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Research Article

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Long-Term Risk for Noncervical Anogenital Cancer in Women with Previously Diagnosed High-Grade Cervical Intraepithelial Neoplasia: A Danish Nationwide Cohort Study

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Abstract

Background: High-risk human papillomavirus (HPV) is essential for developing high-grade cervical intraepithelial neoplasia (CIN2 and CIN3) and has also been associated with noncervical anogenital cancers. However, limited knowledge exists about the long-term risk for anal, vulvar, and vaginal cancer following CIN2 or CIN3 diagnosis.

Methods: In a nationwide cohort study, we followed nearly 2.8 million women born in 1918–1990 who were recorded as living in Denmark between January 1, 1978 and December 31, 2012. The cohort was linked to multiple nationwide registers to obtain information on cancer diagnoses and confounders. Follow-up started when the women reached 18 years, date of immigration, or January 1978, and continued until emigration, death, December 31, 2012, or the date of first diagnosis of anogenital or rectal cancer.

Results: Women with a history of CIN2 or CIN3 had higher risks for subsequent anal, vulvar, and vaginal cancer than women with no such history. The relative risks were higher for CIN3 than CIN2. No excess risk was found for rectal cancer. Analyses in which time since first CIN3 was taken into account showed increased relative risks for anal [HR = 4.8; 95% confidence interval (CI), 3.3–7.0], vulvar (HR = 3.2; 95% CI, 2.0–5.3), and vaginal (HR = 5.5; 95% CI, 2.4–12.3) cancers ≥25 years after CIN3 diagnosis.

Conclusion: Women with a history of CIN2 or CIN3 have a long-term increased relative risk for developing anal, vulvar, and vaginal cancer due to an impaired ability to control a persistent HPV infection.

Impact: This finding adds to our understanding of the relation between HPV infection and noncervical anogenital cancer. *Cancer Epidemiol Biomarkers Prev; 25(7); 1090–7. ©2016 AACR.*

Introduction

It is well established that persistent genital infection with high-risk human papillomavirus (HPV) is an essential factor for the development of cervical cancer and high-grade cervical intraepithelial neoplasia (CIN; refs. 1–3). CIN grades 2 and 3 (CIN2 and CIN3) are regarded as high-grade precursor lesions; it is estimated that high-risk HPV is responsible for approximately 10 million cases of CIN2 or CIN3 each year globally (4).

High-risk HPV has also been causally linked to other anogenital cancers, including anal, vulvar, and vaginal cancers. HPV DNA is found in 83%–95% of anal cancers, 60%–65% of vaginal cancers, and 20%–50% of all types of vulvar cancer (5). Other risk factors for anogenital cancers include factors such as lower socioeconomic status and smoking (6–8).

Thus, high-grade CIN and anogenital cancer share a common etiology. Still, there is relatively limited knowledge about the long-term risk for noncervical anogenital cancer in women with a previous diagnosis of CIN2 or CIN3. Some studies have shown that women with high-grade CIN have increased risks for anal, vulvar, and vaginal cancers (9–14). Several of these studies, however, have limitations, such as small study populations, limited follow-up time, limited number of outcomes and, especially, lack of adjustment for potential confounders. Further information in the area would increase understanding of the natural history of genital HPV infection and its consequences and could also indicate if there should be an increased awareness on the subsequent risk for other anogenital cancers in women previously diagnosed with high-grade CIN.

Thus, we conducted a population-based, nationwide cohort study in which we followed nearly 2,800,000 women for up to 34 years. The aim of the study was to assess the long-term risks in women with a history of CIN2 or CIN3 for primary noncervical, HPV-associated anal, vulvar, and vaginal cancers. As a comparison, we also investigated their risk for rectal cancer, which is not related to HPV infection.

Materials and Methods

Study population

All Danish citizens are assigned a unique personal identification number, which is used in national population and

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health databases and allows accurate linkage of data between registers. The Danish Civil Registration System contains the personal identification number and variables such as date of birth, death, and immigration (15). Initially, we identified 2,791,389 women born between 1918 and 1990, who were alive and recorded as living in Denmark between January 1, 1978 and December 31, 2012. We excluded 21,588 women from the study population because a cancer diagnosis (except nonmelanoma skin cancer) was registered in the Danish Cancer Registry before the start of follow-up. The final study population consisted of 2,769,801 women.

