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Risk of CIN3 or worse with persistence of 13 individual oncogenic HPV types

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Human papillomavirus (HPV) is essential in cervical carcinogenesis, however, less is known about the carcinogenic potential of individual HPV types. Our aim was to examine the risk of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) after persistence of 13 individual oncogenic HPV types. Liquid-based cervical samples (n = 40,399) collected in 2002–2005 were tested for HPV by hybrid capture 2 and genotyped with INNO-LiPAv2. Persistence was defined as having the same genotype twice 1–4.5 years apart. The absolute risk of CIN3+ was estimated by the Aalen-Johansen estimator and Cox proportional hazard regression was used to compare the rates of CIN3+ according to HPV type adjusting for age and time between HPV tests. Of 2,875 oncogenic HPV-positive women, 874 had persistence of one or more types and 761 persisted for one oncogenic HPV type only. Persistent HPV16 infection was associated with the highest risk of CIN3+, with an 8-year absolute risk of 55% (95% CI: 45%–66%), followed by HPV33 (33% (95% CI: 20%–50%)), HPV18 (32% (95% CI: 20%–48%)) and HPV31 (31% (95% CI: 21%–46%)). Other HPV types, including HPV52 and HPV45, were also associated with high risks. Persistent HPV56 had the lowest 8-year absolute risk of CIN3+ (3% (95% CI: 0.4%–20%)). In Cox analyses, a similar pattern remained after adjustment for age and time between tests. Our results add knowledge about the varying carcinogenic potential of individual persistent oncogenic HPV types, which may have implications for the clinical use of HPV testing.

Key words: human papillomavirus, type-specific persistence, prospective cohort study, cervical neoplasia, cervical cancer

Abbreviations: CI: confidence interval; CIN3+: cervical intraepithelial neoplasia grade 3 or worse; CIN3: cervical intraepithelial neoplasia grade 3; HC2: hybrid capture 2; HPV: human papillomavirus; HR: hazard ratio; IARC: International Agency for Research on Cancer; PIN: personal identification number

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Background

Persistent infection with oncogenic human papillomavirus (HPV) is a prerequisite for the development of cervical intraepithelial neoplasia grade 3 (CIN3) and cervical cancer.[1] In the most recent evaluation by the International Agency for Research on Cancer (IARC), 12 genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) were classified as carcinogenic and HPV68 was classified as probably carcinogenic to humans (group 2A)[2] on the basis of their high prevalence in large case series of cervical cancer. Thus, several HPV types have been characterized as oncogenic, however, they do not all appear to have the same carcinogenic potential; notably HPV16 has shown a unique carcinogenic potential.

A limited number of studies have examined the risk of CIN after persistence of several individual HPV types. It has previously been found that persistence of HPV16, 18, 31 and 33 conveys high absolute risks of CIN2+ or CIN3+.[3–6] These studies are, however, limited by small numbers of persistent HPV infections and outcomes. In addition, several studies had a relatively short follow-up time, examined only a limited number of persistent HPV types, or did not distinguish between multiple and single persistent HPV types in the analyses.[3–8]

What's new?

While the critical role of human papilloma virus (HPV) infection in cervical cancer is well established, less is known about the cancer risk associated with individual HPV subtypes. Here the authors performed a large population-based study including 40,000 Danish women. They show large differences in absolute risk for high-grade cervical intraepithelial neoplasia (CIN3+) development linked to persistent infection with 13 oncogenic HPV types. They suggest that these differences may inform clinical use of type-specific HPV testing in the future.

We conducted a large, prospective, population-based cohort study of more than 40,000 Danish women ranging widely in age. The aim of the study was to examine their risk of CIN3+ after type-specific persistence of 13 individual oncogenic HPV types.

Material and Methods

The study was based on a cohort established in Copenhagen, Denmark, during 2002–2005 of the results of cervical cytology or histology and HPV DNA analysis of more than 40,000 Danish women of a wide age range. The cohort has previously been described in detail.[9–11] The study protocol was approved by the Danish Scientific Ethics Committee and the Danish Data Protection Agency.

Sample collection

In Denmark, cervical screening is recommended every third year for women aged 23–49 years and every fifth year for women aged 50–64 years. Opportunistic screening is common, including among women under 23 years of age. In the Greater Copenhagen area, cervical samples are routinely collected by general practitioners or gynecologists using the SurePath liquid-based cytology system (BD Diagnostics, Tripath, Burlington, NC), and all cervical cytologic and histologic samples collected during both organized and opportunistic screening are analyzed at the Department of Pathology at Copenhagen University Hospital of Hvidovre. Cell material remaining after cytologic examinations was collected weekly by the Unit of Virus, Lifestyle and Genes at the Danish Cancer Society Research Center and sent to the Medical Virology Department, University Hospital of Tübingen, Germany, for HPV DNA testing.

