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Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study

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Abbreviations: ADC: adenocarcinoma; AIS: adenocarcinoma *in situ*; ASC: adenosquamous carcinoma; CGN: cervical glandular neoplasia; CI: confidence intervals; CIN: cervical intraepithelial neoplasia; H&E: haematoxylin and eosin; HG-CIN: high grade cervical intraepithelial neoplasia; HPV: human papillomavirus; HR: high risk; ICC: invasive cervical cancer; LR: low risk; NOS: not otherwise specified; PCR: polymerase chain reaction; SCC: squamous cell carcinoma; SMILE: stratified mucin-producing intraepithelial lesion

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

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Cervical glandular neoplasias (CGN) present a challenge for cervical cancer prevention due to their complex histopathology and difficulties in detecting preinvasive stages with current screening practices. Reports of human papillomavirus (HPV) prevalence and type-distribution in CGN vary, providing uncertain evidence to support prophylactic vaccination and HPV screening. This study [108288/108290] assessed HPV prevalence and type-distribution in women diagnosed with cervical adenocarcinoma *in situ* (AIS, *N* = 49), adenosquamous carcinoma (ASC, *N* = 104), and various adenocarcinoma subtypes (ADC, *N* = 461) from 17 European countries, using centralised pathology review and sensitive HPV testing. The highest HPV-positivity rates were observed in AIS (93.9%), ASC (85.6%), and usual-type ADC (90.4%), with much lower rates in rarer ADC subtypes (clear-cell: 27.6%; serous: 30.4%; endometrioid: 12.9%; gastric-type: 0%). The most common HPV types were restricted to HPV16/18/45, accounting for 98.3% of all HPV-positive ADC. There were variations in HPV prevalence and ADC type-distribution by country. Age at diagnosis differed by ADC subtype, with usual-type diagnosed in younger women (median: 43 years) compared to rarer subtypes (medians between 57 and 66 years). Moreover, HPV-positive ADC cases were younger than HPV-negative ADC. The six years difference in median age for women with AIS compared to those with usual-type ADC suggests that cytological screening for AIS may be suboptimal. Since the great majority of CGN are HPV16/18/45-positive, the incorporation of prophylactic vaccination and HPV testing in cervical cancer screening are important prevention strategies. Our results suggest that special attention should be given to certain rarer ADC subtypes as most appear to be unrelated to HPV.

What's new?

Cervical cancer occurs in several different types; while Pap smears do a fine job of preventing one type, they fall short when it comes to another. Cervical adenocarcinoma (ADC) can look very different from case to case, and is more difficult to identify. This study characterized the HPV subtypes most commonly associated with ADC. Most commonly found were HPV 16, 18, and 45; thus, many of these cancers could be prevented by vaccination. The rarer subtypes of ADC had less of a link to HPV infection, and these cancers warrant special attention to develop better screening tools.

Invasive cervical cancer (ICC) is the fourth most common cancer in incidence and cancer-related deaths in women worldwide.[1] Approximately 67,400 new cases and 28,000 deaths from ICC were reported in Europe in 2012.[2] The overall incidence of ICC in Europe has decreased substantially over recent decades, but there are large variations in incidence and mortality by ICC subtypes and between the member states of the European Union.[1–3] Decreased incidence of ICC is mainly attributable to the early detection of squamous cell carcinoma (SCC) precursors through cytological screening and subsequent early treatment.

In contrast, the incidence of cervical adenocarcinoma (ADC) has been steadily increasing, especially in younger women, with its rate approaching 20 to 25% of all ICC in some countries.[4–7] Despite marked variation between regions in the incidence of ADC, there is evidence for an absolute increase in ADC.[8] This fact partly reflects the increase in ADC relative to SCC, the inherently poor identification of adenocarcinoma *in situ* (AIS) by routine cytology and colposcopy, and the variation in the quality and coverage of cytological screening.[6,7,9–11]

ADC comprises a heterogeneous group of histological subtypes including the most common usual-type ADC with the variants of endocervical, intestinal, signet-ring cell and villoglandular.[12] Other rarer ADC subtypes include clear-cell, mesonephric, serous, endometrioid, and gastric-type (including minimal deviation ADC).[12] There are also glandular cancers that exhibit variable squamous differentiation, termed adenosquamous carcinoma (ASC).

AIS is recognised as an immediate precursor of usual-type ADC. Both AIS and usual-type ADC are aetiologically related to infection with high-risk (HR)-human papillomavirus (HPV) types.[12] The precursor tumours of other ADC subtypes have a poorly defined natural history, and are rare and difficult to detect.[12,13] It is unclear whether some rare ADC subtypes (gastric-type, mesonephric and clear-cell) are associated with HPV infection,[14–17] as the few relevant studies report inconsistent results.[14,17,18] In the largest cross-sectional study of ADC cases to-date, using standardised methodology, only 62.8% were HPV-positive. The possibility that some of these cases have arisen independent of HPV infection was suggested. The authors also considered that some false negative results could be attributable to reduced quality and increased age of some specimens.[17]

HPV16, HPV18 or HPV45 are detected in the majority of HPV-positive ADC cases worldwide, but proportions vary by country.[19–22] HPV-typing of ADC subtypes has been conducted in several studies,[12,17,18,23] but published data on HPV type-distribution by ADC subtypes in Europe are scarce.

