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Time trends of human papillomavirus types in invasive cervical cancer, from 1940 to 2007

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Abbreviations: 95% CIs: 95% confidence intervals; ADC: adenocarcinoma; ADSCC: adenosquamous cell carcinoma; DEIA: deoxyribonucleic enzyme immunoassay; DNA: deoxyribonucleic acid; FFPE: formalin-fixed paraffin-embedded; H&E: hematoxylin and eosin; HPV: human papillomavirus; ICC: invasive cervical cancer; LiPA: line probe assay; OD: optical density; PCR: polymerase chain reaction; RC: relative contribution; SCC: squamous cell carcinoma; SD: standard deviation; SPF: short primer fragment; ULR: unconditional logistic regression

Additional Supporting Information may be found in the online version of this article.

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Contribution over time of human papillomavirus (HPV) types in human cancers has been poorly documented. Such data is fundamental to measure current HPV vaccines impact in the years to come. We estimated the HPV type-specific distribution in a large international series of invasive cervical cancer (ICC) over 70 years prior to vaccination. Paraffin embedded ICC cases diagnosed between 1940 and 2007 were retrieved from eleven countries in Central-South America, Asia and Europe. Included countries reported to have low-medium cervical cancer screening uptake. Information on age at and year of diagnosis was collected from medical records. After histological confirmation, HPV DNA detection was performed by SPF-10/DEIA/LiPA25 (version1). Logistic regression models were used for estimating the adjusted relative contributions (RC) of HPV16 and of HPV18 over time. Among 4,771 HPV DNA positive ICC cases, HPV16 and HPV18 were the two most common HPVs in all the decades with no statistically significant variations of their adjusted-RC from 1940–59 to 2000–07 (HPV16—from 61.5 to 62.1%, and HPV18—from 6.9 to 7.2%). As well, the RC of other HPV types did not varied over time. In the stratified analysis by histology, HPV16 adjusted-RC significantly increased across decades in adenocarcinomas. Regarding age, cases associated to either HPV16, 18 or 45 were younger than those with other HPV types in all the evaluated decades. The observed stability on the HPV type distribution predicts a high and stable impact of HPV vaccination in reducing the cervical cancer burden in future vaccinated generations.

What's new?
Evaluation of the success or failure of human papillomavirus (HPV) vaccination programs depends in part on knowledge of the historical contribution of the different HPV types to human cancer. The present study analyzed HPV type-specific relative contributions to invasive cervical cancer (ICC) over a 70-year period prior to the implementation of HPV vaccination. The relative contributions of different HPV types, including those for which a vaccine is now available, were found to be constant across decades. The findings indicate that HPV vaccination will have a high, stable impact on cervical cancer reduction.

Human papillomavirus (HPV) prophylactic vaccines against the two HPV types most frequently causing cervical cancer (HPV16 and HPV18) have been recently introduced in some national immunization programmes, mainly in countries from developed regions. There are two available vaccines, namely: Cervarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium)—a bivalent vaccine against HPVs 16/18, and Gardasil TM (Merck, Whitehouse Station, NJ)—a quadrivalent against HPVs 6/11/16/18. These vaccines have demonstrated to be highly immunogenic, safe and efficacious representing an important step forward for the control of invasive cervical cancer (ICC).[1] Long term surveillance of vaccinated populations will be required to fully evaluate the impact of vaccination over time. This surveillance will elucidate partially unanswered questions such as duration of vaccine protection and potential appearance of type replacement phenomenon.[2] While we await these long-term outcomes, information on the variation of HPV type relative contributions in ICC prior to the introduction of HPV vaccines can be obtained retrospectively. This information will provide valuable baseline data for monitoring post-vaccination type distributions and predicting vaccine impact.