HPV testing

The HPV testing method has been described in detail elsewhere.[9–11]

Hydrid capture 2 testing. In brief, samples were tested with Qiagen Hybrid Capture 2 (HC2), which combines the low-risk probe that detects HPV 6, 11, 42, 43 and 44 and the high-risk probe for detection of HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. All testing was done according to the manufacturer's instructions. Samples were considered positive if they attained or exceeded the threshold approved by the United States Food and Drug Administration of 1.0 pg/mL of HPV DNA.

HPV genotype testing. HPV genotyping was performed on HC2-positive samples. Total DNA was isolated from the remaining samples and analyzed with the INNO-LiPA v2HPV prototype assay (Innogenetics, Ghent, Belgium) according to the manufacturer's instructions. All PCR manipulations were performed in a separate room, according to ISO 15189 diagnostic guidelines. An aliquot of the amplified PCR product was hybridized to LiPA hybridization stripes with an auto-LiPA device. The resulting stripes were analyzed on a flatbed scanner with LiRAS prototype software, which shows the patterns and intensity of positive bands as gray tones values between 0.1 and 1.0. The LiPA test can identify 24 genotypes (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70 and 74). HPV testing was performed blind: samples were marked only with a study number, and the laboratory did not have demographic or clinical information related to the cervical sample.

Definition of type-specific HPV persistence

HPV status was categorized on a type-specific basis according to our findings at enrollment and second examination.[12] Women were defined as having type-specific persistent HPV infection if they were positive for the same HPV type in two separate cervical samples. As most HPV infections are cleared within 6–12 months,[13] we defined persistent infections as those lasting ≥1 year. Thus, we required that the women were positive for the same HPV type on the cytology at enrollment and on a subsequent cytology 1–4.5 years later. If a woman tested positive for the same HPV type on the two occasions but had one or more cervical samples that were negative for the specific HPV type between the two samples, she was considered to have cleared the HPV type.

Identification of the study population

A total of 40,399 women were enrolled in the study (Fig. 1). Of these, 5,528 women were oncogenic HPV positive by both HC2 and LiPA and had normal cytology at enrollment. We excluded 2,598 women who did not have a second cervical sample within 1–4.5 years after the first sample and 55 women who underwent conization between the first and second HPV test. Of the remaining 2,875 oncogenic HPV positive women, 900 had a type-specific persistent HPV infection with at least one of the 13 HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) classified as oncogenic by IARC. Of these, 26 women were excluded because they had no new