We have previously reported the HPV prevalence and type-distribution by diagnosis in a large series of cervical intraepithelial neoplasia (CIN) and ICC specimens from women in 17 European countries from the HERACLES and SCALE studies.[20] The present article describes additional analyses specifically focused on ADC (and its subtypes), ASC and AIS not covered in the previous publication. Centralised expert histopathological review confirmed 461 ADC, 104 ASC and 49 AIS cases on which HPV-typing for 25 HR- and low risk (LR)-HPV types was performed. These data are unique and important to better understand the role of HPV in cervical glandular neoplasia (CGN) and to understand the potential benefit of HPV vaccination and HPV-based screening in preventing this group of tumours.

Material and Methods

Study population

This retrospective cross-sectional study [108288/108290] was conducted in 17 European countries in medical centres that maintained an archive of cervical biopsy, excision or resection specimens representative of their total population, as previously described.[20] The majority of centres were located in areas with cervical screening programs. From each participating country, approximately 290 consecutive archived formalin-fixed paraffin-embedded cervical specimens were selected. These specimens were from women aged 18 years or older, diagnosed with high-grade precancer (including AIS) or ICC (including ADC, ASC) since 2001. Specimens were selected sequentially in reverse chronological order starting, depending on country, between November 2006 and August 2008 (Supporting Information Figs. *a* and *b*). The sample collection procedures were standardised across the countries.

For a woman to be included in the study, relevant cervical specimens on which the diagnosis was made (prior to any chemotherapy or radiotherapy) had to be available. If several blocks were available for a case, the specimen containing the most advanced tumour was chosen. The specimens had to be of appropriate size (<2 cm across), and be adequately preserved (formalin-fixed and paraffin-embedded) (Supporting Information Figs. *a* and *b*). For each case, age at and country of diagnosis, as well as the initial histological diagnosis were collected as previously described.[20]

Anonymised cervical specimens were shipped to a central laboratory (DDL Diagnostic Laboratory, Rijswijk, The Netherlands) for histopathological review. An experienced gynaecological histopathologist, blinded to the original diagnosis in the country of origin, reviewed all cases and categorised them according to an agreed predefined classification based on a modification of the WHO histological classification of tumours of the uterine cervix (http://screening.iarc.fr/atlasclassifwho.php). The specific categories were AIS (either pure AIS or AIS with coexistent high-grade cervical intraepithelial neoplasia (HG-CIN)), ASC and ADC (including usual-type, clear-cell, serous, gastric-type, endometrioid and ADC "not otherwise specified" (NOS)) (Supporting Information Figs. *a* and *b*). It should be noted that in this study, mesonephric and clear-cell ADC were categorised together, despite the fact that histogenesis of these two tumours is different. This was decided because only a single haematoxylin and eosin (H&E) stained section was available for reviewing. The paraffin blocks were selected for polymerase chain reaction (PCR) subtyping so there was no possibility to perform immunohistochemistry. Thus, it was not possible for the reviewers to reliably differentiate these tumour subtypes in every case.

As an additional quality control measure, all ADC, ASC and AIS sections were further (independently and blinded) reviewed by two expert histopathologists (E.C.P., W.G.McC.). Finally, a third expert (M.W.) reviewed those cases for which there was no diagnostic agreement. A simple majority rule was applied to make the diagnosis. If all three experts disagreed, the case was not included in the study.

All protocols were approved by the appropriate Institutional Review Board and/or Independent Ethics Committee

in each participating country and were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Where required, written informed consent was obtained from participating subjects prior to any study procedure on clinical specimens.

HPV testing

Cervical specimens with a confirmed histopathological diagnosis were analysed at DDL for the presence of HPV DNA. Paraffin sections were systematically obtained from each block using a sandwich method (4 μm sections for H&E staining were taken immediately before and after the sections used for HPV DNA analysis). Total DNA was extracted using a proteinase K lysis procedure.[19] HPV testing was performed using the L1-based SPF_{10}-DEIA/$LiPA_{25}$-PCR system (SPF_{10}-$LiPA_{25}$ version 1, Labo Biomedical Products, Rijswijk, Netherlands, based on licensed Innogenetics technology).[24] The SPF_{10}-DEIA assay has the potential to amplify and recognize by hybridization with a cocktail of nine conservative probes at least 54 individual HPV types. If positive by SPF_{10}-DEIA the amplimers can be genotyped by $LiPA_{25}$, which enables simultaneous genotyping of 14 HR (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68/73) and 11 LR (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, 74) HPV types.[24,25] Each run contained positive and negative controls to monitor DNA isolation, PCR amplification, HPV detection and genotyping procedures. If the sample tested negative for HPV, the DNA was diluted ten-fold and the testing was repeated.

Statistical analysis

All women with an expert-confirmed diagnosis of ADC, ASC or AIS were included in the analysis. Levels of agreement between original country diagnoses and expert diagnoses were assessed by computing simple Cohen's kappa coefficients (95% confidence interval (CI)). Characteristics of subjects with AIS, ASC, ADC and its subtypes (as determined by final expert diagnosis) were summarised by median age (95% CI) at diagnosis, HPV-positivity, HPV type-prevalence overall and per country. Median age (95% CI) was also computed per HPV type, and diagnosis. Statistical analyses were performed using Statistical Analysis Software (SAS version 9.1).

Results

Cervical adenocarcinoma and subtypes

From a total of 3,626 women originally diagnosed with ICC, 514 (14.2%) were diagnosed with ADC. Expert pathology review confirmed the ADC diagnosis in 411 of these 514 women. A further 50 women originally diagnosed with non-ADC ICC were given an expert-confirmed ADC diagnosis. These women comprised 10.9% of all 461 expert-confirmed ADC cases (Supporting Information Fig. *a*). Analysis of the level of agreement between original diagnoses and expert diagnoses yielded a kappa coefficient of 0.82 (95% CI: 0.79–0.85). Among the 461 women with confirmed ADC (representing 14.6% of all expert-confirmed ICC), usual-type ADC, clear-cell ADC, serous ADC, gastric-type ADC, endometrioid ADC and ADC NOS accounted for 364 (79.0%), 29 (6.3%), 23 (5.0%), 7 (1.5%), 31 (6.7%) and 7 (1.5%) cases, respectively (Tables 1 and 2).