It has been largely described that HPV16 and HPV18 are the two most frequent types involved in ICC worldwide, both accounting for about 70% of ICC cases. HPVs 31, 33, 35, 45, 52 and 58 have been identified as the next most common HPV types.[3,4] Some differences in the frequency of these last HPV types have been described across world regions (*e.g.*, HPV33 ranked third in Europe, while HPV45 ranked third in most of other world regions, or HPVs 52 and 58 observed in a higher proportion in Asia as compared to other regions).[4] However, variation on HPV type-specific distributions over time has not received much attention until HPV vaccines started to be implemented. There are scarce epidemiological studies on HPV genotype patterns over time and those published so far show diverse results. No variation on HPV type-specific distributions in ICC has been found in some studies,[5] whereas others have found a decrease in HPV16 and an increase of HPV types heterogeneity[6–8]; and still, some have suggested an increase in HPV16 over time.[3,9,10] Variation in HPV detection methods, population study sizes, geographical regions covered or relatively short evaluated study periods could partially explain the wide spectrum of results.

The present analysis is part of a larger study[4] which represents an international effort to collect and analyze, following a common protocol, ICC specimens in order to explore the HPV type-specific relative contributions in these lesions.

Table 1. Characteristics of ICCs included in the study by region and country

Region	Country	ICC cases (*n*)	Time at diagnosis (years)	Mean age at diagnosis-years[1] (SD)	Histological diagnosis			
					SCC (*n*; %)	ADC (*n*; %)	ADSCC (*n*; %)	Other (*n*; %)
Europe	Czech Republic	113	1953–1995	56.6 (15.3)	69 (61.1%)	41 (36.3%)	2 (1.8%)	1 (0.9%)
	Portugal	332	1950–2004	57.5 (14.3)	314 (94.6%)	12 (3.6%)	3 (0.9%)	3 (0.9%)
	Spain	1,043	1949–2007	56.2 (15.4)	912 (87.4%)	100 (9.6%)	20 (1.9%)	11 (1.1%)
	Total	*1,488*	*1949–2007*	*56.4 (15.1)*	*1,295 (87.0%)*	*153 (10.3%)*	*25 (1.7%)*	*15 (1.0%)*
Central-South	Argentina	384	1940–2005	44.9 (12.6)	328 (85.4%)	30 (7.8%)	18 (4.7%)	8 (2.1%)
America	Brazil	540	1974–2003	54.3 (13.6)	455 (84.3%)	71 (13.1%)	5 (0.9%)	9 (1.7%)
	Chile	258	1949–2003	51.4 (14.5)	225 (87.2%)	23 (8.9%)	6 (2.3%)	4 (1.6%)
	Colombia	623	1954–1999	49.9 (13.7)	557 (89.4%)	47 (7.5%)	6 (1.0%)	13 (2.1%)
	Mexico	573	1965–2003	48.1 (12.5)	505 (88.1%)	40 (7.0%)	22 (3.8%)	6 (1.0%)
	Paraguay	432	1961–2004	47.7 (11.6)	401 (92.8%)	16 (3.7%)	14 (3.2%)	1 (0.2%)
	Peru	770	1955–2003	53.0 (13.5)	767 (99.6%)	2 (0.3%)	1 (0.1%)	0 (0.0%)
	Total	*3,580*	*1940–2005*	*50.7 (13.4)*	*3,238 (90.4%)*	*229 (6.4%)*	*72 (2.0%)*	*41 (1.1%)*
Asia	Korea, South	669	1958–1999	47.7 (11.6)	620 (92.7%)	42 (6.3%)	5 (0.7%)	2 (0.3%)
	Total	*669*	*1958–1999*	*47.7 (11.6)*	*620 (92.7%)*	*42 (6.3%)*	*5 (0.7%)*	*2 (0.3%)*
Total		*5,737*	*1940–2007*	*51.2 (13.7)*	*5,153 (89.8%)*	*424 (7.4%)*	*102 (1.8%)*	*58 (1.0%)*

[1]Age at diagnosis: Available information for 4,482 cases among the 5,737 cases. Abbreviations: ICC: invasive cervical cancer; SD: standard deviation; SCC: squamous cell carcinoma; ADC: adenocarcinoma; ADSCC: adenosquamous cell carcinoma; other: Other histological diagnosis which includes mainly undifferentiated and neuroendocrine carcinomas; %: row percent.

Particularly in this report, we evaluated the potential changes in the relative contributions of HPV types in a subset of the ICC series including 11 countries over an extended time period of 70 years prior to HPV vaccine implementation (from 1940 to 2007).