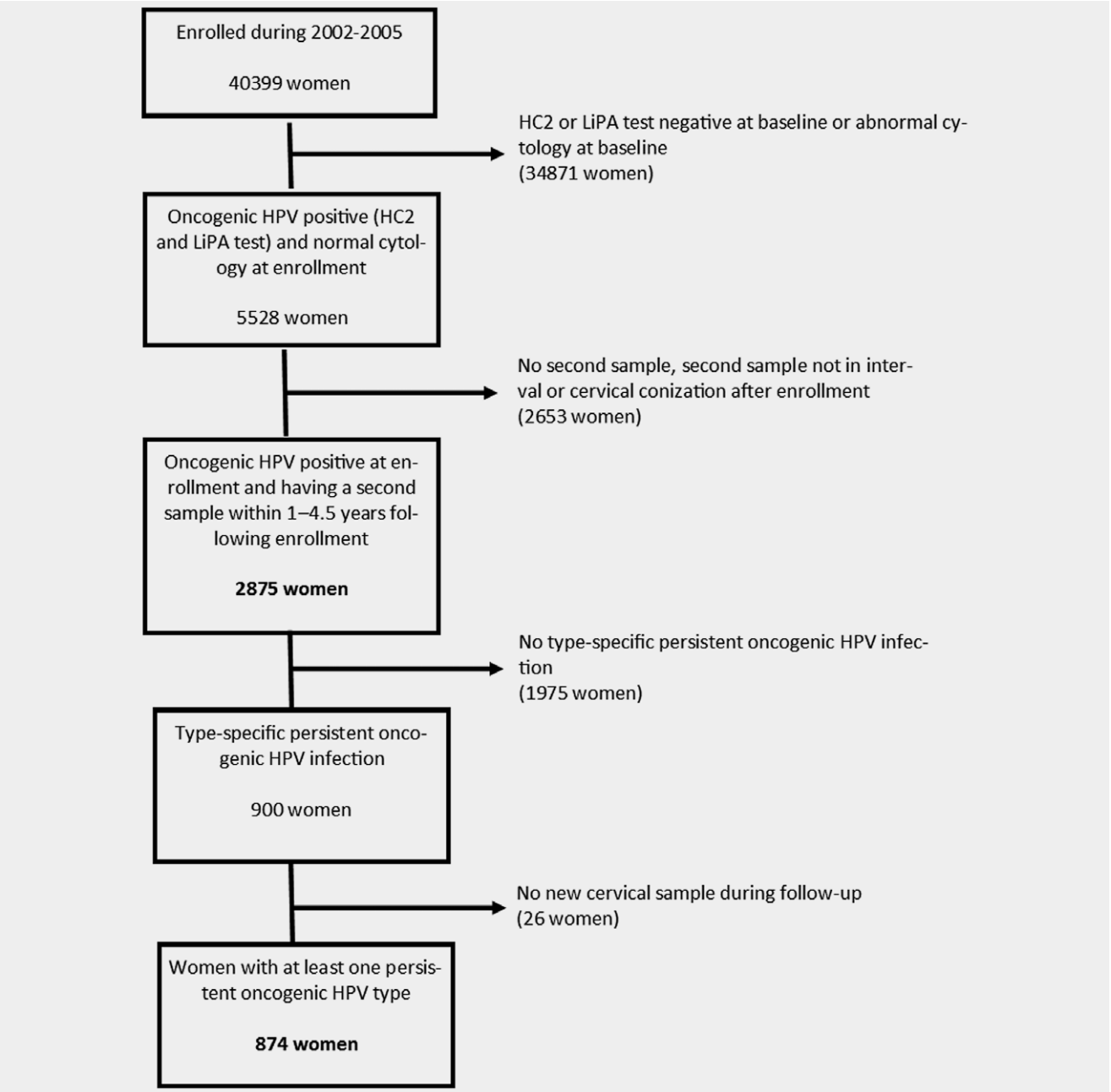


Figure 1. Women included in the study.

cervical sample during the follow-up period until February 2015, leaving 874 women with one or more persistent oncogenic HPV types. The median age of the study population at the second examination was 31 years (range: 18–65 years). In addition, the median time between the first and second HPV test was 3.1 years.

Ascertainment of outcome

The women were followed through nationwide Danish registers. Since 1968, all Danish citizens have been assigned a unique personal identification number (PIN), which is used in all national population and health databases and allows accurate linkage of data among registers. The PINs are registered in the Danish Civil Registration System.[14] We linked the cohort with the Danish Pathology Databank, a nationwide computerized pathology register that contains information on all cervical cytology and histology examinations performed in Denmark.[15] Histologic diagnoses of severe dysplasia, CIN3, cancer *in situ* and cervical cancer were categorized as CIN3+. The women were followed in the Danish Pathology Databank until February 2015.

Statistical analysis

Women were followed from the date of the second examination until diagnosis of CIN3+, conization or last cervical examination with a diagnosis of CIN2 or less. Date of CIN3+ was defined as the midpoint between the diagnosis or CIN3+ and the preceding examination to account for interval censoring. We calculated simple proportions of women who developed the CIN3+ during the entire follow-up period without taking into account person-time of follow-up.

We estimated the absolute risk of CIN3+ in relation to follow-up time with conization as a competing event by use of the Aalen-Johansen estimator.[16] Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were estimated by Cox proportional hazards regression with time since second examination as underlying timescale to compare the rates of CIN3+ according to HPV type. The analyses were adjusted for women's age at persistence and time between HPV tests (defining persistence). To investigate to what extend the association with CIN3+ among women with a persistent HPV type other than HPV16 was explained by either having cleared or acquired a HPV16 infection between the first and second examination, in two separate analyses we furthermore adjusted for HPV16 status at the first and second HPV test respectively among those being persistent for a non-HPV16 type. The proportional hazards assumption was tested using scaled Schoenfeld's residuals. All analyses were performed with R version 3.3.3[17] packages etm[18] and survival.[19]