Exposure assessment

By linking the women's personal identification numbers to the Danish Cancer Registry and the Pathology Data Bank, we obtained information on histologically verified CIN2 (i.e., moderate dysplasia) or CIN3 (i.e., severe dysplasia and cancer *in situ* of the cervix) at individual level.

The Danish Cancer Registry was established in 1943 as a nationwide registry; it contains information about precancerous lesions, cancer types, topography, and morphology and is supplemented by linkage to the Causes of Death Registry and the National Patient Registry to ensure complete registration (16). The Pathology Data Bank contains information on all cytologic and histologic examinations, including topography and morphology, from all Danish pathology departments since the mid-1990s. In addition, most pathology departments have transferred information to the Pathology Data Bank from the mid-1990s back to 1978, but data from this period are not entirely complete (17). By using both registers, we increased completeness of data. Women who were registered in both registers were included only when CIN2 or CIN3 was first diagnosed. In addition, CIN3 lesions were divided into the histologic subtype; adenocarcinoma *in situ* (AIS) and squamous cell lesions.

A total of 52,135 women were diagnosed with CIN2 and 104,155 women had a history of CIN3.

Ascertainment of outcome

To obtain information on subsequent anogenital cancer including anal, vulvar, and vaginal cancer, by the ICD-10 codes C20.9, C21, C51, and C52, the cohort was linked to the Danish Cancer Registry. The cancers were limited to invasive squamous cell carcinomas, defined by morphologic subtypes 8050–8084 and 8120–8131, which are associated with HPV infection. The women were also followed for rectal cancer (ICD-10 code C20) and all histologic types except squamous cell carcinomas were considered as rectal cancer.

Ascertainment of educational level and smoking status

From Statistics Denmark, we extracted information on education level from 1981. It was reported as basic, vocational, higher, or unknown. We also obtained information on smoking from the Danish Medical Birth Register for a subcohort. This registry contains information on all women who have given birth in Denmark since 1973; from 1997, information on smoking status was added, as registered during antenatal care. The subcohort consisted of 515,814 women, of whom 30,225 had a history of CIN3. It was only possible to adjust for smoking habits for CIN3 as exposure because of too few events following CIN2 diagnosis.

Statistical analysis

Follow-up started when the women reached 18 years, date of first immigration or January 1978, whichever occurred latest. Women were followed until emigration, death, December 31, 2012 or the date of the first diagnosis of anogenital or rectal cancer; only first diagnosis was used because treatment might change the risk for subsequent anogenital cancer. Sensitivity analyses were conducted without censoring the women at the first anogenital cancer. As the results were similar, only results following site-specific censoring are described. As a history of CIN2 or CIN3 was treated as a time-dependent covariate, the women contributed person–years as unexposed until their first registered CIN2 or CIN3 diagnosis.

Cox proportional hazard model was used to estimate HRs and corresponding 95% confidence intervals (CI) for the association between CIN2 or CIN3 and anal, vulvar, vaginal, and rectal cancer. In all analyses, attained age was the underlying time scale, and all were stratified by birth year in 1-year intervals. Time since exposure was analyzed in strata, defined as time since first CIN3 diagnosis (<1 year, 1–4 years, 5–9 years, 10–14 years, 15–20 years, 20–24 years, $\geq$25 years, and $\geq$1 year). Because of fewer events following CIN2 diagnosis, wider time intervals were used (anal, vulvar, and rectal cancer: 0–9 years, 10–19 years, $\geq$20 years, and $\geq$1 year; and vaginal cancer: 0–9 years, $\geq$10 years, and $\geq$1 year). It was not possible to stratify by birth cohort in this analysis for vaginal cancer due to lack of convergence. Calendar period (1978–1989, 1990–2001, and 2002–2012), age at exposure ($\leq$28 years, 29–36 years, and $\geq$37 years) and attained age (18–39 years, 40–49 years, 50–59 years, and $\geq$60 years) were all treated as time-dependent covariates. Age-specific cancer risk was visualized on graphs of the incidence rates of anal, vulvar, vaginal, and rectal cancer in age-specific groups (18–39, 40–49, 50–59, and $\geq$60 years) for women with and without a history of CIN2 or CIN3. The incidence rates and CIs were calculated on the basis of the assumption that the expected number of cancer cases follows a Poisson distribution. All analyses for CIN3 as exposure were also conducted with the follow-up period starting in 1995 where information from the Pathology Databank is known to be complete. As the results were virtually identical to the original analysis, the results are only presented with the longer follow-up (i.e., starting in 1978).