Methods

Study design and materials

Materials and methods of the study have been previously described.[4] A cross-sectional retrospective study based on archival specimens was designed. Large collections of formalin-fixed paraffin-embedded (FFPE) blocks of ICC specimens were identified fom 38 countries in five continents. The overall contribution consisted of 15,084 ICC and controls, with varied time periods of diagnosis across participating centers, covering altogether the 1940–2007 timespan. For the present analyses, we selected only the centers that contributed with at least ten consecutive cases per decade, for a minimum of four decades (Supporting Information Fig. 1). The time periods of diagnosed cases covered by country are shown in Table 1. Collaborating centers were largely public medical institutions representing referral management centers for gyneacological cancers at least at a regional level. No wide organized cervical cancer screening programs were available in the included countries in the time-span of cases recruitment. The represented regions were Central-South America (Argentina, Brazil, Chile, Colombia, Mexico, Paraguay, Peru); Europe (Czech Republic, Portugal, Spain); and Asia (South Korea).

Information on age at and year of diagnosis and original histopathological diagnosis was collected from medical records. All specimens were received anonymized and the processing was centralized at the Institut Català d'Oncologia (ICO), in Barcelona. The respective local and ICO ethics committees approved all protocols and the study progress was overseen by an international steering committee specifically constituted for the project.

FFPE tissue blocks processing and histopathological evaluation

At least four paraffin sections were obtained from each block (sandwich method). First and last sections were used for histopathological evaluation after Hematoxylin and Eosin (H&E) staining. The in-between sections of the blocks were kept in Eppendorf tubes for HPV DNA testing. FFPE blocks were processed under strict conditions to avoid potential contamination (*i.e.*, a tissue-free paraffin block was cut after processing each study block to detect any HPV carry-over, the microtome was exhaustively cleaned and a new blade was used from block to block). To further control for possible sources of contamination blocks containing non-HPV related tissue processed at the same time as the ICC specimens in the local pathology lab were blindly included (5% of the total amount of ICC samples).

The reassessment of the histopathological diagnosis was done by a panel of three pathologists at ICO and was performed following a protocol based on the World Health Organization classification of the uterine cervix tumors.[11] All

slides were read blind to the local pathology diagnosis by one pathologist at ICO. All doubtful diagnosis, all discordant with the local diagnosis cases, and an additional random sample of cases were verified by a second pathologist. In case of differences between evaluations a final lecture involving a third pathologist was conducted and a consensus diagnosis was reached. The cases diagnosed as adenocarcinoma were revised by all pathologists for confirmation. A block was determined to be adequate for further testing if ICC was observed in the two H&E stained sections of the specimen, guaranteeing the presence of tumor cells in the in-between sections used for the HPV DNA testing.

HPV DNA detection and typing

Nearly 250 µl of freshly Proteinase K solution was used to extract DNA. SPF-10 PCR[12,13] was performed using 10 µl of the DNA extract in a final reaction volume of 50 µl. All samples were run with a 1:10 dilution. The amplified PCR products were tested for the presence of HPV DNA using a hybridization probe in a microtiter plate format with a cocktail of conservative probes recognizing, at least, 54 mucosal Alpha HPV genotypes. Optical densities (OD450) were read on a microtiter plate reader. HPV DNA positive samples were subsequently analysed by $LiPA_{25}$ (version 1: Labo Biomedical Products, Rijswijk, The Netherlands), a reverse hybridization technique that detects 25 high-risk and low-risk HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68–73, 70, 74). Specimens that were HPV DNA positive but did not hybridize with any of the 28 probes were coded as HPV type X (undetermined type). HPVX and HPV68or73, HPV39or68or73 cases were further analyzed by sequencing of the SPF-10 amplicon as previously described.[4,14] A random sample of the HPV DNA negative specimens was examined in depth using other testing procedures to exclude false negative results (*e.g.*, dilutions of the target DNA to minimize inhibitors and type-specific PCRs for HPV16 and 18). These procedures were performed at DDL Diagnostic Laboratories (DDL, Voorburg, Netherlands) and ICO facilities. Quality controls of laboratory procedures, between the two labs, were regularly performed including systematic cross-testing of randomly selected cases and regular review of the results with uncertain interpretation.

