern Asia where HPV45 and HPV58 are more prevalent. Additionally, the positivity of HPV45 in ICC is particularly high in Western/Central Asia and Africa, whereas HPV31 and HPV52 are more prevalent in Eastern Asia. These findings highlight the importance of understanding the distribution of HPV types in different regions to inform public health strategies and target interventions. The analysis also underscores the potential role of HPV16 and HPV18 in the development of ICC, particularly in regions with lower positivity for these types. The data further support the need for regionalized approaches to HPV screening and vaccination programs, taking into account the local prevalence of HPV types and the associated risk of ICC.

Figure 2. Positivity (±1.96 SE) for human papillomavirus (HPV) types 16, 18 and 45 as a proportion of HPV-positive samples by cervical disease grade. Abbreviations: ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia grade; ICC: invasive cervical cancer; SE: standard error, calculated as described in Material and Methods section.
that differ largely in their carcinogenic potential and could merit differential management in HPV-based screening programs. We judged the ratio of positivity in HPV-positive ICC to that in HPV-positive normal cytology to be particularly informative as (i) the available data cluster at these two extremes of the disease spectrum, and (ii) this measure captures the complete carcinogenic process and avoids variations due to different quality standards of cytology and histology for intermediate lesions. However, the ratio of positivity in HPV-positive ICC to that in HPV-positive CIN3 is also helpful for interpreting how the results of screening and vaccination studies that rely on CIN3 as the worst outcome are relevant to ICC prevention.

The steady rise in HPV16 positivity through the classification of cervical disease from normal cytology to ICC in all world regions confirms results from cohort studies showing an increased long-term risk for CIN3\textsuperscript{18–21} and ICC\textsuperscript{22} following infection with HPV16. Although there was regional heterogeneity in the fraction of lesions positive for HPV16, HPV16 nevertheless remains the cause of more than half of ICC in all world regions.

HPV18 and HPV45 were also enriched, or similarly represented, in ICC compared to lower grades of diagnosis, suggesting a higher carcinogenic potential relative to all other non-HPV16 types. This corroborates the higher risk for developing CIN3 reported for HPV18 in prospective studies.\textsuperscript{19–21} However, the relative importance of HPV18 and HPV45 increased even more between CIN3 and ICC, suggesting that studies using CIN3 as the principal outcome underestimate the relative carcinogenic potential of HPV18

Figure 3. Positivity ($\pm 1.96 \text{ SE}$) for human papillomavirus (HPV) types (a) 33, 58, 31, 52 and 35 and (b) HPV types 39, 59, 51, 56, 68 as a proportion of HPV-positive samples by cervical disease grade. Abbreviations: ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia grade; ICC: invasive cervical cancer; SE: standard error, calculated as described in Material and Methods section.
and 45 relative to other HR types. In addition, the closely related types HPV39 and HPV59 also showed some evidence of under-representation in CIN3 compared to ICC in a sensitivity analysis restricted to CIN3 tested for HPV from biopsies/tissue. Of note, HPV18, 45 and other types in the alpha-7 species have a particular propensity for endocervical

**Figure 4.** Positivity (±1.96 SE) for human papillomavirus (HPV) types 16, 18, 45, 33, 58, 31, 52 and 35 as a proportion of HPV-positive samples by cervical disease grade* and region†. *Low- and high-grade include diagnoses by cytology and/or histology (see Material and Methods section). †Graphs are not shown for regions with less than 100 women in any cervical disease grade, namely Western/Central Asia. #Standard error not calculable for 20 high-grades tested for HPV52 (0 positive) in W/C Asia. Abbreviations: ICC: invasive cervical cancer; W/C Asia: Western/Central Asia; S/C America: South/Central America; SE: standard error, calculated as described in Material and Methods section.
Table 2. Positivity for high-risk human papillomavirus (HPV) types in HPV-positive invasive cervical cancer (ICC) and histologically confirmed cervical intraepithelial neoplasia 3 (CIN3) by source of DNA used for HPV testing (cells versus biopsies/tissue)\(^1\)

