

SEXUALLY TRANSMITTED INFECTION AS A CAUSE OF ANAL CANCER

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ABSTRACT

Background The incidence of anal cancer has increased in recent decades, particularly among women. To identify underlying risk factors, we conducted a population-based case-control study in Denmark and Sweden.

Methods We conducted telephone interviews with 324 women and 93 men in whom invasive or in situ anal cancer was diagnosed between 1991 and 1994, 534 controls with adenocarcinoma of the rectum, and 554 population controls. The interviews covered a wide spectrum of possible risk factors for anal cancer. Odds ratios were calculated by logistic regression. Specimens of anal-cancer tissue and samples of rectal adenocarcinomas were tested for human papillomavirus (HPV) DNA with the polymerase chain reaction.

Results Multivariate analysis revealed consistent and statistically significant associations between measures of sexual promiscuity and the risk of anal cancer in both men and women. There was a significant trend toward an association between higher numbers of partners of the opposite sex in women ($P < 0.001$) and men ($P < 0.05$) and strong associations with a variety of venereal diseases. In women, receptive anal intercourse, particularly before the age of 30 years, and venereal infections in the partner were also associated with an increased risk (odds ratios, 3.4 and 2.4, respectively). Fifteen percent of the men with anal cancer reported having had homosexual contact, as compared with none of the controls ($P < 0.001$). High-risk types of HPV, notably HPV-16, were detected in 84 percent of the anal-cancer specimens examined, whereas all rectal-adenocarcinoma specimens tested were negative for HPV.

Conclusions Our study provides strong evidence that a sexually transmitted infection causes anal cancer. The presence of high-risk types of HPV, notably HPV-16 (which is known to cause cancer of the cervix), in the majority of anal-cancer tissue specimens suggests that most anal cancers are potentially preventable. (N Engl J Med 1997;337:1350-8.)

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EPIDERMOID anal cancer is a rare neoplasm of the epithelium lining the anal canal and perianal skin. The incidence of epidermoid anal cancer has increased considerably in recent decades,¹⁻³ most notably among women, unmarried men, and persons living in or near large cities.^{2,3} Early observations suggested that anal cancer was as-

sociated with sexually transmitted diseases or male homosexual contact.⁴ Subsequent case reports^{5,6} and case-control studies⁷⁻⁹ supported these suggestions. A number of observations also link anal cancer to cervical cancer,¹⁰⁻¹² a neoplasm now considered to be caused by certain types of sexually transmitted human papillomaviruses (HPV).¹³ However, apart from the strong association between anal cancer and male homosexual contact or a history of genital warts,^{7,9} there are only inconsistent data on other factors, such as age at first sexual intercourse, number of sexual partners of the opposite sex, receptive anal intercourse, partner's characteristics, and sexually transmitted diseases other than genital warts. We conducted a population-based case-control study in Denmark and Sweden to determine the role of sexual practices and venereal diseases, with a particular emphasis on the role of HPV, in the development of anal cancer.

METHODS

Patients with Anal Cancer

We searched national cancer registries in Denmark and Sweden to identify all incident cases of histologically verified invasive or in situ anal or rectal epidermoid carcinoma (hereafter referred to as anal cancer) reported during the period from 1991 to 1994 (with five cases reported in 1995). Danish patients were considered eligible for the study if the topography code for the tumor was 1541 (rectum), 1542 (anal canal), 1543 (anus), 1548 (anorectum), or 1735 (perianal skin) and if the histology code was 80512 or 80513, between 80702 and 80763, 80812, 80943, between 81203 and 81243, or 85603 (variants of invasive and in situ epidermoid carcinoma), according to the *International Classification of Diseases for Oncology*.¹⁴ Swedish patients were eligible if the topography code was 1541 (anus) or 1540 (rectum) and the histology code was 146 (invasive squamous-cell carcinoma), 144 (in situ squamous-cell carcinoma), or 126 (basaloid carcinoma), according to a national extension of the *International Classification of Diseases, 7th Revision*, coding system.¹

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Controls

We also included two control groups. One group consisted of patients with adenocarcinoma of the rectum, who were chosen as controls to minimize any possible bias due to differential recall between patients with anal cancer and controls. The other control group consisted of persons from the general population. For the men, we tried to match two patients with rectal adenocarcinoma and two population controls to each patient with anal cancer, and for the women, we sought a 1:1:1 match. Controls were frequency-matched within each country for sex and age (in five-year intervals, except for the youngest patients in the cancer control group, because of the rarity of rectal cancer in young persons), and the patients with rectal adenocarcinoma were matched for the year of the diagnosis.