Statistical analysis

Data analysis was performed with the Statistical Package STATA 10 (Stata, College Station, TX). For categorical variables proportions and 95% Confidence Intervals (95% CIs) using the binomial exact test were estimated, while for quantitative variables mean and standard deviation (SD) were obtained (Table 1). Age and categorical variables distribution over time were assessed by ANOVA or Chi-squared linear trend tests when appropriate (Table 2).

Unless otherwise specified, HPV type-specific relative contributions (RC) were estimated among HPV DNA positive ICC cases and counting multiple HPV infections following a previously described weighting algorithm.[4,15,16]

Table 2. Characteristics of ICCs included in the study by time at diagnosis

	Global ($n = 5,737$)	1940–59 ($n = 647$)	1960–69 ($n = 961$)	1970–79 ($n = 1,244$)	1980–89 ($n = 1,114$)	1990–99 ($n = 1,137$)	2000–07 ($n = 634$)
Age at diagnosis (mean;SD)[1]							
Age (years)	51.2 (13.7)	47.9 (12.1)	46.3 (11.0)	50.6 (13.3)	52.1 (13.8)	53.1 (14.3)	54.4 (14.9)
Region (n;%)							
Europe	1,488 (25.9%)	163 (25.2%)	212 (22.1%)	278 (22.3%)	307 (27.6%)	188 (16.5%)	340 (53.6%)
Central-South America	3,580 (62.4%)	451 (69.7%)	570 (59.3%)	810 (65.1%)	651 (58.4%)	804 (70.7%)	294 (46.4%)
Asia	669 (11.7%)	33 (5.1%)	179 (18.6%)	156 (12.5%)	156 (14.0%)	145 (12.8%)	0 (0.0%)
Histological diagnosis (n;%)							
Squamous cell carcinoma	5,153 (89.8%)	582 (90.0%)	895 (93.1%)	1,119 (90.0%)	1,022 (91.7%)	1,008 (88.7%)	527 (83.1%)
Adenocarcinoma	424 (7.4%)	47 (7.3%)	47 (4.9%)	93 (7.5%)	61 (5.5%)	94 (8.3%)	82 (12.9%)
Adenosquamous cell carcinoma	102 (1.8%)	9 (1.4%)	13 (1.4%)	19 (1.5%)	22 (2.0%)	23 (2.0%)	16 (2.5%)
Other tumors	58 (1.0%)	9 (1.4%)	6 (0.6%)	13 (1.0%)	9 (0.8%)	12 (1.1%)	9 (1.4%)
HPV positivity (n;%)							
HPV positive ICCs[2]	4,771 (83.2%)	538 (83.2%)	743 (77.3%)	999 (80.3%)	960 (86.2%)	983 (86.5%)	548 (86.4%)
HPV single infections[3]	4,464 (93.6%)	498 (92.6%)	695 (93.5%)	950 (95.1%)	896 (93.3%)	908 (92.4%)	517 (94.3%)
HPV multiple infections[3]	281 (5.9%)	32 (5.9%)	40 (5.4%)	45 (4.5%)	61 (6.4%)	72 (7.3%)	31 (5.7%)
HPV undetermined[3]	26 (0.5%)	8 (1.5%)	8 (1.1%)	4 (0.4%)	3 (0.3%)	3 (0.3%)	0 (0.0%)

[1]Age at diagnosis: Available information for 4,482 cases among the 5,737 cases.
[2]% among ICCs HPV analyzed.
[3]% among HPV DNA positive ICC cases; All distribution and mean comparisons over time $p < 0.05$. Abbreviations: ICC: invasive cervical cancer; SD: standard deviation.