In selected analyses, we adjusted for education level and smoking status. Education level was used as a time-dependent covariate, which changed at three age intervals. The education level at age 18 was used for the age range 18–24, that at age 25 for ages 25–29 years, and that at 30 years for ages $\geq$ 30 years. If information about education level was missing for 1 year, the next year within the age interval was searched. Information about smoking habits was included as a binary variable.

The tests were conducted as Wald tests, and *P* values below 5% were considered statistically significant. All analyses were performed in R version 3.0.1 (18). The study protocol was approved by the Danish Data Protection Agency.

Results

The study population consisted of 2,769,801 women. CIN2 was diagnosed in 52,135 women and 104,155 women had a history of CIN3, contributing a total follow-up time of 597,467 person–years and 1,529,564 person–years, respectively. The total follow-up time of women with no history of CIN2 or CIN3 was

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Table 1. Number of person–years of follow-up according to characteristics of the study population

	No history of CIN2/3 (2,769,801 women)	Previous CIN2 (52,135 women)	Previous CIN3 (104,155 women)
	Person–years of follow-up		
Total years of follow-up	62,926,843	597,467	1,529,569
Educational level			
Basic	10,260,251	97,512	227,784
Vocational	36,603,714	363,533	974,273
Higher	9,490,451	120,488	283,152
Unknown	3,163,697	9,958	30,608
Calendar period			
1978–1989	18,560,834	67,688	186,804
1990–2001	22,338,414	211,399	523,176
2002–2012	22,027,595	318,380	819,589
Attained age (years)			
18–39	27,160,340	246,049	566,036
40–49	11,913,251	175,005	465,723
50–59	10,620,652	113,194	309,606
≥60	13,232,600	63,219	188,204

62,926,843 person–years. The mean age at diagnosis of CIN2 was 33.8 years (range, 9.0–92.6) and for CIN3 it was 34.0 years (range, 10.7–92.6). The follow-up times for exposed and unexposed women according to level of education, calendar period, and attained age are presented in Table 1. In the subcohort analysis, in which information on smoking was available, more women with a history of CIN3 reported that they were former or current smokers (36.6%) than women with no history of CIN3 (21.2%).

A relevant anogenital cancer (anal, vulvar, and vaginal cancer) developed in 66 women with CIN2 (32 anal, 23 vulvar, and 11 vaginal cancer) and in 293 women with CIN3 history (125 anal, 103 vulvar, and 65 vaginal cancer). For women in the unexposed group 2,621 developed an anogenital cancer (125 anal, 103 vulvar, and 65 vaginal cancer). In the total group of 1,369 vulvar squamous cell carcinomas, the majority (1,224 = 89%) were registered as vulvar cancer not specified and only 118 and 27 vulvar cancers were registered as, respectively, keratinizing, or nonkeratinizing tumors. Rectal cancer was diagnosed in 68 women with a history of CIN2, 200 women with a CIN3 history and 10,639 unexposed women.

Among the 104,155 women diagnosed with CIN3 a total of 101,407 women had a squamous cell lesion and 2,748 were diagnosed with AIS. No women with AIS subsequently developed anal cancer or vulvar cancer. Less than 5 women developed vaginal cancer after being diagnosed with AIS.

We estimated the age-specific incidence rates of anal, vulvar, vaginal, and rectal cancer in women with and without a history of CIN2 or CIN3 (Fig. 1). Generally, women with a history of CIN2 and especially CIN3 had a higher age-specific crude incidence of all HPV-related cancers than women without such a history. The incidence of anal, vulvar, and vaginal cancer was higher for women diagnosed with CIN3 compared with CIN2. In women ages ≥60 years, the incidences of anal cancer were 24.7 for women with a history of CIN3 and 4.2 for unexposed women, those of vulvar cancer were 11.8 and 5.9, respectively, and those of vaginal cancer were 18.8 and 1.4, respectively. For women with a history of CIN2, the incidences in the age category ≥60 years were 16.0, 6.4, and 9.6 for anal, vulvar, and vaginal cancer, receptively. Women with a history of CIN2 or CIN3 did not have a significantly higher age-specific crude incidence of rectal cancer than women with no such history.