Three hundred fifty-one (76.1%) ADC specimens were HPV-positive. The majority of usual-type ADC (90.4%) were HPV-positive, whereas lower positivity rates were observed for other ADC subtypes (30.4% for serous, 27.6% for clear-cell and 12.9% for endometrioid). All seven gastric-type ADC were HPV-negative (Table 1). Infection with a single HPV type was present in 94.2%, 62.5%, 85.7%, and 100.0% of HPV-positive usual-type, clear-cell, serous, and endometrioid ADC, respectively. The most prevalent single HPV types in ADC were HPV16 (50.5%), HPV18 (39.8%), HPV45 (8.0%), HPV33 (0.6%), and one case each of HPV31, HPV39 and HPV51. HR-HPV types were present in all singly-infected usual-type ADC (HPV16 (50.3%), HPV18 (40.6%), HPV45 (7.4%) and other HR-HPV (1.7%)) (Table 1). Multiple HPV infections were present in only 4.3% of usual-type ADC. Multiple infections were more frequent in clear-cell (25.0%) and serous (14.3%) ADC. There were no multiple HPV infections in cases with endometrioid ADC (Table 1). The most prevalent HPV types in multiple infections were HPV16 + other (41.2%), HPV45 + other (35.3%) and HPV18 + other (29.4%).

The median age (95%CI) of women with ADC was 45 (44.0–48.0) years. Median age appeared to be lower for usual-type ADC (43 (42.0–45.0) years), and higher for the other ADC subtypes (65 (53.0–76.0) years for clear-cell, 66 (53.0–72.0) years for serous, 60 (41.0–74.0) years for gastric-type, and 57 (49.0–68.0) years for endometrioid). The majority (58%) of women diagnosed with usual-type ADC were younger than 46 years of age (Table 1). When stratified by HPV status, median age for women with HPV-positive ADC was lower (43 years) than for women with the same histological diagnosis but with a HPV-negative ADC (61 years). The median ages at diagnosis for women with ADC positive for HPV16, 18, and 45 were 43 (95% CI: 41.0–45.0), 43 (41.0–44.0), and 44 (39.0–50.0) years, respectively. In comparison, median ages for women with ADC positive for HPV31, 33 and "other" types were 48 (36.0–76.0), 55 (42.0–71.0), and 54 (22.0–72.0) years, respectively (Table 1).

The number of women diagnosed with ADC as a proportion of women with ICC varied by country, with the highest proportion being in Portugal (19.9%) and lowest in Romania (6.3%) (Table 2). In all countries, the majority of women diagnosed with ADC had usual-type ADC, with the proportion being highest in Denmark (90.5%) and lowest in Romania (43.8%). The relative frequency of ADC subtypes (clear-cell, serous, gastric-type, and endometrioid) by country were generally very low, apart from Romania where 38% of ADC cases were clear-cell and serous types (Table 3).

The HPV-positivity rates for any ADC also varied by country with the highest rates observed in Denmark (95.2%) and the lowest in Romania (43.8%). These country-specific HPV-positivity rates tended to align with the country-specific prevalence rates of usual-type ADC (Table 2). Country-specific prevalence of the most common HPV types in usual-

Table 1. Histology, human papillomavirus (HPV) status and age at diagnosis

		AIS	ASC	ADC overall	ADC by sub-type					
					Usual-type	Clear-cell	Serous	Gastric	Endo	NOS
All	*N* (%)	49	104	461	364 (79.0)[1]	29 (6.3)[1]	23 (5.0)[1]	7 (1.5)[1]	31(6.7)[1]	7 (1.5)[1]
	Median age (95% CI)[2] at date of specimen collection (yr)	37 (33.0–41.0)	50.5 (45.0–54.0)	45 (44.0–48.0)	43 (42.0–45.0)	65 (53.0–76.0)	66 (53.0–72.0)	60 (41.0–74.0)	57 (49.0–68.0)	54 (35.0–80.0)
	Age class *n* (%) (yr)									
	≤25	4 (8.2)	0 (0)	8 (1.7)	6 (1.7)	2 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)
	>25–45	32 (65.3)	42 (40.4)	223 (48.4)	204 (56.0)	5 (17.2)	3 (13.0)	2 (28.6)	6 (19.4)	3 (42.8)
	>45–65	13 (26.5)	44 (42.3)	154 (33.4)	120 (33.0)	8 (27.6)	8 (34.8)	2 (28.6)	14 (45.2)	2 (28.6)
	>65	0 (0)	18 (17.3)	76 (16.5)	34 (9.3)	14 (48.3)	12 (52.2)	3 (42.9)	11 (35.5)	2 (28.6)
HPV positive	*N* (%) HPV positive	46 (93.9)	89 (85.6)	351 (76.1)	329 (90.4)	8 (27.6)	7 (30.4)	0	4 (12.9)	3 (42.9)
	Single HPV infection (% of HPV+ women)	37 (80.4)	81 (91.0)	327 (93.2)	310 (94.2)	5 (62.5)	6 (85.7)	NA	4 (100)	2 (66.7)
	Multiple HPV infection (% of HPV+ women)	9 (19.6)	6 (6.7)	18 (5.1)	14 (4.3)	2 (25.0)	1 (14.3)	NA	0	1 (33.3)
	Unknown HPV type (% of HPV+ women)	0	2 (2.2)	6 (1.7)	5 (1.5)	1 (12.5)	0	NA	0	0
	N (% of single infected women)									
	Any single HR	37 (100.0)	81 (100.0)	326 (99.7)	310 (100.0)	5 (100.0)	5 (83.3)	NA	4 (100.0)	2 (100.0)
	Single HPV16	21 (56.8)	28 (34.6)	165 (50.5)	156 (50.3)	3 (60.0)	3 (50.0)		3 (75.0)	0
	Single HPV18	14 (37.8)	39 (48.1)	130 (39.8)	126 (40.6)	2 (40.0)	0		1 (25.0)	1 (50.0)
	Single HPV-31	0	2 (2.5)	1 (0.3)	1 (0.3)	0	0		0	0
	Single HPV-33	1 (2.7)	2 (2.5)	2 (0.6)	2 (0.6)	0	0		0	0
	Single HPV-45	1 (2.7)	7 (8.6)	26 (8.0)	23 (7.4)	0	2 (33.3)		0	1 (50.0)
	Single HR HPV other	0	3 (3.7)	2 (0.6)	2 (0.7)	0	0		0	0
	Any single LR	0	0	1 (0.3)	0	0	1 (16.7)		0	0
	Median age (95% CI)[2] in single and multiple infected women, by HPV type (yr)									
	Any HPV	36.5 (33.0–41.0)	49 (45.0–54.0)	43 (41.0–44.0)	42 (41.0–44.0)	44 (22.0–74.0)	51 (43.0–72.0)	NA	57 (43.0–68.0)	39 (35.0–76.0)
	HPV16	35 (30.0–43.0)	56 (46.0–63.0)	43 (41.0–45.0)	42 (40.0–45.0)	56 (36.0–74.0)	51 (43.0–70.0)	–	57 (43.0–68.0)	–
	HPV18	37 (32.0–48.0)	44 (41.0–49.0)	43 (41.0–44.0)	42 (40.0–44.0)	45 (44.0–45.0)	–	–	57	35