Results

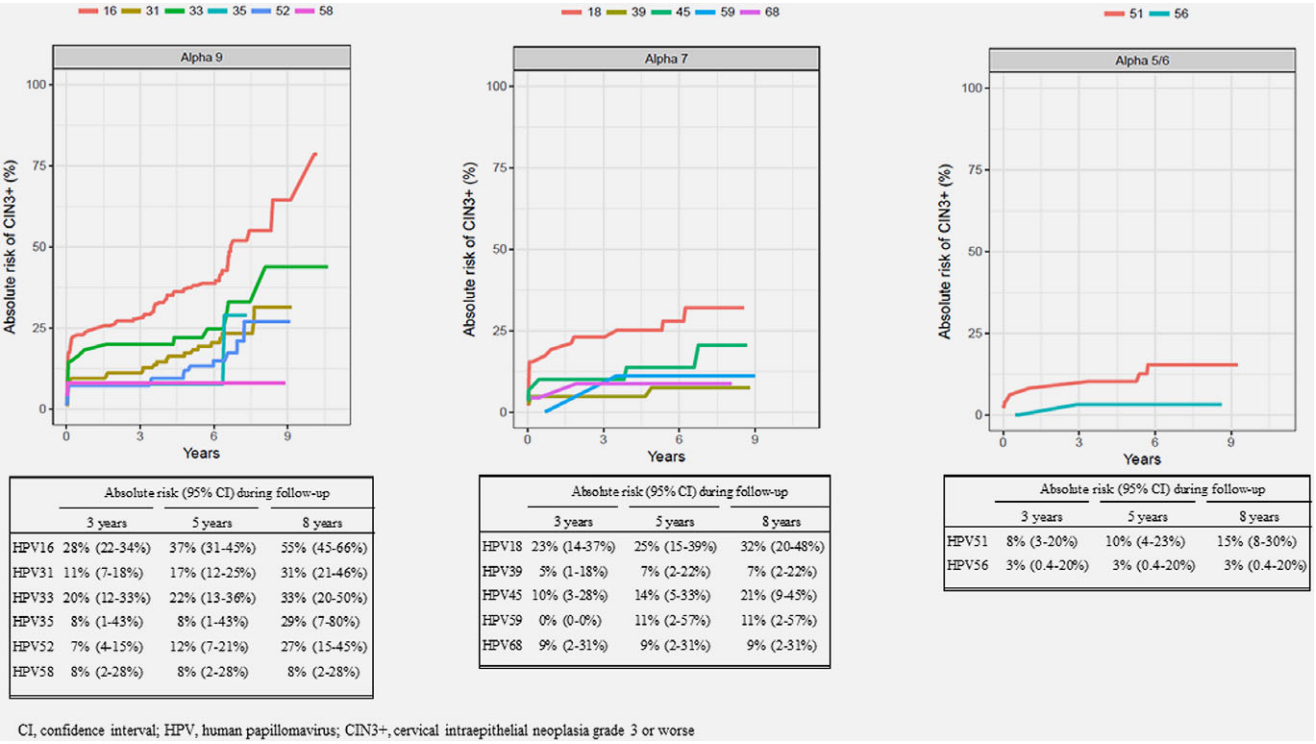
Proportions of women developing CIN3+ during follow-up after persistence for specific oncogenic HPV types

Among the 2,875 oncogenic HPV positive women, HPV16 was the most common type followed by HPV52 and HPV31. HPV16 was also the most persistent type followed by HPV31, HPV33, HPV52 and HPV18 (Table 1). Of the 874 women with one or more persistent oncogenic HPV infections, 239 were diagnosed with CIN3+ during the entire follow-up period (234 women had CIN3 and 5 women had cervical cancer). Table 1 also displays the proportion of women who, given persistence of a given HPV type, developed CIN3+ during the follow-up period up to 10 years. Women with HPV16 persistence had the highest likelihood of subsequently developing CIN3+ (46.2%). The likelihood of progression after persistence was also high for HPV18 (35.1%), HPV33 (32.5%), and HPV31 (27.5%). In contrast, the likelihood of progression

Table 1. Number and proportions of persistent HPV types and of women who developed CIN3 or worse during the entire follow-up period among women with one or more persistent HPV types and among women with only one persistent HPV type