The age-adjusted HRs for anal cancer (HR = 2.9; 95% CI, 2.0–4.1), vulvar cancer (HR = 2.5; 95% CI, 1.6–3.8), and vaginal cancer (HR = 8.1; 95% CI, 4.4–15.0) were all significantly higher in women with a history of CIN2 than in those without (Table 2). For women diagnosed with CIN3, the relative risks for anal (HR = 4.2; 95% CI, 3.4–5.0), vulvar (HR = 4.0; 95% CI, 3.2–4.9), and vaginal (HR = 17.1; 95% CI, 12.8–22.9) cancers all tended to be higher than those for women with CIN2. In contrast, no significant increase in risk was seen for rectal cancer for women with a CIN2 (HR = 1.0; 95% CI, 0.8–1.3) or CIN3 (HR = 1.0; 95% CI, 0.9–1.2) history compared with unexposed women. The relative risk estimates were virtually unchanged after additional adjustment for educational level.

We also estimated risk according to follow-up time since the CIN2 or CIN3 diagnosis (Table 3). For all cancers except rectal cancer, there were strongly increased risks within the first year after CIN3 diagnosis (anal cancer: HR = 7.0; 95% CI, 2.6–18.8; vulvar cancer: HR = 11.1; 95% CI, 5.3–23.5; vaginal cancer: HR = 89.1; 95% CI, 45.0–176.4) compared with unexposed women. However, the risks for these cancers did not differ from the overall relative risk estimates presented in Table 2 after exclusion of the first year after diagnosis. Although the risk estimates in the subsequent periods for time since CIN3 diagnosis were lower, they were still high and significantly increased. Twenty-five years or more after a CIN3 diagnosis, the HRs for anal, vulvar, and vaginal cancer were 4.8 (95% CI, 3.3–7.0), 3.2 (95% CI, 2.0–5.3), and 5.5 (95% CI, 2.4–12.3), respectively. Analyses of time since diagnosis of CIN2 showed the same risk pattern as that of women with CIN3, but the relative risks were lower. The relative risk for anal cancer (HR = 3.5; 95% CI, 2.0–5.9) and vulvar cancer (HR = 2.6; 95% CI, 1.3–5.2) was increased for ≥20 years after CIN2 diagnosis and for vaginal cancer (HR = 5.0; 95% CI, 2.2–11.2) the risk was increased for ≥10 years after diagnosis of CIN2. The relative risk estimates for rectal cancer were virtually unchanged when time since diagnosis of CIN2 or CIN3 was taken into account.

Furthermore, we analyzed the time trend of risk for anogenital cancers and rectal cancer after diagnosis of CIN2 or CIN3 since