| HPV type | Cells | % positive (±1.96 SE) | Biopsies/tissue | % positive (±1.96 SE) | ICC | % positive (±1.96 SE) | CIN3 cells | CIN3 biopsies/tissue |
|----------|-------|----------------------|----------------|----------------------|-----|----------------------|------------|----------------------|
| HPV16    | 7,634 | 59.3 ± 3.0           | 2,160          | 54.5 ± 15.7          | 22,677 | 64.7 ± 3.6          | 1.08       | 1.17                 |
| HPV18    | 7,595 | 7.8 ± 1.3            | 2,115          | 4.9 ± 1.6            | 22,725 | 16.5 ± 2.9          | 2.13       | 3.39                 |
| HPV45    | 6,603 | 3.9 ± 1.2            | 1,903          | 1.7 ± 0.7            | 17,221 | 4.3 ± 0.7           | 1.06       | 2.39                 |
| HPV33    | 7,388 | 9.0 ± 1.1            | 2,015          | 11.0 ± 3.2           | 20,203 | 5.1 ± 1.3           | 0.55       | 0.45                 |
| HPV58    | 6,944 | 8.4 ± 2.7            | 1,928          | 10.8 ± 8.6           | 18,793 | 5.5 ± 3.9           | 0.66       | 0.51                 |
| HPV31    | 7,388 | 12.1 ± 1.8           | 2,039          | 10.7 ± 3.1           | 19,595 | 3.5 ± 0.6           | 0.30       | 0.34                 |
| HPV52    | 6,927 | 10.5 ± 3.7           | 1,926          | 10.9 ± 7.4           | 18,493 | 3.7 ± 1.6           | 0.37       | 0.36                 |
| HPV35    | 6,758 | 3.6 ± 1.4            | 1,929          | 3.1 ± 1.0            | 17,357 | 1.2 ± 0.4           | 0.37       | 0.43                 |
| HPV39    | 6,564 | 4.0 ± 1.9            | 1,826          | 1.5 ± 0.8            | 16,507 | 1.5 ± 0.6           | 0.37       | 0.98                 |
| HPV59    | 6,564 | 2.7 ± 1.3            | 1,690          | 0.8 ± 1.0            | 17,171 | 1.3 ± 0.5           | 0.52       | 1.67                 |
| HPV51    | 6,630 | 6.2 ± 2.1            | 1,827          | 3.5 ± 2.5            | 16,234 | 0.8 ± 0.3           | 0.14       | 0.24                 |
| HPV56    | 6,800 | 2.9 ± 1.3            | 1,827          | 1.5 ± 0.8            | 15,986 | 0.9 ± 0.3           | 0.30       | 0.56                 |
| HPV68    | 5,020 | 2.4 ± 1.1            | 1,707          | 1.1 ± 1.0            | 14,246 | 0.6 ± 0.3           | 0.25       | 0.54                 |
| Multiple HPV\(^2\) | 4,810 | 31.5 ± 10.7 | 896            | 15.8 ± 7.1           | 16,096 | 11.9 ± 2.8           | 0.38       | 0.75                 |

\(^1\)Studies from Africa, Western/Central Asia and South/Central America excluded due to inadequate numbers of CIN3. \(^2\)Overall prevalence of multiple infections was available only for a subset of studies and may include low-risk types.

Abbreviation: SE: standard error, calculated as described in Material and Methods section.

Glandular lesions and cervical adenocarcinoma (ADC),\(^{23}\) which are known to be less efficiently detected and prevented by cytological screening.\(^{5,6,24}\) Nevertheless, ADC constituted less than 10% of all ICCs in the present analysis (and only 3% of African ICC, where the enrichment of HPV45 in ICC was the strongest) so that the ICC:normal ratios in this meta-analysis are driven by findings for SCC.

HPV31, 33 and 58 have each been reported to confer higher absolute risks for CIN3 than other non-HPV16 HR types.\(^{19–22,25,26}\) This meta-analysis confirmed these findings, showing strong enrichment of these types between normal cytology and CIN3, a pattern that was seen also for HPV52 and HPV35. However, the relative importance of these types dropped between CIN3 and ICC, an effect that was robust in a sensitivity analysis restricted to ICC with HPV tested from biopsies/tissue. This suggests that their relative carcinogenic potential in comparison to HPV16/18/45 might be overestimated based on CIN3. Of note, the ICC:normal ratio for HPV33 was consistently higher than for other non-HPV16/18/45 types and was even slightly higher than HPV45 in some regions.