Table 1. Histology, human papillomavirus (HPV) status and age at diagnosis (Continued)

		AIS	ASC	ADC overall	ADC by sub-type					
					Usual-type	Clear-cell	Serous	Gastric	Endo	NOS
	HPV31	35.5 (35.0–36.0)	60 (45.0–74.0)	48 (36.0–76.0)	42 (36.0–48.0)	–	–	–	–	76
	HPV33	33.5 (28.0–39.0)	73 (54.0–92.0)	55 (42.0–71.0)	44 (42.0–65.0)	71	–	–	–	–
	HPV45	33 (30.0–36.0)	61 (32.0–67.0)	44 (39.0–50.0)	44 (39.0–50.0)	–	57 (48.0–65.0)	–	–	39
	HR HPV other	38	49 (44.0–90.0)	54 (22.0–72.0)	54 (43.0–64.0)	22	72	–	–	
HPV negative	*N* (%) HPV negative	3 (6.1)	15 (14.4)	110 (23.9)	35 (9.6)	21 (72.4)	16 (69.6)	7 (100.0)	27 (87.1)	4 (57.1)
	Median age (95% CI)[2]	51 (32.0–53.0)	51 (43.0–67.0)	61 (55.0–67.0)	57 (50.0–64.0)	72 (54.0–81.0)	70 (63.0–75.0)	60 (41.0–74.0)	55 (49.0–73.0)	56 (44.0–80.0)

[1]Percentage of all ADC.
[2]95%CI calculations of median age are *post hoc* analyses.
Abbreviations: AIS: adenocarcinoma *in situ* (includes AIS and AIS + any high-grade cervical intraepithelial neoplasia (CIN)); ASC: cervical adenosquamous carcinoma; ADC: cervical adenocarcinoma; usual-type: usual-type ADC; clear-cell: clear-cell ADC; serous: serous ADC; gastric: gastric ADC (incl. minimal deviation ADC); endo: endometrioid ADC; NOS: ADC not otherwise specified; *N*: total number of subjects in a given group; HPV+: HPV DNA-positive; HR: high-risk; LR: low-risk; clear-cell ADC includes one expert-confirmed case of mesonephric ADC; 95% CI: lower bound of 95%CI – upper bound 95%CI; NA: not applicable.

type ADC ranged from 15.8% to 75.0% for HPV16, 25.0% to 68.4% for HPV18 and 0.0% to 16.7% for HPV45. In the majority of countries, HPV16 was the most prevalent type, although HPV18 was most prevalent in Denmark, Germany and Greece. HPV45 was the third most prevalent type in ADC in all countries, except for Belgium (HPV-31) and Portugal (other HR-HPV) (Fig. 1a).

Adenocarcinoma *in situ*

From a total of 3,979 women originally diagnosed with high-grade cervical pre-cancer, 65 cases (1.6%) were diagnosed with AIS, with or without coexistent squamous HG-CIN. Expert pathology review confirmed the AIS diagnosis in 26 of these 65 women. A further 23 women originally diagnosed with HG-CIN with non-AIS were given an expert-confirmed AIS diagnosis. These women comprised 46.9% of all 49 expert-confirmed AIS cases (Supporting Information Fig. *b*). Analysis of the level of agreement between original diagnoses and expert diagnoses yielded a kappa coefficient of 0.45 (95% CI: 0.33–0.56). Among the 49 women diagnosed with AIS, 28 (57.1%) had AIS with coexistent CIN and 21 (42.9%) had pure AIS.