		All women with one or more persistent oncogenic HPV types[1]				All women with a single persistent oncogenic HPV type			
	Total no. of oncogenic HPV positive at baseline	No. of women persistent for a given HPV type 1–4.5 years after baseline (proportion among HPV positive at baseline)		No. of women diagnosed with CIN3+ (proportion among women with persistence)		No. of women persistent only for the given HPV type (proportion among HPV positive at baseline)		No. of women diagnosed with CIN3+ (proportion among women with persistence)	
Alpha 9									
HPV16	743	264	(35.5)	122	(46.2)	206	(27.7)	90	(43.7)
HPV31	575	160	(27.8)	44	(27.5)	127	(22.1)	28	(22.0)
HPV33	244	65	(26.6)	21	(32.5)	55	(22.5)	16	(29.1)
HPV35	128	18	(14.1)	3	(16.7)	13	(10.2)	2	(15.4)
HPV52	613	133	(21.7)	33	(24.8)	97	(15.8)	16	(16.5)
HPV58	175	32	(18.3)	3	(9.4)	25	(14.3)	2	(8.0)
Alpha 7									
HPV18	349	74	(21.2)	26	(35.1)	52	(14.9)	15	(28.8)
HPV39	351	56	(16.0)	11	(19.6)	42	(12.0)	3	(7.1)
HPV45	308	44	(14.3)	9	(20.5)	30	(9.7)	5	(16.7)
HPV59	159	11	(6.9)	1	(9.1)	9	(5.7)	1	(11.1)
HPV68	335	28	(8.4)	3	(10.7)	23	(6.9)	2	(8.7)
Alpha 5/6									
HPV51	488	72	(14.8)	16	(22.2)	49	(10.0)	7	(14.3)
HPV56	288	37	(12.8)	2	(5.4)	33	(11.5)	1	(3.0)

Abbreviations: CIN3, cervical intraepithelial neoplasia grade; HPV, human papillomavirus.
[1]Women with multiple persistent HPV infections are counted for each HPV type separately.



	Absolute risk (95% CI) during follow-up		
	3 years	5 years	8 years
HPV16	28% (22-34%)	37% (31-45%)	55% (45-66%)
HPV31	11% (7-18%)	17% (12-25%)	31% (21-46%)
HPV33	20% (12-33%)	22% (13-36%)	33% (20-50%)
HPV35	8% (1-43%)	8% (1-43%)	29% (7-80%)
HPV52	7% (4-15%)	12% (7-21%)	27% (15-45%)
HPV58	8% (2-28%)	8% (2-28%)	8% (2-28%)

	Absolute risk (95% CI) during follow-up		
	3 years	5 years	8 years
HPV18	23% (14-37%)	25% (15-39%)	32% (20-48%)
HPV39	5% (1-18%)	7% (2-22%)	7% (2-22%)
HPV45	10% (3-28%)	14% (5-33%)	21% (9-45%)
HPV59	0% (0-0%)	11% (2-57%)	11% (2-57%)
HPV68	9% (2-31%)	9% (2-31%)	9% (2-31%)

	Absolute risk (95% CI) during follow-up		
	3 years	5 years	8 years
HPV51	8% (3-20%)	10% (4-23%)	15% (8-30%)
HPV56	3% (0.4-20%)	3% (0.4-20%)	3% (0.4-20%)

CI, confidence interval; HPV, human papillomavirus; CIN3+, cervical intraepithelial neoplasia grade 3 or worse

Figure 2. Absolute risks of CIN3+ following single persistent infection with one of 13 individual HPV types. The figures are supplemented by tables showing the absolute risks of CIN3+ at 3, 5 and 8 years after single persistent infection with the HPV types. The HPV types are grouped according to alpha 9, alpha 7 and alpha 5/6 species.

to CIN3+ was much lower for HPV58 (9.4%), HPV 59 (9.1%) and HPV56 (5.4%).

To avoid possible confounding between HPV types, we chose to focus only on women with a single persistent HPV infection (n = 761) in the following analysis of the carcinogenic potential of individual HPV types (Table 1). During the follow-up, 63 women were treated with conization for ≤CIN2. In addition, during the follow-up period, starting at the earliest 1 year after enrollment, 188 women with single persistent HPV infection were diagnosed with CIN3+ (183 with CIN3 and 5 with cervical cancer). Three of the five women who developed cervical cancer had persistent HPV16 infection, and two had persistent HPV18 infection. Among women with single-type oncogenic HPV persistence, HPV16 still conveyed the highest likelihood of progression (43.7%) during follow-up up to 10 years, followed by HPV33 (29.1%) and HPV18 (28.8%).