The pattern for HPV58 was different in Eastern Asia than elsewhere. Not only was the HPV58 proportion consistently elevated across all cervical disease grades, accounting for 10.2% ± 3.9% of all HPV-positive ICC, but also the ICC:normal ratio was elevated in comparison to other regions. The ICC:CIN3 ratio for HPV58, however, remained similar as for other regions. A recent prospective study in Taiwan suggested that the long-term risk for ICC was higher for HPV58 than other non-HPV16 types.\(^{22}\) Conversely, HPV45 appeared to be relatively rare in Eastern Asian populations, but especially frequent in ICC in Africa (11.0% ± 2.2%).

HR types found in <2% of ICC (HPV35, 39, 59, 51, 56 and 68) were present in an important proportion of low- and high-grade lesions; however, low ICC:normal ratios suggest that such types have relatively low carcinogenic potential.

No clear difference was evident in HR-type distribution between HPV-positive normal cytology, ASCUS or LSIL (or even with CIN1), most clearly seen in the lack of enrichment of HPV16. This suggests that the cytological diagnoses of ASCUS or LSIL have limited utility for risk-stratification of HPV-positive women and, together with CIN1, are predominantly proxies for HPV infection rather than true cancer precursors.\(^{27}\) Indeed, a diagnosis of ASCUS and/or LSIL has been shown to add little long-term risk stratification for CIN3 in comparison to HR HPV testing.\(^{8,19}\) The HPV-type distribution among CIN2 was in between that of normal/ASCUS/LSIL and ICC, with an important over-representation of non-16/18/45 HR types in comparison to ICC. This supports other findings that HPV16-positive CIN2 is more likely to indicate a true precancerous state that non-HPV16-positive CIN2.\(^{27}\)
The major strength of our present meta-analysis is the assessment of 369,186 women from all world regions of whom 31.4% were HPV-positive. The detailed stratification by cytological and histological diagnoses is also an innovative contribution to the knowledge of HPV natural history. Although the large numbers involved make random variations of little concern, they cannot, however, rule out systematic biases. General limitations associated with such cross-sectional meta-analyses include the variation in PCR-based HPV detection protocols, age ranges and quality of diagnosis and cervical screening practices across included countries and studies. All these potential problems are mitigated, however, by restriction of HPV type-specific comparisons to women testing HPV-positive with a limited number of broad-spectrum consensus PCR primers. Although differences in type-specific detection have been reported even for these primers and are also sensitive to post DNA-amplification genotyping protocols, adjustment of ICC: normal ratios for PCR primers did not materially affect main findings (data not shown).

Lastly, because of the lack of individual-level data on the type-specific breakdown of multiple infections, type-specific estimates include types present in multiple HPV infections. However, this is not expected to systematically bias relative differences between HR HPV types in our current approach, as all types are treated equally. Indeed, on restriction to CIN3 tested from biopsies/tissue only, which has the effect as all types are treated equally. Indeed, on restriction to differences between HR HPV types in our current approach, type-specific breakdown of multiple infections, type-specific shown).

3. Bulkmans N, Berkhof J, Rozendaal L, van

References

1. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Gona N, Freeman C, Galilich L, Cogliano V. A review of human carcinogens, Part B: Biological agents. Lancet Oncol 2009;10:321–2.

2. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM. Human papillomavirus type distribution in 30,484 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. Int J Cancer 2011;128:927–35.

3. Bulkmans N, Berkhof J, Rozendaal L, van Kemnade FJ, Boele A, Bulk S, Voorhorst F, Verheijen R, van Groningen K, Boon M, Ruitinga W, van Ballegooijen M, et al. Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. Lancet 2007;370:1764–72.

4. Naulec P, Ryd W, Tornberg S, Strand A, Wadell G, Elgren K, Radberg T, Strander B, Forslund O, Hansson BG, Hagmar B, Johansson B, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst 2009;101:88–99.

5. Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Palma PD, Del Mistro A, Ghirotto B, Girlando S, Gillio-Tos A, De Marco L, Naldoni C, Pierotti P, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol 2010;11:249–57.

6. Catki HA, Kinney WK, Fetterman B, Lorey T, Poitras NE, Cheung L, Doherty F, Schiffman M, Wacholder S, Castle PF. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice. Lancet Oncol 2011;12:663–72.

7. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Mwonge R, Budukh AM, Hingmire S, Malvi SG, Thorat R, Kohari A, Chinoy R, Kelkar R, et al. HPV screening for cervical cancer in rural India. N Engl J Med 2009;360:1385–94.

8. Castle PE, Stoler MH, Wright TC Jr, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol 2011;12:880–90.

9. Einstein MH, Martens MG, Garcia FA, Ferris DG, Mitchell AI, Day SP, Olson MC. Clinical validation of the Cervista HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. Gynecol Oncol 2010;118:116–22.

10. Carozzi FM, Burroni E, Bisanzi S, Puliti D, Confortini M, Giorgi RP, Sani C, Scaliisi A, Chini F. Comparison of clinical performance of Abbott RealTime High Risk HPV test with that of hybrid capture 2 assay in a screening setting. J Clin Microbiol 2011;49:1446–51.

11. Schweizer J, Lu PS, Mahoney CW, Berard-Bergery M, Ho M, Ramasamy V, Silver JE, Bish A, Labiad Y, Peck RB, Lom J, Jerosnin J, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. J Clin Microbiol 2010;48:4646–8.

12. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.

Acknowledgements

Peng Guan and Ni Li, while at the International Agency for Research on Cancer (IARC) (where the major part of this work was performed), were both supported by the IARC International Postdoctoral Fellowships, and Rebecca Howell-Jones by a postdoctoral fellowship from Fondation Innovations en Infectiologie (FINOVIC). Rebecca Howell-Jones, while at the Health Protection Agency, was also funded from the Department of Health, United Kingdom. The authors are grateful to Prof. Joakim Dillner for critical comments to the manuscript, and to Veronique Chabanis for technical assistance. They also thank those authors who made additional data available from their published studies.

References
Appendices

Table A1. Number of included HPV-positive women tested for (N) and positive for (n) individual HR HPV types by cervical disease grade

| Normal | ASCUS | LSIL | HSIL | CIN1 | CIN2 | CIN3 | ICC |
|--------|-------|------|------|------|------|------|-----|
| N      | n     | N    | n    | N    | n    | N    | N   |
| HPV16  | 33,154| 6,767| 6,810| 1,562| 13,480| 3,387| 6,616| 3,142| 8,106| 2,235|
| HPV18  | 32,964| 2,787| 6,795| 614  | 13,478| 1,168| 6,606| 636  | 8,058| 729  |
| HPV45  | 30,359| 1,462| 5,819| 338  | 11,466| 492  | 5,093| 230  | 5,984| 251  |
| HPV33  | 31,121| 1,476| 6,152| 366  | 12,874| 784  | 6,383| 536  | 7,557| 461  |
| HPV56  | 30,961| 1,959| 5,955| 463  | 11,596| 824  | 5,290| 417  | 6,167| 594  |
| HPV31  | 31,130| 2,490| 6,119| 577  | 12,827| 1,217| 6,302| 694  | 7,515| 852  |
| HPV52  | 29,660| 2,386| 5,538| 559  | 10,460| 849  | 4,664| 446  | 5,982| 826  |
| HPV35  | 29,279| 985  | 5,439| 306  | 10,499| 493  | 4,868| 274  | 7,268| 298  |
| HPV59  | 29,260| 1,521| 5,601| 430  | 10,455| 706  | 4,552| 189  | 5,781| 395  |
| HPV58  | 29,163| 932  | 5,302| 306  | 10,204| 539  | 4,685| 136  | 5,713| 294  |
| HPV51  | 28,259| 1,994| 5,692| 501  | 10,110| 1,190| 4,799| 321  | 5,887| 636  |
| HPV56  | 29,485| 1,535| 5,690| 365  | 10,474| 898  | 4,622| 158  | 5,811| 465  |
| HPV68  | 28,192| 869  | 5,004| 171  | 9,512| 257  | 4,231| 85   | 5,836| 191  |