Forty-six (93.9%) AIS cases were HPV-positive (all 21 cases of pure AIS and 25 (89.3%) of AIS with coexistent CIN) and 37 (80.4%) were infected with a single HPV type. The most common single HPV types in AIS were HPV16 (56.8%) and HPV18 (37.8%) (Table 1). In the nine women with AIS infected with multiple HPV types, HPV16 and HPV18 were the most common HPV types (each type present in four cases).

The median age of women diagnosed with AIS was 37 (95% CI: 33.0–41.0) years. Women with HPV-positive AIS had a median age of 36.5 years compared to 51 years in women with HPV-negative AIS. Median ages of women diagnosed with AIS positive for HPV16 and HPV18 were 35 (30.0–43.0) and 37 (32.0–48.0) years, respectively (Table 1).

Country-specific prevalence of the most common HPV types in AIS ranged from 33.3 to 66.7% for HPV16 and 16.7 to 66.7% for HPV18. In most countries HPV16 was the most prevalent type. HPV18 was most prevalent in Ireland while both HPV16 and 18 showed equal prevalence in Poland and Hungary (Fig. 1b).

Adenosquamous carcinomas

From a total of 3,626 women originally diagnosed with ICC, 97 (2.7%) were diagnosed with ASC. Expert pathology review confirmed the ASC diagnosis in 27 of these 97 women. A further 77 women originally diagnosed with non-ASC ICC were given an expert-confirmed ASC diagnosis. These women comprised 74.0% of all 104 expert-confirmed ASC cases (Supporting Information Fig. *a*). Analysis of the level of agreement between original diagnoses and expert diagnoses yielded a low kappa coefficient of 0.25 (95% CI: 0.17–0.33). The majority (85.6%) of ASC were HPV-positive, the most prevalent single HPV types being HPV16, 18 and 45 representing 34.6%, 48.1% and 8.6% of the single infected ASC cases, respectively (Table 1).

Table 2. Histology and human papillomavirus (HPV) status by country

	HG-CIN	AIS		ICC	ASC		ADC		Usual-type	
Country	*N*	% of HG-CIN (95% CI)[1]	% HPV +	*N*	% of ICC (95% CI)[1]	% HPV+	% of ICC (95% CI)[1]	% HPV+	% of ADC (95% CI)[1]	% HPV+
Denmark	276	3.6 (1.8-6.6)	100.0	261	3.4 (1.6-6.4)	100.0	16.1 (11.9-21.1)	95.2	90.5 (77.4-97.3)	97.4
Greece	259	1.2 (0.2-3.3)	100.0	291	2.1 (0.8-4.4)	66.7	16.2 (12.1-20.9)	63.8	76.6 (62.0-87.7)	80.6
Portugal	253	2.8 (1.1-5.6)	100.0	321	2.8 (1.3-5.3)	88.9	19.9 (15.7-24.7)	75.0	76.6 (64.3-86.2)	87.8
Norway	259	1.9 (0.6-4.4)	100.0	338	4.7 (2.7-7.6)	81.3	18.0 (14.1-22.6)	83.6	86.9 (75.8-94.2)	92.5
Hungary	230	3.0 (1.2-6.2)	85.7	226	0.9 (0.1-3.2)	100.0	8.8 (5.5-13.3)	70.0	70.0 (45.7-88.1)	92.9
Germany	NC	–	–	177	7.3 (4.0-12.2)	100	15.3 (10.3-21.4)	74.1	77.8 (57.7-91.4)	90.5
Scotland	NC	–	–	260	3.5 (1.6-6.5)	77.8	13.1 (9.2-17.8)	79.4	85.3 (68.9-95.0)	89.7
Wales	NC	–	–	332	2.4 (1.0-4.7)	75.0	16.9(13.0-21.3)	76.8	76.8 (63.6-87.0)	95.3
Poland	205	1.0 (0.1-3.5)	100.0	193	6.2 (3.3-10.6)	75.0	7.8 (4.4-12.5)	73.3	66.7 (38.4-88.2)	100.0
Czech Republic	272	1.5 (0.4-3.7)	75.0	254	3.5 (1.6-6.6)	100.0	18.1 (13.6-23.4)	84.8	84.8 (71.1-93.7)	94.9
Romania	169	0.0 (0.0-2.2)	–	254	2.0 (0.6-4.5)	80.0	6.3 (3.6-10.0)	43.8	43.8 (19.8-70.1)	57.1
Belgium	NC	–	–	255	2.4 (0.9-5.1)	83.3	12.9 (9.1-17.7)	63.6	75.8 (57.7-88.9)	84.0
Austria	209	2.4 (0.8-5.5)	100.0	NC	–	–	–	–	–	–
Estonia	250	0.0 (0.0-1.5)	–	NC	–	–	–	–	–	–
Spain	265	0.8 (0.0-2.7)	100.0	NC	–	–	–	–	–	–
Ireland	241	1.7 (0.5-4.2)	75.0	NC	–	–	–	–	–	–
Russia	215	0.0 (0.0-1.7)	–	NC	–	–	–	–	–	–
Overall	3,103	1.6 (1.2-2.1)	93.9	3,162	3.3 (2.7-4.0)	85.6	14.6 (13.4-15.9)	76.1	79.0 (74.9-82.6)	90.4

[1]95%CI calculations are *post hoc* analyses.
Abbreviations: HG-CIN: any high-grade cervical intraepithelial neoplasia (includes any adenocarcinoma *in situ* (AIS)); AIS: adenocarcinoma *in situ* (includes AIS and AIS+ any high-grade cervical intraepithelial neoplasia (HG-CIN)); ICC: invasive cervical cancer including squamous cell carcinoma (SCC), cervical adenocarcinoma (ADC), cervical adenosquamous carcinoma (ASC), and other; usual-type: Usual-type ADC; HPV: human papillomavirus; NC: Not collected in the country; 95% CI: lower bound of 95%CI - upper bound 95%CI.