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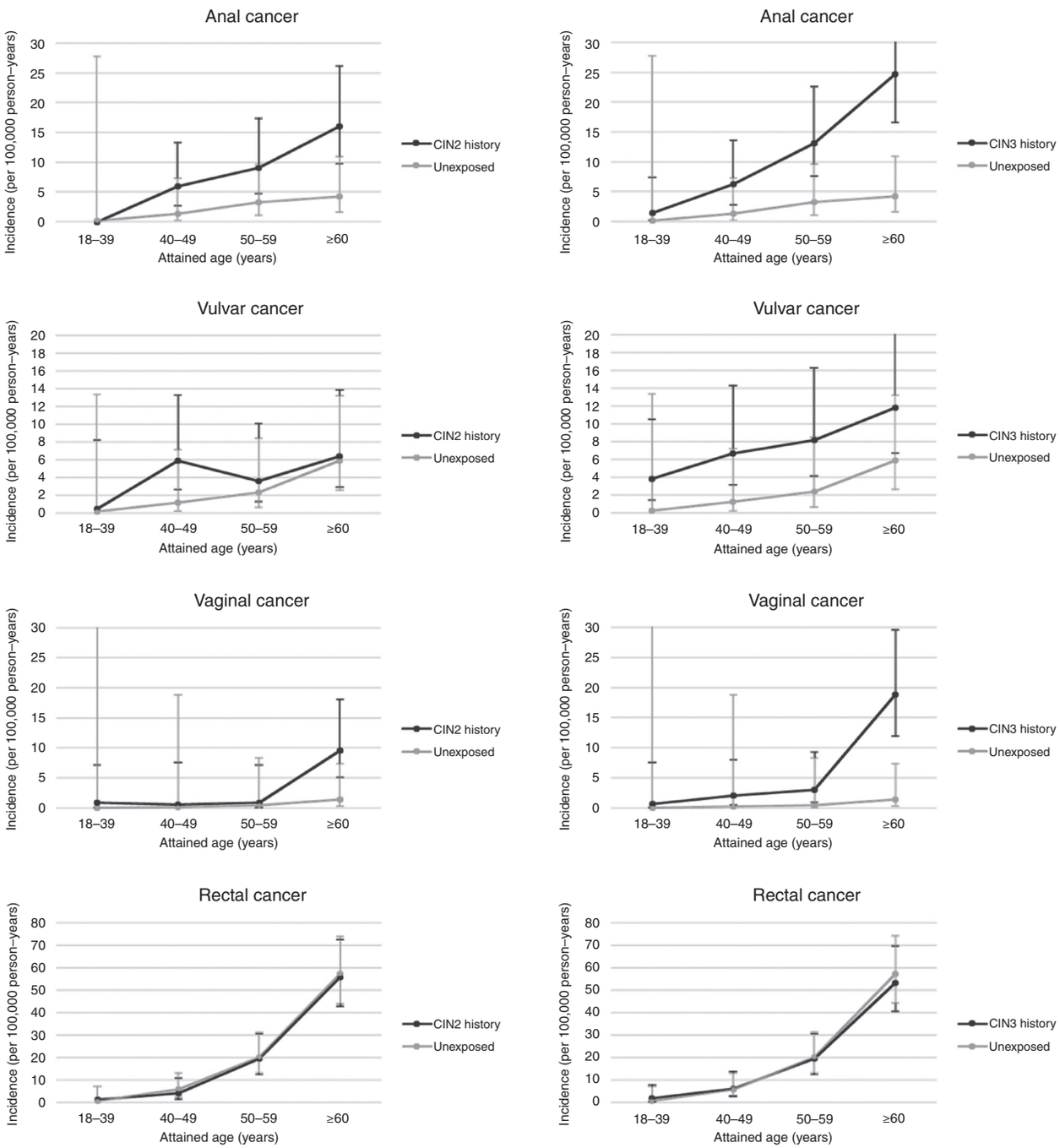

Figure 1.
Age-specific crude incidences of anal, vulvar, vaginal, and rectal cancers plotted against attained age of women with and without a history of CIN2 or CIN3. Note that the *y*-axis scale for the graphs differs for the anogenital cancers.

1978. The HR was high in all calendar periods, and no significant difference or pattern was seen for any of the anogenital cancers or rectal cancer (data not shown). We also considered the effect of age at diagnosis of CIN2 or CIN3 on the relative risks for anal, vulvar, and vaginal cancer. HR was high regardless of age at diagnosis, and no marked variation was observed (data not shown).

Finally, we looked at the subcohort for whom adjustment for smoking habits was possible (i.e., women who had delivered a child in or after 1997). In this subcohort, anal cancer developed in 31 women, vulvar cancer in 29, and rectal cancer in 129; as fewer than 5 women had vaginal cancer, we could not analyze the effect of smoking habits on this cancer. After adjustment for smoking, a history of CIN3 remained significantly associated with anal cancer

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Table 2. HRs for anal, vulvar, vaginal, and rectal cancer according to history of CIN2 or CIN3

	No. of women in the study population	No. of events	HR (95% CI)	
			Adjusted for age	Adjusted for age and education
Anal cancer				
No CIN2/3 history	2,626,551	1,105	1.00	1.00
CIN2	52,135	32	2.9 (2.0–4.1)	2.8 (2.0–4.1)
CIN3	104,155	125	4.2 (3.4–5.0)	4.1 (3.4–5.0)
Vulvar cancer				
No CIN2/3 history	2,626,551	1,243	1.00	1.00
CIN2	52,135	23	2.5 (1.6–3.8)	2.5 (1.6–3.7)
CIN3	104,155	103	4.0 (3.2–4.9)	3.9 (3.2–4.8)
Vaginal cancer				
No CIN2/3 history	2,626,551	273	1.00	1.00
CIN2	52,135	11	8.1 (4.4–15.0)	8.3 (4.5–15.5)
CIN3	104,155	65	17.1 (12.8–22.9)	17.4 (13.0–23.2)
Rectal cancer				
No CIN2/3 history	2,626,551	10,639	1.00	1.00
CIN2	52,135	68	1.0 (0.8–1.3)	1.0 (0.8–1.3)
CIN3	104,155	200	1.0 (0.9–1.2)	1.0 (0.9–1.2)