Absolute risk of CIN3+ according to years of follow-up among women with single type persistence for specific oncogenic HPV types

We estimated the absolute risks of CIN3+ for each HPV type in relation to follow-up time in women with a single persistent HPV type (Fig. 2). The overall absolute risk of CIN3+ in relation to persistence of a single oncogenic HPV type was 15% (95% CI: 13%–18%) after 3 years, 20% (95% CI: 18%–23%) after 5 years and 31% (95% CI: 27%–36%) after 8 years (data not shown). Generally, single persistent HPV types in the alpha 9 group were more likely to progress to CIN3+ than HPV types in the alpha 7 or alpha 5/6 group. Especially HPV16, but also HPV33 and HPV31, in the alpha 9 group were associated with a high absolute risk of CIN3+. The absolute risks of CIN3+ at 3, 5 and 8 years after HPV16 persistence were 28% (95% CI: 22%–34%), 37% (95% CI: 31%–45%) and 55% (95% CI: 45%–66%), respectively. After 8 years of follow-up, the absolute risks of CIN3+ were 33% (95% CI: 20%–50%) after persistent HPV33 and 31% (95% CI: 21%–46%) after persistent HPV31. The absolute risk of CIN3+ after HPV52 was also relatively high (27%; 95% CI: 15%–45%), whereas that after persistent HPV58 was the lowest in the alpha 9 group (8%; 95% CI: 2%–28%) after 8 years. In the alpha 7 group, persistent HPV18 was associated with the highest absolute risk of CIN3+ after 8 years of follow-up (32%; 95% CI: 20%–48%), followed by HPV45 (21%; 95% CI: 9%–45%). In the alpha 5/6 group, persistent HPV56 and HPV51 had an absolute risk of 3% (95% CI: 0.4%–20%) and 15% (95% CI: 8%–30%), respectively, after 8 years of follow-up.

Comparison of the carcinogenic potential between HPV types taking woman's age and time between HPV tests into account

Table 2 presents the HRs of CIN3+ for 12 single persistent oncogenic HPV types as compared to single persistent

Table 2. Relative risk of CIN3+ unadjusted and adjusted for age at persistence and time between HPV tests for 12 single persistent oncogenic HPV types compared to single persistent HPV16

	Hazard ratio (95% CI)			
HPV type	Unadjusted		Adjusted for age and time between HPV tests	
Alpha 9				
HPV16	1 (Reference)		1 (Reference)	
HPV31	0.41	(0.27–0.63)	0.43	(0.28–0.66)
HPV33	0.58	(0.34–0.99)	0.61	(0.36–1.03)
HPV35	0.28	(0.07–1.14)	0.28	(0.07–1.12)
HPV52	0.31	(0.18–0.53)	0.32	(0.19–0.54)
HPV58	0.15	(0.04–0.62)	0.16	(0.04–0.65)
Alpha 7				
HPV18	0.60	(0.34–1.03)	0.61	(0.36–1.05)
HPV39	0.12	(0.04–0.38)	0.13	(0.04–0.40)
HPV45	0.28	(0.11–0.69)	0.28	(0.11–0.69)
HPV59	0.17	(0.02–1.25)	0.21	(0.03–1.52)
HPV68	0.15	(0.04–0.62)	0.15	(0.04–0.61)
Alpha 5/6				
HPV51	0.24	(0.11–0.51)	0.25	(0.12–0.55)
HPV56	0.05	(0.01–0.38)	0.05	(0.01–0.37)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; CIN3+, cervical intraepithelial neoplasia grade 3 or worse.

HPV16, both unadjusted and adjusted for age at persistence and time between HPV tests defining persistence. In the unadjusted analyses, all the persistent oncogenic HPV types were associated with a lower rate of subsequent CIN3+ than persistent HPV16 and the HRs were significantly decreased for the majority of HPV types. When adjusting for age and time between HPV tests the HRs were virtually unchanged. The adjusted HR was 0.61 (95% CI: 0.36–1.03) for HPV33, 0.61 (95% CI: 0.35–1.05) for HPV18, 0.43 (95% CI: 0.28–0.66) for HPV31 and 0.32 (95% CI: 0.19–0.54) for HPV52. The group of HPV types associated with the lowest rate of CIN3+ as compared to persistent HPV16 included HPV68 (HR = 0.15; 95% CI: 0.04–0.61), HPV39 (HR = 0.13; 95% CI: 0.04–0.40) and HPV56 (HR = 0.05; 95% CI: 0.01–0.37).