Discussion

This large study assessed HPV prevalence and type-distribution in women diagnosed with CGN from 17 European countries, using standardised and rigorous centralised pathology review and HPV PCR testing. We observed that ADC is histologically heterogeneous, with usual-type ADC being the most prevalent subtype. The majority of CGN were HPV-positive and particularly AIS, usual-type ADC and ASC were strongly related to HPV16, 18 and 45. This finding was consistent across most countries. Women diagnosed with HPV-positive ADC presented at a younger age than women with HPV-negative disease of the same histological type. We also observed variation in HPV-positivity by country. Nearly all AIS were associated with HPV16 or 18 infections. Taken together, these points confirm the importance of HPV types 16/18/45 in relation to the incorporation of prophylactic vaccines and HPV testing in primary screening to prevent CGN.

HPV prevalence in cervical glandular neoplasia

We observed high HPV-positivity rates for usual-type ADC (90.4%), ASC (85.6%) and AIS (93.9%) cases. HPV16/18/45 were detected in 98.3%, 91.3% and 97.3% of HPV-positive usual-type ADC, ASC and AIS cases, respectively, highlighting the restricted distribution of HPV types in these CGN. In previous smaller studies, HPV16/18/45 were reported as present in 79% to 96% of all HPV-positive ADC with an average of 90%.[14,17,26–29] While the overall proportion of combined HPV16/18-positive ADC cases in this study was similar to, or higher than that seen in previous studies, we observed geographical variations in the individual prevalence of these two HPV types. HPV16 was the most common type in the majority of countries, but HPV18 was predominant in Denmark, Greece and Germany, while in Norway HPV16 and 18 were equally frequent. Other international studies also suggest geographical variability, with HPV16 predominant in North Africa and South America, but HPV18 predominant in South East Asia.[17,23,26,28]

HPV prevalence in different histological cervical adenocarcinoma subtypes

Our results support previous evidence that HPV is not a necessary cause of gastric-type ADC, and the majority of clear-cell, serous and endometrioid ADC are also causally unrelated to HPV. We observed that the rate of HPV-positivity

Table 3. Number of cases of cervical adenocarcinoma (ADC) and its subtypes, and human papillomavirus status (HPV) by country

	ADC	Usual-type	Clear-cell			Serous			Gastric			Endo			NOS		
Country	*N*	*N*	*N*	% of ADC	% HPV+	*N*	% of ADC	% HPV+	*N*	% of ADC	% HPV+	*N*	% of ADC	% HPV+	*N*	% of ADC	% HPV+
Denmark	42	38	0	0.0	–	1	2.4	0.0	0	0.0	–	2	4.8	100.0	1	2.4	100.0
Greece	47	36	0	0.0	–	5	10.6	20.0	0	0.0	–	6	12.8	0.0	0	0.0	–
Portugal	64	49	8	12.5	50.0	3	4.7	33.3	2	3.1	0.0	1	1.6	0.0	1	1.6	–
Norway	61	53	5	8.2	20.0	2	3.3	50.0	0	0.0	–	1	1.6	0.0	0	0.0	–
Hungary	20	14	1	5.0	0.0	3	15.0	33.3	0	0.0	–	2	10.0	0.0	0	0.0	–
Germany	27	21	2	7.4	50.0	0	0.0	–	2	7.4	0.0	2	7.4	0.0	0	0.0	–
Scotland	34	29	1	2.9	0.0	1	2.9	0.0	1	2.9	0.0	2	5.9	50.0	0	0.0	–
Wales	56	43	4	7.1	0.0	4	7.1	25.0	0	0.0	–	3	5.4	33.3	2	3.6	–
Poland	15	10	1	6.7	0.0	0	0.0	–	1	6.7	0.0	2	13.3	0.0	1	6.7	100.0
Czech Republic	46	39	2	4.3	50.0	1	2.2	100.0	0	0.0	–	4	8.7	0.0	0	0.0	–
Romania	16	7	3	18.8	33.3	3	18.8	33.3	0	0.0	–	2	12.5	0.0	1	6.3	100.0
Belgium	33	25	2	6.1	0.0	0	0.0	–	1	3.0	0.0	4	12.1	0.0	1	3.0	–
Overall	461	364	29	6.3	27.6	23	5.0	30.4	7	1.5	0.0	31	6.7	12.9	7	1.5	42.9

Abbreviations: ADC: any cervical adenocarcinoma; usual-type: usual-type ADC; clear-cell: clear-cell ADC; serous: serous ADC; gastric: minimal deviation/gastric ADC; Endo: endometrioid ADC; NOS: ADC not otherwise specified.

varied between ADC subtypes, with high prevalence in usual-type (90.4%) and much lower prevalence in other ADC subtypes, ranging from 30.4% in serous ADC down to 0% in gastric-type ADC. These results are consistent with those of another, recently published, large series of ADC cases, which also clearly indicated a high prevalence of HPV in usual-type ADC (71.8%) and much lower HPV prevalence in unusual ADC subtypes.[17]

HPV prevalence in cervical adenocarcinomas by country

Very few prior studies undertaken in Europe have investigated substantial numbers of ADC cases. In a study from the Netherlands, the overall ADC HPV-positivity was reported as 94% ($n = 77$).[26] Other studies have reported the prevalence of HPV16 and 18 only as 85% in Italy ($n = 138$), 85% in Germany ($n = 54$), and 57% in Scotland ($n = 97$).[30–32] In the present study, we observed marked variation in HPV-positivity by country, from 95.2% in Denmark to 43.8% in Romanian cases. Several factors may contribute to the variation in HPV-positivity and histology, including differences in tissue fixation protocols, the use of unbuffered formalin, and the age of specimens (although in this study, none of the specimens had been stored for more than 8 years). Another important factor might have been variation in country-specific diagnostic criteria and procedures, referral patterns and risk factors, as well as possible selection bias. For instance, Denmark routinely employs p16 immunostaining in the diagnosis of ADC, and showed the highest rates of HPV-positivity. Conversely, Romania had high proportions of rarer ADC subtypes, with corresponding low HPV-positivity rates while even usual-type ADC from Romania showed a relatively low rate of HPV-positivity (57.1%).