Abbreviations: ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; CIN: cervical intraepithelial neoplasia grade; ICC: invasive cervical cancer.
Table A2. Number of included HPV-positive women tested for \( N \) and positive for \( n \) individual HR HPV types by cervical disease grade and region

|          | Normal |         | Low-grade |         | High-grade |         | ICC |         |
|----------|--------|---------|-----------|---------|------------|---------|-----|---------|
|          | \( N \) | \( n \) | \( N \)   | \( n \) | \( N \)    | \( n \) | \( N \) | \( n \) |
| HPV16    |        |         |           |         |            |         |      |         |
| Africa   | 2,221  | 290     | 517       | 87      | 251        | 76      | 2,402| 1,275   |
| Eastern Asia | 6,313  | 1,071   | 2,790     | 588     | 3,693      | 1,401   | 9,670| 5,968   |
| Western/Central Asia | 977    | 288     | 237       | 73      | 79         | 54      | 2,076| 1,515   |
| Europe   | 14,636 | 3,344   | 14,319    | 3,704   | 8,348      | 4,540   | 9,723| 6,484   |
| North America | 3,057  | 804     | 5,532     | 1,366   | 4,598      | 2,610   | 2,497| 1,528   |
| South/Central America | 5,201  | 838     | 2,692     | 675     | 1,896      | 1,002   | 6,488| 3,861   |
| Oceania  | 749    | 132     | 385       | 95      | 924        | 498     | 787  | 493     |
| HPV18    |        |         |           |         |            |         |      |         |
| Africa   | 2,221  | 184     | 517       | 43      | 251        | 23      | 2,402| 476     |
| Eastern Asia | 6,259  | 572     | 2,790     | 231     | 3,693      | 272     | 9,630| 1,520   |
| Western/Central Asia | 950    | 60      | 237       | 16      | 79         | 5       | 2,076| 313     |
| Europe   | 14,636 | 1,286   | 14,256    | 1,297   | 8,214      | 634     | 9,811| 1,609   |
| North America | 2,958  | 281     | 5,532     | 524     | 4,598      | 441     | 2,497| 489     |
| South/Central America | 5,191  | 323     | 2,690     | 184     | 1,886      | 178     | 6,488| 825     |
| Oceania  | 749    | 62      | 385       | 33      | 924        | 89      | 787  | 167     |
| HPV45    |        |         |           |         |            |         |      |         |
| Africa   | 1,972  | 117     | 432       | 19      | 245        | 10      | 2,402| 264     |
| Eastern Asia | 5,858  | 157     | 2,549     | 30      | 3,148      | 64      | 6,751| 202     |
| Western/Central Asia | 539    | 34      | 189       | 4       | 28         | 2       | 1,736| 99      |
| Europe   | 13,434 | 805     | 10,472    | 485     | 6,244      | 234     | 7,425| 346     |
| North America | 3,057  | 159     | 5,415     | 301     | 4,397      | 211     | 2,417| 132     |
| South/Central America | 4,750  | 162     | 2,166     | 98      | 1,669      | 80      | 6,089| 371     |
| Oceania  | 749    | 28      | 385       | 25      | 924        | 39      | 628  | 33      |
| HPV33    |        |         |           |         |            |         |      |         |
| Africa   | 2,221  | 113     | 517       | 44      | 235        | 21      | 2,402| 86      |
| Eastern Asia | 5,858  | 282     | 2,790     | 138     | 3,693      | 367     | 8,522| 458     |
| Western/Central Asia | 568    | 23      | 210       | 15      | 31         | 3       | 1,843| 93      |
| Europe   | 13,842 | 726     | 13,186    | 875     | 7,844      | 735     | 8,652| 447     |
| North America | 3,003  | 83      | 5,317     | 225     | 4,598      | 351     | 2,439| 83      |
| South/Central America | 4,880  | 220     | 2,517     | 189     | 1,776      | 115     | 6,161| 221     |
| Oceania  | 749    | 29      | 385       | 22      | 924        | 73      | 590  | 10      |
| HPV58    |        |         |           |         |            |         |      |         |
| Africa   | 2,221  | 238     | 517       | 56      | 251        | 28      | 2,221| 29      |
| Eastern Asia | 6,259  | 472     | 2,781     | 376     | 3,647      | 715     | 8,767| 897     |
| Western/Central Asia | 610    | 21      | 189       | 12      | 28         | 3       | 1,827| 52      |
| Europe   | 13,436 | 735     | 10,489    | 698     | 6,278      | 362     | 7,068| 102     |
| North America | 2,956  | 173     | 5,213     | 395     | 4,397      | 299     | 2,368| 34      |
| South/Central America | 4,740  | 278     | 2,220     | 152     | 1,589      | 151     | 6,107| 170     |
| Oceania  | 749    | 42      | 385       | 35      | 924        | 54      | 590  | 5       |
Table A2. Number of included HPV-positive women tested for (N) and positive for (n) individual HR HPV types by cervical disease grade\(^1\) and region (Continued)