(HR = 4.1; 95% CI, 3.4–5.0) and vulvar cancer (HR = 2.7; 95% CI, 1.0–7.1). No substantial change in the relative risk for rectal cancer was seen after adjustment for smoking (HR = 0.7; 95% CI, 0.3–1.8).

Discussion

In this nationwide cohort study, we found that women with a diagnosis of CIN2 or CIN3 are at increased risk for subsequent noncervical HPV-associated cancers of the anogenital region (anal, vulvar, and vaginal cancer). Importantly, this increased risk appears to be long lasting, as it persisted for 25 years or more after the CIN3 diagnosis. In contrast, a CIN2 or CIN3 diagnosis was not related to the subsequent risk of rectal cancer, which may be expected, as this cancer is not associated with HPV infection.

Several studies have addressed the risk for anogenital cancers among women with CIN (9–14); however, in most, standardized incidence ratios were used to compare the risk for anogenital cancers in cohorts of women with cervical neoplasia with that of the general population (9, 11–14). Such comparisons control for potential confounding by age and calendar time but do not take into account common risk factors such as socioeconomic status and smoking habits. Furthermore, many of the studies were limited by small study populations and a short follow-up time. Our results are consistent with those of a Swedish cohort study, the only other study in which adjustment was made for confounders such as smoking and socioeconomic status (10). In that study, the adjusted risks for anal, vulvar, and vaginal cancer were increased after 10 or more years of follow-up after a CIN3 diagnosis (10). A similar pattern is described in other studies (9, 12–14), but the results are not consistent (11).

High-risk HPV infection is an essential factor for the development of both CIN and certain anogenital cancers (1, 5), and the presence of high-grade CIN is a good proxy for persistent high-risk HPV infection. This strong common etiologic factor probably explains why women with a history of CIN2 or CIN3 are at significantly increased risk for anal, vulvar, and vaginal cancer. CIN2 is, however, a more heterogeneous lesion than CIN3 and has more equivocal carcinogenic potential (19). This would explain why the relative risk for anogenital cancers was higher in women diagnosed with CIN3. In noncervical anogenital cancer, HPV16 is the most frequently detected HPV type. HPV16 is also more frequent and more likely persistent in CIN3 compared with CIN2 (19, 20) and could therefore contribute to the higher relative risk for anal, vulvar, and vaginal cancer following a CIN3 diagnosis observed in the study. In cervical high-grade lesions of squamous cell type, HPV16 is also dominating, whereas in AIS, HPV18 is the most frequent type. In line with this, we found that all anal and vulvar cancers developed in women with high-grade squamous cell lesions and none where observed among women diagnosed with AIS. Women diagnosed with CIN2 or CIN3 were not at increased risk for rectal cancer, in agreement with previous reports of lack of an association between HPV infection and this type of cancer (21, 22).

Various mechanisms might explain why the risks for these cancers are increased. HPV infection is a common sexually transmitted infection (23), but most infections are transient, and the great majority of women clear their infection within 1 to 2 years of acquisition. A small proportion of women, however, are unable to clear the virus (24). It has been suggested that women with persistent high-risk HPV infection might have an immune dysregulation that results in an inadequate immune response to HPV infection, leading to an increased risk for persistence of the virus. This, together with the fact that studies in both women and men have shown that genital infection with HPV often affects the entire anogenital region (25, 26), could lead to a long-term increased risk for anogenital cancers. Thus, it is not unexpected that, even if the persistent infection responsible for the precursor lesion is usually removed by treatment, HPV might still be present in the anogenital region and could give rise to other anogenital cancers. The fact that the risk following CIN3 is more substantial than following CIN2 taken together with the finding of no increased risk for subsequent rectal cancer points to an impaired ability to control a persistent HPV infection and are likely not more sensitive to develop other non-HPV–associated cancers.