Relation of age to cervical adenocarcinoma subtype and HPV status

Median age at diagnosis differed between ADC subtypes. Usual-type ADC was diagnosed more often in younger women relative to the rarer ADC subtypes which were more commonly diagnosed in older women. A younger age at diagnosis was also observed for HPV-positive relative to HPV-negative usual-type ADC. The modest number of cases of rarer histological types of ADC limited the potential to demonstrate a relationship between HPV status and age at diagnosis in these types.

Age at diagnosis appeared to be related to HPV type. Younger age at diagnosis was often observed in ADCs related to HPV16, 18, 45 relative to ADCs associated with other HPV types. Although the confidence intervals were wide and often overlapped, reflecting the small numbers of cases of some HPV types (*e.g.,* HPV31 ($n = 1$), HPV33 ($n = 2$) and HPV "other" ($n = 2$)) which hampers the interpretation, similar observations have been previously reported.[20,33] Associations between younger age at diagnosis and infection with HPV16, 18 and 45 are consistent with faster progression, possibly due to the higher levels of genomic instability associated with these types.[34]

Adenocarcinoma *in situ*

Nearly all women with AIS were HR-HPV-positive. The most prevalent type was HPV16 (56.8%) followed by HPV18

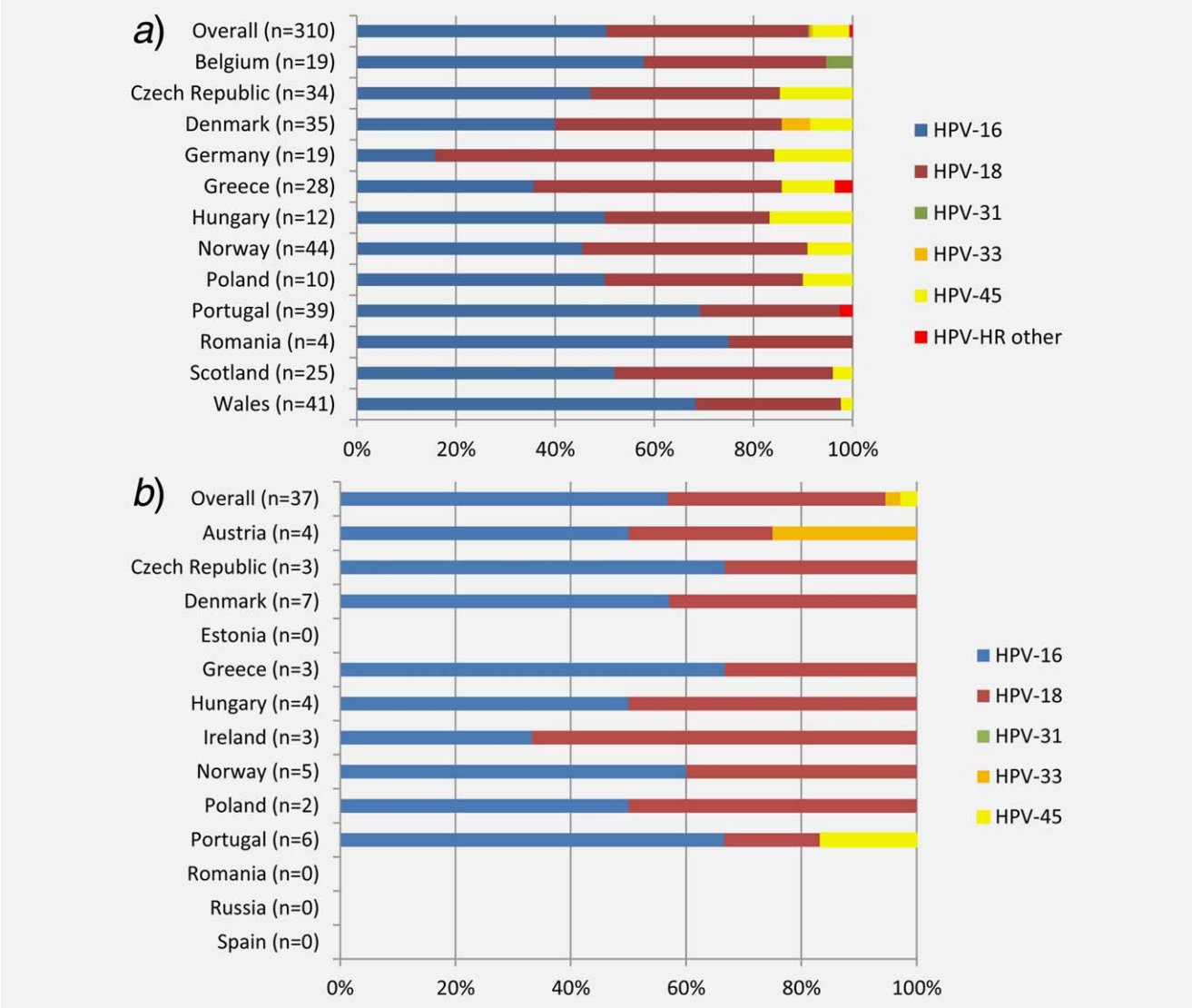

Figure 1. Overall and country-specific HPV type-distribution in (*a*) usual-type adenocarcinoma and (*b*) adenocarcinoma *in situ* (AIS). Data are based on HPV type-distribution in cases infected with a single HPV type. AIS: adenocarcinoma *in situ* (includes AIS and AIS + any high-grade cervical intraepithelial neoplasia (CIN)); HPV: human papillomavirus; HPV+: HPV positive; HPV-HR other: includes HPV-39, 51, 52, 56 and 59; usual-type ADC: usual-type cervical adenocarcinoma.