| HPV | Region                  | Normal | Low-grade | High-grade | ICC |
|-----|-------------------------|--------|-----------|------------|-----|
|     |                         | N      | n         | N          | n   | N    | n    |
| HPV31 |                         |        |           |            |     |       |      |
|      | Africa                  | 1,986  | 129       | 432        | 28  | 245  | 20   | 2,215 | 57  |
|      | Eastern Asia            | 5,804  | 214       | 2,552      | 113 | 3,596| 249  | 8,205 | 254 |
|      | Western/Central Asia    | 620    | 37        | 229        | 10  | 63   | 15   | 1,970 | 81  |
|      | Europe                  | 13,877 | 1,586     | 13,166     | 1,647| 7,930| 980  | 8,319 | 347 |
|      | North America           | 3,057  | 186       | 5,532      | 490 | 4,598| 600  | 2,481 | 91  |
|      | South/Central America   | 5,037  | 282       | 2,504      | 130 | 1,837| 194  | 6,157 | 365 |
|      | Oceania                 | 749    | 56        | 385        | 51  | 924  | 114  | 590   | 14  |
| HPV52 |                         |        |           |            |     |       |      |
|      | Africa                  | 1,986  | 170       | 432        | 51  | 245  | 27   | 2,221 | 76  |
|      | Eastern Asia            | 6,259  | 679       | 2,624      | 478 | 3,469| 740  | 8,532 | 520 |
|      | Western/Central Asia    | 539    | 23        | 72         | 2   | 20   | 0    | 1,629 | 41  |
|      | Europe                  | 12,474 | 1,019     | 9,577      | 798 | 6,067| 437  | 7,003 | 121 |
|      | North America           | 2,903  | 234       | 4,839      | 519 | 4,205| 435  | 2,368 | 64  |
|      | South/Central America   | 4,750  | 194       | 2,127      | 108 | 1,587| 100  | 6,107 | 168 |
|      | Oceania                 | 749    | 67        | 385        | 44  | 924  | 113  | 590   | 9   |
| HPV35 |                         |        |           |            |     |       |      |
|      | Africa                  | 1,972  | 130       | 432        | 34  | 245  | 29   | 2,221 | 108 |
|      | Eastern Asia            | 6,259  | 166       | 2,713      | 41  | 3,113| 58   | 6,801 | 73  |
|      | Western/Central Asia    | 487    | 22        | 185        | 8   | 26   | 1    | 1,763 | 36  |
|      | Europe                  | 12,264 | 476       | 9,922      | 479 | 6,015| 194  | 7,578 | 105 |
|      | North America           | 3,057  | 92        | 5,443      | 354 | 4,598| 299  | 2,388 | 36  |
|      | South/Central America   | 4,491  | 76        | 2,202      | 52  | 1,598| 81   | 5,975 | 114 |
|      | Oceania                 | 749    | 23        | 385        | 12  | 924  | 35   | 590   | 13  |

\(^1\)Low- and high-grade include diagnoses by cytology and/or histology (see Material and Methods section). Abbreviation: ICC: invasive cervical cancer.