To assess time trends in HPV16 and in HPV18, adjusted-RC and 95% CIs were estimated by unconditional logistic regression (ULR) (Fig. 1). RC values were adjusted for region, histological diagnosis, age and time at diagnosis as categorical covariates (region: Europe, Central-South America and Asia; histology: squamous cell carcinoma [SCC], adenocarcinoma [ADC], adenosquamous cell carcinoma [ADSCC], other histologies; age: ≤39, 40–49, 50–59, 60–69 and ≥70 years; time

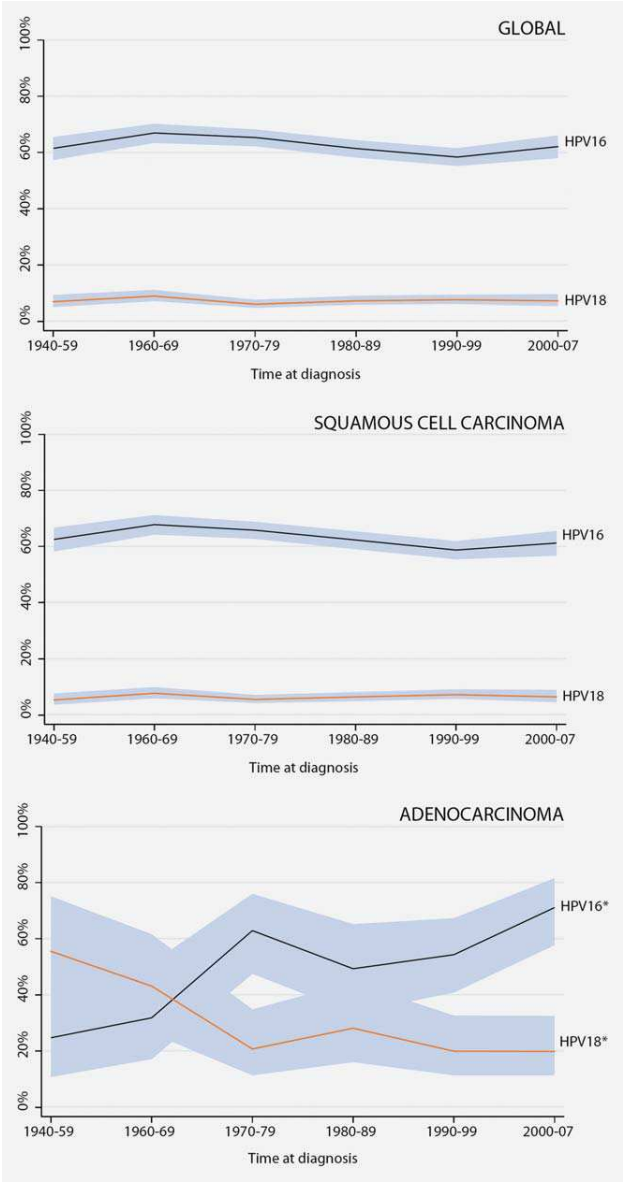

Figure 1. Adjusted-RC (%-solid lines and 95%CI-blue shadow) of HPV16 and of HPV18 in ICCs globally, in squamous cell carcinomas, and in adenocarcinomas; by time at diagnosis. Footnote: "ICC": Invasive Cervical Cancer; "Adjusted-RC": Adjusted relative contribution by region, age at diagnosis, histology and time at diagnosis; RC: % is calculated among HPV DNA positive ICC cases, and multiple infections are proportionally attributed as explained in the methods; "*" p value of trend <0.05; "95%CI": 95% Confidence Interval.

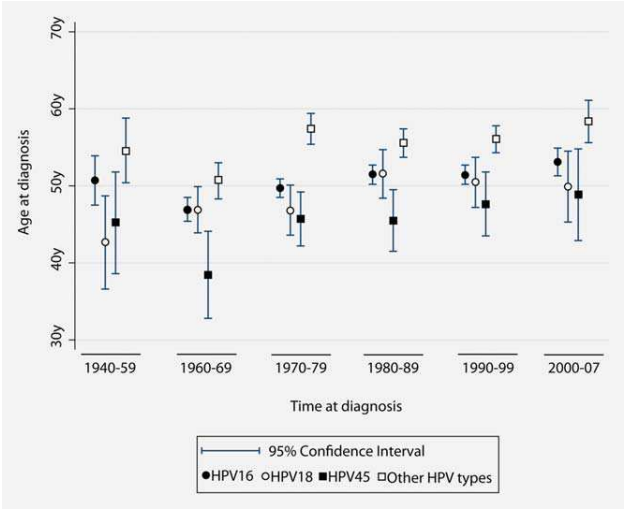

Figure 2. Mean age at diagnosis and 95%CI of ICCs with an HPV16, 18, 45 or Other HPV types by time at diagnosis. Footnote: "ICC": Invasive Cervical Cancer; Adjusted mean age by region and histology, stratified by decade. Only single HPV infections are included in the analysis.