Increased surveillance of women with newly diagnosed CIN3 could explain the particularly high risks for anal, vulvar, and vaginal cancers in the first year after CIN3 diagnosis; however, the risk was still significantly increased after exclusion of the first year after the diagnosis in our study. Therefore, surveillance bias cannot explain our results. In addition, the risk for an anogenital cancer remained high after 25 years or more of follow-up after CIN3 diagnosis.

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Table 3. HRs adjusted for age and education for anal, vulvar, vaginal, and rectal cancers stratified by time since CIN2 or CIN3 diagnosis

CIN2				CIN3			
Time since diagnosis	**Follow-up (pyrs)**	**No. of events**	**HR (95% CI)**	**Time since diagnosis**	**Follow-up (pyrs)**	**No. of events**	**HR (95% CI)**
Anal cancer				Anal cancer			
≥1 year	555,788	30	2.7 (1.9–3.9)	≥1 year	1,432,075	121	4.1 (3.3–4.9)
0–9 years	317,761	5	1.7 (0.7–4.2)	<1 year	97,494	4	7.0 (2.6–18.8)
10–19 years	189,169	13	3.0 (1.7–5.2)	1–4 years	350,050	8	3.0 (1.5–6.0)
≥20 years	90,536	14	3.5 (2.0–5.9)	5–9 years	354,265	19	4.3 (2.7–6.8)
				10–14 years	276,198	11	2.0 (1.1–3.6)
				15–19 years	202,326	29	4.8 (3.3–7.0)
				20–24 years	134,332	26	4.9 (3.3–7.3)
				≥25 years	114,904	28	4.8 (3.3–7.0)
Vulvar cancer				Vulvar cancer			
≥1 year	555,788	19	2.1 (1.3–3.3)	≥1 year	1,432,075	96	3.7 (3.0–4.6)
0–9 years	317,761	8	2.9 (1.5–5.9)	<1 year	97,494	7	11.1 (5.3–23.5)
10–19 years	189,169	7	2.0 (0.9–4.2)	1–4 years	350,050	14	4.8 (2.8–8.2)
≥20 years	90,536	8	2.6 (1.3–5.2)	5–9 years	354,265	11	2.6 (1.4–4.7)
				10–14 years	276,198	16	3.3 (2.0–5.4)
				15–19 years	202,326	18	3.7 (2.3–6.0)
				20–24 years	134,332	21	5.1 (3.3–7.9)
				≥25 years	114,904	16	3.2 (2.0–5.3)
Vaginal cancer				Vaginal cancer			
≥1 year	555,788	10	7.9 (4.1–15.0)	≥1 year	1,432,075	56	15.4 (11.3–20.9)
0–9 years	317,761	5	10.0 (4.1–24.5)	<1 year	97,494	9	89.1 (45.0–176.4)
≥10 years	279,705	6	5.0 (2.2–11.2)	1–4 years	350,050	10	22.5 (11.8–43.1)
				5–9 years	354,265	12	18.2 (10.1–33.0)
				10–14 years	276,198	8	10.5 (5.1–21.3)
				15–19 years	202,326	13	15.9 (9.1–28.0)
				20–24 years	134,332	7	9.1 (4.3–19.3)
				≥25 years	114,904	6	5.5 (2.4–12.3)
Rectal cancer				Rectal cancer			
≥1 year	555,788	67	1.0 (0.8–1.3)	≥1 year	1,432,075	196	1.0 (0.9–1.2)
0–9 years	317,761	11	0.7 (0.4–1.2)	<1 year	97,494	4	1.1 (0.4–3.1)
10–19 years	189,169	29	1.2 (0.9–1.8)	1–4 years	350,050	24	1.5 (1.0–2.3)
≥20 years	90,536	28	1.0 (0.7–1.5)	5–9 years	354,265	16	0.6 (0.4–1.0)
				10–14 years	276,198	31	1.0 (0.7–1.4)
				15–19 years	202,326	43	1.2 (0.9–1.7)
				20–24 years	134,332	31	0.9 (0.6–1.3)
				≥25 years	114,904	51	1.1 (0.8–1.4)

Abbreviation: pyrs, person–years.