We estimated the HRs of CIN3+ additionally taking into account HPV16 status at first and second examination (Table 3). Of the 555 women with one persistent non-HPV16 oncogenic HPV type, 44 women were positive for HPV16 at the first test, and of these 8 developed CIN3+ during follow-up. In addition, 33 women persistent for a non-HPV16 type were also positive for HPV16 at the second test of which only three women developed CIN3+ during follow-up. Among women with a non-HPV16 persistent infection who were positive for HPV16 at the first HPV examination the HRs for HPV18, HPV33, HPV31 and HPV52 were 0.67 (95% CI: 0.28–1.63), 0.66 (95% CI: 0.29–1.07), 0.47 (95% CI: 0.21–1.07) and 0.35 (95% CI: 0.15–0.81), respectively. The types associated with the lowest rates were HPV58 (HR = 0.18; 95% CI: 0.04–0.82), HPV68 (HR = 0.17; 95% CI: 0.03–0.81), HPV39 (HR = 0.14; 95% CI: 0.04–0.54) and HPV56 (HR = 0.06; 95% CI: 0.01–0.46). Taking into account the HPV16 status at the second examination, we found no difference in the pattern between the HPV types in relation to rate of progressing to CIN3 + .

Discussion

This large, population-based prospective study, with a follow-up time up to 10 years, provides estimates of the absolute risk of CIN3+ after type-specific persistent infection with 13 individual oncogenic HPV types. HPV16 was the most prevalent type at enrollment, conveyed the highest risk of persistence[12] and HPV16 persistence also implied the highest risk of subsequent progression to CIN3+, the absolute risk estimated to 55% after 8 years of follow-up. Persistent HPV33, 18 and 31 were also associated with a high risk of CIN3+, followed by a medium-risk group including e.g. HPV52 and HPV45. Our outcome CIN3+ was dominated by CIN3 lesions. However, for HPV types where there is an enrichment of that particular HPV type in CIN3 compared to cervical cancer, which is the case for e.g. HPV 31, 33 and 58, we will tend to overestimate the carcinogenic potential in our study. In contrast, the carcinogenic potential of persistent HPV18 and 45 could be even higher than estimated in the present study, as these types are often more prevalent in cervical cancer than CIN3.[20] Persistent infection with HPV39, 56, 58, or 68 was associated with a lower risk of progression to CIN3+, with an absolute risk of <10% for each genotype after 8 years of follow-up. Some geographical differences may exist as e.g. a study from Taiwan

Table 3. Relative risk of CIN3+ for 12 single persistent oncogenic HPV types compared to single persistent HPV16 taking into account HPV16 status at first and second examination

	Hazard ratio (95% CI)			
HPV type	Adjusted for age, time between HPV tests, and HPV16 status at the first HPV test[1]		Adjusted for age, time between HPV tests, and HPV16 status at the second HPV test[2]	
Alpha 9				
HPV16	1 (Reference)		1 (Reference)	
HPV31	0.47	(0.21–1.07)	0.25	(0.08–0.83)
HPV33	0.66	(0.29–1.54)	0.35	(0.10–1.21)
HPV35	0.30	(0.06–1.41)	0.16	(0.03–0.96)
HPV52	0.35	(0.15–0.81)	0.18	(0.05–0.64)
HPV58	0.18	(0.04–0.82)	0.09	(0.02–0.56)
Alpha 7				
HPV18	0.67	(0.28–1.63)	0.36	(0.10–1.22)
HPV39	0.14	(0.04–0.54)	0.07	(0.02–0.37)
HPV45	0.31	(0.10–0.96)	0.16	(0.04–0.68)
HPV59	0.23	(0.03–1.88)	0.12	(0.01–1.18)
HPV68	0.17	(0.03–0.81)	0.09	(0.01–0.52)
Alpha 5/6				
HPV51	0.28	(0.10–0.77)	0.15	(0.04–0.57)
HPV56	0.06	(0.01–0.46)	0.03	(0.00–0.29)

Abbreviations: CI, confidence interval; HPV, human papillomavirus; CIN3+, cervical intraepithelial neoplasia grade 3 or worse.
[1]Women with a non-HPV16 persistent type who are HPV16 positive at the first HPV test compared to HPV16 persistence.
[2]Women with a non-HPV16 persistent type who are HPV16 positive at the second HPV test compared to HPV16 persistence.

reported a higher risk of cervical cancer after persistent HPV58 compared to other non-HPV16 oncogenic types.[4] This was confirmed in a meta-analysis by Guan *et al.*,[20] who also found that the prevalence of HPV52 in cervical cancer is higher in Africa, than in other parts of the world.[20]

In a model comparing the risk of CIN3+ for the different oncogenic HPV types with that of persistent HPV16, we found that the non-HPV16 oncogenic types all had a lower risk compared to persistent HPV16 when adjusting for age and time between HPV tests defining persistence.