in decades: 1940–59, 1960–69, 1970–79, 1980–89, 1990–99, 2000–07). Because of the small number of subjects in the first two decades, cases from 1940 to 1959 were grouped. For each HPV type, a separate model in which type-specific positivity was contrasted with all other HPV DNA positive cases was estimated. Cases with multiple infections including the evaluated types (*i.e.*, 16/18) were included both in the type-specific and in the comparison group, weighting the model as previously described[15,16] (so after weighting, the total number of cases in the model was the total number of HPV DNA positive ICCs). Adjusted-RC by time at diagnosis were estimated using the adjust command in STATA based on probability estimates from the regression models and the statistical significance of the trend was estimated by including the time as a continuous variable in the ULR. Other statistical methods were explored such as joint point analysis and smoothing methods; however, in view of the available data (no major changes over time and low number of time points-nodes), ULR was selected as the most suitable and parsimonious approach for this analysis.

To assess type-specific mean ages at diagnosis by decade, adjusted average age was estimated using a linear regression model taking into account the effect of the region and histological diagnosis. For this age-based analysis only single infections were included (Fig. 2).

For all models, the best fitting ones were selected by use of the logarithm-likelihood ratio test and stratified analysis by histological groups and for individual countries were also performed. Statistical significance for all analyses was set at the two-sided 0.05 level, and Bonferroni correction for multiple comparisons was used whenever necessary.

Results

Of the 10,575 ICC cases included in the global study,[4] 5,737 fulfilled the criteria established for this time trend analysis

(Supporting Information Fig. 1). A description of the samples in terms of country of origin, age at and time of diagnosis and histological diagnoses are presented in Table 1. Table 2 summarizes the distribution of the main characteristics of ICC cases by time at diagnosis. Samples from the earliest and the most recent decades were less represented. The mean age of cases increased from 47.9 years in 1940–59 to 54.4 years in 2000–07, $p < 0.001$. The study had a higher representation of Central-South America compared to the other regions. Although the most frequent histological type identified in all time periods was SCC, the proportion of ADC slightly increased over time (SCC *vs.* ADC, p value for trend<0.001).

The overall crude HPV DNA positivity was 83.2% (Table 2). The proportion of HPV positivity slightly increased across decades (p value for trend<0.001). As previously described,[4] ADC and older age at diagnosis were associated with HPV negativity in almost all time periods (data not shown). More than 90% of the HPV positive cases had single infections over all decades. Multiple HPV type infections were rarely detected and no variations over time were observed (single *vs.* multiple, p value for trend 0.224). Undetermined HPV types were also identified in a low proportion but a slight decrease was found across decades (p value for trend<0.001).

HPV types 16 and 18 were respectively the first and second types in frequency identified among HPV positive cases in all time periods (Supporting Information Table 1). Figure 1 shows the adjusted-RC of these HPV types globally and by the two main histological types (SCC and ADC) over the study period. No statistically significant time variations in HPV16 and in HPV18 adjusted-RC were observed: HPV16 ranged from 61.5% in 1940–59 to 62.1% in 2000–07 (p value for trend 0.342); and HPV18 from 6.9 to 7.2% (p value for trend 0.810). In the stratified analysis by histological diagnosis, no time trend was observed for the SCCs; whereas, for ADC an increasing trend was observed for HPV16 ranging from 24.7% in 1940–59 to 71.1% in 2000–07 (p value for trend 0.001); and a decreasing trend for HPV18 from 55.5 to 19.8% (p value for trend 0.003). Stratification by country did not modify our results with the exception of Peru, where the adjusted-RC of HPV16 decreased in the two most recent decades (Supporting Information Fig. 2). HPV variations over time in histology categories were only observed in Mexico and Colombia.