Anogenital cancer and high-grade CIN have other common risk factors, such as socioeconomic status and smoking habits (6–8). The relative risk remained high and statistically significant for all anogenital cancers in the analysis adjusted for socioeconomic status and changed only slightly after further adjustment for smoking habits. Therefore, smoking appeared to explain only a minor part of the effect. As information on smoking habits was available for only a subgroup of women who gave birth between 1997 and 2012, the results should be interpreted with caution and might not be generalizable to all women.

The treatment modalities for high-grade CIN have changed over time. A previous study found increased risks for vaginal and cervical cancer after high-grade CIN in the calendar period 1996–2002 (9), but we found no difference in the relative risk for anogenital cancers after taking calendar period into account. This indicates that a difference in treatment modality is not strongly related to subsequent development of anal, vulvar, or vaginal cancer.

We found that the risk for anal, vulvar, or vaginal cancer was increased regardless of the age at diagnosis of high-grade CIN. In addition, women with a history of CIN2 or CIN3 had a higher age-specific incidence of all HPV-related cancers than women without such a history. This might suggest that women of all ages previously diagnosed with high-grade CIN could potentially benefit from an increased awareness from the general practitioners and gynecologists on other HPV-associated anogenital cancers because they seem to be at higher risk compared with women with no such history. Still, more studies are needed to clarify whether a special follow-up strategy to detect early stages of anogenital cancer would be relevant for women previously diagnosed with high-grade CIN. The follow-up programmer might depend on the grade of CIN, HPV vaccination status and other risk factors; however, cost-effectiveness studies are necessary to define an optimal follow-up strategy.

The strengths of our nationwide cohort study are the large study population, the prospective design and the long follow-up time. As all Danish citizens have a unique personal identification number that is registered in all nationwide registers, we had virtually complete follow-up. In addition, we consider that the validity of our outcome is high because the Danish Cancer Registry has virtually complete, accurate diagnoses (27, 28). In addition, by combining the Pathology Data Bank and the Danish

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Cancer Registry, we increased the completeness of our exposure data. Furthermore, in the sensitivity analysis in which follow-up was started in 1995, the results were virtually identical, indicating minimal misclassification at the beginning of the study period. Another strength of the study is that we adjusted for confounding from smoking habits and socioeconomic status.

The limitations include the fact that registration of exposure is not entirely complete from all Danish pathology departments until the mid-1990s; therefore, older women in the study population could have had a diagnosis of CIN2 or CIN3 before this time which had not been registered, which would tend to an underestimated risk, as the exposed women would be misclassified as unexposed. In addition, we had no information on the HPV type in CIN2, CIN3, or anogenital tumor tissues; therefore, we did not have direct evidence that HPV was the causal factor of subsequent anal, vulvar, and vaginal cancers in women with CIN2 or CIN3. This would, however, appear to be the likely explanation of the observed effect, and it is credible that HPV16, which is the most frequent type in cervical neoplasia, also played a crucial role in the increased cancer risk observed. This HPV type is targeted by prophylactic HPV vaccines (29), which might imply that HPV vaccination could also be used in the prevention of noncervical anogenital cancers. In Denmark, the quadrivalent HPV vaccine has been offered free of charge to 12-year-old girls since January 2009 as part of the children's vaccination program, and catch-up vaccination programs have been initiated to cover 13- to 15-year-old girls (since October 2008) and, most recently, women up to 27 years of age (from August 2012; ref. 30).

In conclusion, our study provides further evidence that women with a history of CIN2 or CIN3 have a long-lasting increased risk for the noncervical HPV-related anal, vulvar, and vaginal cancers, which, in the current study, persisted for 25 years and more after the first recorded diagnosis of CIN3.

Disclosure of Potential Conflicts of Interest

C. Munk was invited by Sanofi Pasteur MSD Denmark to participate in EUROGIN 2013 and 2015. Sanofi Pasteur MSD paid for registration, accommodation, and transportation. S.K. Kjær reports receiving commercial research grants from Merck and is a consultant/advisory board member for Merck and Sanofi Pasteur MSD. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: F.L. Sand, C. Munk, M.F. Svahn, K. Frederiksen, S.K. Kjær

Development of methodology: M.F. Svahn, K. Frederiksen

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Munk, M.F. Svahn, S.K. Kjær

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F.L. Sand, C. Munk, S.M. Jensen, M.F. Svahn, K. Frederiksen, S.K. Kjær

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