Based on the unique carcinogenic potential of HPV16, we investigated to what extend the association with CIN3+ among women with a persistent HPV type other than HPV16 was influenced by either having cleared an HPV16 infection between the first and second examination or acquired an HPV16 infection at the second examination. We found that the hierarchical order of the relative carcinogenicity of the persistent non-HPV16 types was virtually unchanged when taking into account if the women had the ability to clear an HPV16 infection between the first and second HPV test or were HPV16 positive at the second examination.

Previous studies have confirmed the critical role of type-specific HPV persistence in predicting the subsequent risk of CIN3+, but few studies have examined the risk of cervical neoplasia or cancer after persistence of a broad spectrum of individual HPV types. Previously, we found in a cohort of young Danish women (20–29 years) that the probability of progression to CIN3+ was greatest for persistent HPV16, followed by HPV33, 31 and 18.[3] The high carcinogenic potential of these HPV types has been reported in other studies.[4–6] In the present study, we followed a larger number of women of all ages with type-specific persistent HPV infection and examined 13 oncogenic HPV types individually. Furthermore, we were able to adjust for age and time between the HPV tests defining persistence, and in addition, when evaluating the risk associated with the non-HPV16 types to take into account the possible effect of having cleared a prevalent HPV16 infection detected at the first HPV test or having acquired an HPV16 infection between the first and second examination. Some of the previous studies had small study populations or examined fewer oncogenic HPV types. Moreover, some studies did not control for potential confounding between HPV types as we were able to by exclusively looking at single type persistent HPV infections.[3–8]

Three prophylactic vaccines against HPV are currently commercially available. The bivalent vaccine protects against HPV types 16 and 18[21] and the quadrivalent vaccine against HPV types 6, 11, 16 and 18.[22] Both vaccines have shown to be highly efficacious against HPV16/18-associated CIN3 in clinical trials,[23,24] and real-world effectiveness has also been documented.[25] In 2006, the quadrivalent vaccine was licensed in Denmark and subsequently implemented in the free-of-charge

general childhood vaccination program for girls aged 12 years. The newest nine-valent HPV vaccine covers the four HPV types included in the quadrivalent vaccine plus five additional carcinogenic types (HPV31, 33, 45, 52 and 58). The HPV types in the nine-valent vaccine are among the HPV types we identified as having the greatest carcinogenic potential. Single persistent infection with HPV16, 18, 31, 33, 45, 52, or 58 together accounted for 172 of the 188 cases (91%) of CIN3 + diagnosed in our study.

The strengths of this population-based cohort study include the large population of women with type-specific HPV persistence, covering a wide age range. Because of this large study population, we were able to restrict our analyses to single persistent HPV infections and thereby limit possible confounding among genotypes. In addition, as the women were followed in the Pathology Databank, which has virtually 100% coverage,[15] we had minimal loss to follow-up. Furthermore, the follow-up period after HPV persistence was relatively long: up to 10 years. The study also had some limitations. Some of the HPV infections that were defined as persistent could have been reinfections with the same HPV type, which might imply an underestimation of the risk of CIN3+ after persistence. Also, we had no information on the HPV genotype that caused the CIN3 or cervical cancer diagnosed during follow-up.

This large prospective cohort study provides unique knowledge about the risk of CIN3+ after persistence of 13 individual HPV types that are currently classified as oncogenic. The carcinogenic potential of the persistent HPV types differed significantly, with an 8-year absolute risk varying from 3% to 55%. HPV16 is not only the most prevalent HPV type; it is also the type with the highest risk of persistence, and given persistence, the type which implies the highest risk of high-grade cervical lesions. Based on our results it was possible to group the HPV types according to their individual carcinogenic potential. Thus, among 13 individual oncogenic HPV types, persistent infection with HPV16 was associated with the by far greatest potential for progression to CIN3+. This was followed by another high-risk group containing HPV18 and HPV33, a medium risk group comprising HPV31, HPV52 and HPV45, and finally a lower risk group containing HPV types 35, 51, 59, 58, 68, 68 and 56. Our results add to the current knowledge about the natural history of HPV genotypes in relation to persistence and progression, and this may inform the clinically use of HPV testing.

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