Additionally, we analyzed the crude RC trends over time of all types separately, and of type combinations by included-vaccine types, by the most frequent types (HPVs 16, 18, 31, 33, 35, 45, 52, 58), and by species (Supporting Information Table 1). We did not found time variations in none of the analysis, neither separately nor combined.

Among cases with multiple infections ($n = 281$), HPV16 and/or HPV18 were identified in 71.2% (200/281); and in 98.6% (277/281) at least one of the eight most common types was detected (HPVs 16, 18, 31, 33, 35, 45, 52, 58). No statistically significant changes over time were observed in these proportions. There were an overall of 102 different combinations of HPV types out of the 281 ICC cases containing more than one HPV type. Numbers by any combination of HPV types were too small to perform any evaluation by time at diagnosis.

HPV16, 18 and 45 positive cases were diagnosed at younger ages compared to that observed among cases positive for other HPV types (Fig. 2). This pattern was consistently observed across decades (Fig. 2), and in almost all histological and regional strata (data not shown).

Discussion

Among 5,737 ICC cases recruited from 11 countries, HPV16 and HPV18 were consistently the two most common types identified in each decade and showed a stable adjusted-RC from 1940 to 2007. As well, the RC of other HPV types did not varied over time. Conversely, in the stratified analysis by histology the adjusted-RC of HPV16 increased while that of HPV18 decreased in ADC diagnosed in recent years. HPV16, 18 and 45 cases were invariably detected at younger ages as compared to ICC cases with other HPV types, in all the study decades.

The stable time pattern of the contribution of HPV16 and HPV18 in ICCs observed in our study is in agreement with that found in a series of 100 ICC cases derived from Australian archives for the period 1920–1980.[5] Moreover, this stability is consistent with our most current knowledge of HPVs characterized as (*i*) being genetically stable DNA viruses with low mutation rates,[17] (*ii*) having a virus-host codivergence at shallow levels[18] and (*iii*) having few descriptions of recombination when compared with other viruses.[19] However, beyond viral genetic characteristics, theoretical factors that could modify the HPV type-specific RCs in ICCs could also originate from virus-host interactions with different environmental pressures over time (Supporting Information Fig. 3), such as (*i*) changes in the HPV background prevalence in general population over time,[20] (*ii*) coinfection with human immunodeficiency virus,[21] (*iii*) increase in life expectancy and finally and (*iv*) probably the most challenging, the introduction of new preventive measures such as screening and in a near future, HPV vaccines. These factors could have affected the specific oncogenic capacity of a given HPV type or/and its equilibrium with other types in ICCs. For example, cervical cancer screening through cervical cytology, could have affected type RCs by censoring differently the lesions associated with different HPVs. Wide implemented screening programmes have been shown to capture HPV16 preneoplastic lesions more frequently than lesions associated with other HPV types, thus reducing the RC of this HPV type in ICCs.[6–8] Indeed, an increase of the relative proportion of ADC (less detectable by cytology) has been observed in many cancer registries from countries with extensive implementation of screening. This should then translate into a higher detection of HPV18 and HPV45 over time, which are more frequently identified in glandular neoplastic lesions compared to SCC.[22,23] The 11 countries included in our analysis have low-medium cervical cancer screening uptakes resulting

in a low effect of this intervention over the time period covered and supporting our findings. Wide implementation of prophylactic HPV vaccines will also modify the genotype distributions in ICCs in the future by preventing HPV16 and 18 infections and related lesions. Active monitoring of HPV type-specific incidence rates in general population and related lesions will be crucial in order to assess potential appearance of type-replacement.[2] This theoretical phenomenon appears to be unlikely given the current evidence regarding the natural history of the infection, and will even turn into insignificant in front of vaccines cross-protection against other types and availability of new broad spectrum vaccines against HPV in the near future.[1,2,24] It is however reassuring that data from the PATRICIA trial in Finland (bivalent vaccine efficacy trial) has reported no increased incidence rate of non-vaccine HPV types in a 4-years follow-up in non vaccinated adolescents compared to the vaccinated, supporting that viral type-replacement is unlikely.[25]