(37.8%), 33 (2.7%) and 45 (2.7%). Similar HPV-positivity rates and type-distribution were observed for usual-type ADC, supporting the recognition of AIS as an immediate precursor of usual-type ADC. These results contrast with those of a Swedish study showing a predominance of HPV18/45 (77%) *versus* HPV16 (27%) among the 95% of AIS tested women who were HR-HPV-positive, although based on only 22 HPV tested women.[35]

In our broader European study of ICC, AIS accounted for only 1.6% of all collected high-grade precancers.[20] AIS hence constitutes a small proportion of cervical premalignant lesions, while usual-type ADC form a much larger proportion of ICC (14.6%). This is consistent with AIS often being missed during cytological-based, colposcopically-verified screening. These limitations could potentially be avoided by use of HPV based cervical screening strategies. Another relevant consideration is the apparently rapid progression of AIS to invasive disease. We observed a difference in median age at diagnosis between AIS and usual-type ADC of 6 years. The corresponding difference in median age between squamous HG-CIN and SCC was approximately 14 years (34 *versus* 48 years).[20] Recently proposed screening recommendations that incorporate cervical cytology and HPV co-testing may help detect premalignant HPV-positive glandular lesions, and so improve their prevention.[36]

High-risk HPV types included in current prophylactic vaccines (HPV16/18) were detected in 94.6% of AIS indicating that vaccination could prevent most of them. In contrast,

the aetiology and precursor tumours for the rarer subtypes of ADC such as clear-cell, endometrioid and serous are currently poorly defined and the screening and prevention of many of these tumours remains a challenge.

Adenosquamous carcinomas

ASC accounted for approximately one-fifth of ICC cases showing glandular differentiation. During the consensus expert pathology review, we observed very low agreement between the original country and expert-confirmed diagnosis of ASC. This probably reflects the diversity in criteria employed to define ASC at the country-level. HPV was detected in 85.6% of ASC cases; HPV18 being the most prevalent (48.1%) followed by HPV16 and HPV45. The histological precursor of ASC has been described and termed as a "stratified mucin-producing intraepithelial lesion (SMILE)", but this diagnosis is not often made and a cytological equivalent has not been characterised.[37] Therefore, screening for ASC precursors is largely nonexistent. However, the high HPV-positivity of this tumour suggests great potential for prevention by vaccination.

Strengths and limitations

Previous international studies evaluating the prevalence of HPV in CGN have been limited in several regards, including small sample size, old/degraded paraffin-embedded histological specimens, possible histological misclassification of different tumour subtypes, and variations in the sensitivity of the methods used to detect HPV DNA.[12,17,19,38] HPV detection in CGN in particular, requires a very sensitive test because of relatively low viral load in these tumours.[14,17] Our study addressed these issues by including a large number of subjects from multiple countries with and without screening programmes, performing standardised specimen collection, undertaking centralised histopathological review, including only recently collected specimens, using a well-validated highly-sensitive method for the detection of HPV in formalin-fixed paraffin-embedded tissue, and carefully investigating HPV-negative cases in order to reduce the potential for pathological misclassification. DNA degradation during tissue preservation (*i.e.,* inadequate DNA quality), storage and processing and/or loss of specific sequences due to mutations in neoplastic cells may be responsible for false negative results in HPV DNA detection and genotyping. However, based on the experience of another research group who utilised the same HPV-genotyping methodology with a set of ADC samples that was similar to, but independent of, those used in the current study, we believe that the rate of HPV DNA false-negative results was marginal in our study.[39] This provides a robust and up-to-date description of the association between CGN and common HPV types in Europe. However, the study was an observational cross-sectional study and, as such, *post hoc* statistical hypothesis testing was not performed. Hence the hypotheses generated from the current data require confirmation in other studies.

Final conclusions

In summary, the high prevalence of HPV16/18/45 infection in CGN indicates that there is good potential to improve the detection of these lesions through the use of HPV DNA testing. Furthermore, the young age at diagnosis and the relatively small difference between age at diagnosis for AIS and usual-type ADC, indicates the importance of timely diagnosis. This could potentially be achieved through screening based on HPV testing which has the potential to detect HPV-positive CGN at the intraepithelial phase. In addition, although there could be as much as 5 to 10% variation either way in HPV positivity and the true impact can only be revealed through real life effectiveness studies, our results also suggest that the great majority of usual-type ADC (83.8%) and ASC (71.2%) are likely to be preventable by HPV vaccination effective against HPV16/18/45. However, amongst the rarer ADC subtypes, a much smaller proportion (clear cell (17.2%), serous (21.7%), and endometrioid (12.9%) ADC) may be preventable. Therefore, special attention should be given to rarer ADC subtypes which are less strongly related to HPV. The impact of replacing cytological screening by HPV-typing for ADC prevention remains unclear. The aetiology of rarer ADC subtypes requires further research but it is likely that most are not HPV-related.

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