It is worth to note that an increase in HPV16 contribution in ICCs over time has been described in Iceland (114 ICCs, time period: 1990–2003),[9] Hong Kong (435 ICCs, 1972–2007)[10] and more remarkably in a large meta-analysis including over 30,000 ICCs from published papers between 1990 and 2010.[3] However, in the report by Chang *et al.*[10] type of tissue preservation and the HPV detection technique used over time changed and this may have affected the results. In that direction the authors made an effort to elucidate potential biased results by performing a comparison between the two techniques used and they showed a high concordance with less multiple infections detected in one of the techniques used. In the meta-analysis[3] the results may also have been affected by the time variable used that was the year of publication and not the year of diagnosis of the cases. Also this particular type of analysis may not fully remove the effect of technical artefacts when HPV detection assays became better and more sensitive over time, however in the stratified analysis by technique the effect remained.

In concordance with that observed for HPV16 and HPV18, we did not find changes over time in the analyses neither in the other types nor in any of the explored combinations of HPV types (vaccine included types, most frequent types, or species). This finding give us more insights in addition to those obtained from natural history studies and coinfection occurrence analyses[24] supporting the low probability of type-replacement occurrence after vaccination and predicting a stable impact in cervical cancer burden reduction of current HPV vaccines in the vaccinated cohorts in countries included. No time variations for the two main HPVs (16/18) were observed across the included countries, except for HPV16 in Peru. It is unknown if this variation could be explained by a wider implementation of screening activities in the country. Further evaluation of less frequent HPV types across geography was limited due to the small numbers.

The crossover of the RC between HPV16 and HPV18 in ADC diagnosed in recent years has been previously described.[3] Li *et al.* linked the finding to a wider coverage of screening programs allowing the detection of concurrent lesions associated to certain HPV types (*i.e.*, joint detection of ADC and squamous intraepithelial lesions, in large part HPV16 positive).[3,26] However, the interpretation of this observation is still unclear and requires further investigation since we cannot exclude low sample size limitation or that other co-factors could be differentially associated in the pathogenesis of ADC being the causes of this observed change over time.

An important result to highlight is the consistent younger mean ages of women with tumors associated to HPV16, 18, 45 compared to those with other HPV types in all study time periods. This consistent pattern is unlikely to be explained by a cohort effect and seems to be related to a differential oncogenic potential between HPV types.[6,27–29] Long-term cohort studies have shown that these types have higher oncogenic capacity, as measured by time to progression to high grade pre-neoplastic lesions and higher integration rates. The progression to invasion of other oncogenic HPV types may be more dependent on host immunogenic background status and other environmental cofactors.

Some of the limitations and strengths of the study have been previously described.[4] The representativeness of tumors analyzed is crucial in this type of analysis. Efforts were made in order to include all consecutive newly histologically diagnosed ICC cases for each of the participating hospitals at a defined period without any additional selection criteria. Access to health care facilities is one of the main limiting selection factor. However, the good concordance in distribution of HPV types with other country-specific reports suggests that the existence of sample selection bias is low or unlikely. Another possible source of bias could be the HPV positivity detection rate, being lower in the oldest samples. As previously described,[4] technical artefacts (*e.g.*, poor fixation, *etc.*) are the most likely causes, not affecting differentially the HPV type-specific positivity. The strengths of our study include its large sample size, the centralized pathologist assessment and HPV DNA detection performance under strict highly sensitive and standardized protocols, and the extended time period covered. Moreover, we included in the analysis only those centers from the whole series that had contributed a minimum of cases and decades in order to minimize the potential bias that could have arisen due to the different contribution of centers across time.

In the 70-years time period and countries covered in the present study, the two HPV types targeted in the current approved prophylactic HPV vaccines, HPV16 and HPV18, were the most common types identified in ICCs and their adjusted-RC remained stable over time. Moreover, the RC of other HPV types separate or combined showed also a stable pattern over time. This result becomes highly relevant when considering potential cross-protection of HPV vaccines. This information provides a robust baseline for monitoring post-vaccination type distributions in ICC, and the observed constant HPV type-specific pattern in ICCs predicts a stable and high impact of HPV vaccines in reducing cervical cancer burden in future vaccinated generations.

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