Data Collection

Identical questionnaires (in Danish and Swedish) were used in the two countries. Measures of sexual behavior and venereal infections were scrutinized in detail. Information on other potential risk factors, such as tobacco consumption and anal inflammatory lesions, was also collected (unpublished data), as was information on marital status; education; height; weight; ethnic background; use of alcohol; travel; gynecologic, hormonal, and reproductive factors; medications; radiographs; obstipation; and inflammatory bowel diseases. For respondents who were married or widowed, we sought information about the current or most recent spouse. For unmarried, separated, or divorced respondents who were in stable heterosexual relationships at the time of the study, identical questions were asked about current partners.

Before inviting subjects to participate in the study, we obtained permission to contact the patients from the physicians responsible for their treatment. All interviews were conducted by medically trained interviewers (medical students and research nurses), who were unaware of the specific study hypotheses. The study was approved by ethics committees in Sweden and Denmark.

Tissue Analyses

Paraffin-embedded specimens of anal cancer and rectal adenocarcinomas were collected from over 60 pathology laboratories in Denmark and Sweden. Tumor specimens (and samples of liver tissue that served as negative controls) were analyzed for HPV with the polymerase-chain-reaction (PCR) assay. From each specimen, 4- μ m sections were digested in 250 μ l of proteinase K mix consisting of 10 mM TRIS-hydrochloride (pH 7.4), 0.45 percent Tween 20, and 100 μ g of proteinase K per milliliter (Boehringer Mannheim, Mannheim, Germany) at 37°C for 20 to 24 hours.¹⁵ Samples were then treated at 100°C for 10 minutes and centrifuged; 10 μ l of the supernatant was used for subsequent PCR. Initially, the samples were subjected to a human globin-specific PCR assay to exclude those without analyzable target DNA for the analysis of HPV. PCR was performed with the use of a general primer (GP5+/GP6+) to amplify approximately 150 base pairs of a broad spectrum of mucosotropic HPV genotypes, as previously described,¹⁶ except that the GP6+ primer was biotinylated.¹⁷ The PCR products were captured on streptavidin-coated microwells, denatured by alkaline treatment, and hybridized to two probe cocktails of digoxigenin-labeled specific oligonucleotides, one comprising 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and the other comprising 6 low-risk HPV types (6, 11, 40, 42, 43, and 44). Samples that were positive for HPV were subjected to individual typing for high-risk HPV types 16, 18, 31, and 33 and for low-risk HPV types 6 and 11.¹⁷

Statistical Analysis

Because of differences in the epidemiology of anal cancer in the two sexes,¹⁻³ men and women were analyzed separately. Relative risks were estimated as odds ratios. First, univariate odds ratios were calculated, with adjustment only for age (<40, 40 to 49, 50

to 59, 60 to 69, 70 to 79, or \geq 80 years), country (Denmark or Sweden), and year of diagnosis (1991, 1992, 1993, or 1994-1995). A pseudo-year of diagnosis was assigned to the population controls according to the distribution of the year of diagnosis among patients of the same sex with anal cancer.

Multivariate logistic regression was performed to evaluate independent predictors of the risk of anal cancer. Because statistically significant univariate odds ratios for the two control groups were generally similar, we combined them in the multivariate analyses to increase the statistical power. The multivariate odds ratio for each variable was adjusted for the lifetime number of partners of the opposite sex (0, 1, 2 or 3, 4 through 9, and \geq 10), smoking status (current smoker, former smoker, or nonsmoker), years of school (<10 or \geq 10) and years of postgraduate education (none, \leq 3, or >3), age at diagnosis (<40, 40 to 49, 50 to 59, 60 to 69, 70 to 79, or \geq 80 years), country (Denmark or Sweden), and year of diagnosis (1991, 1992, 1993, or 1994-1995). Finally, we examined statistically significant associations for possible differences between the two countries by introducing an interaction term in the multivariate model. All regression analyses were performed with the use of likelihood-ratio tests by means of the GENMOD procedure in SAS. In tests for trend, we treated categorized continuous variables as continuous variables, with the median for each category as the category value.¹⁸ The chi-square test was used to test for differences in HPV status between men and women with anal cancer.

RESULTS

We interviewed 417 patients with anal cancer (93 men and 324 women, of whom 6 men and 62 women had in situ anal cancer), 534 controls with adenocarcinoma of the rectum (191 men and 343 women), and 554 population controls (205 men and 349 women). As measured against the total numbers of subjects invited to participate in the study (538 patients with anal cancer, of whom 89 had in situ anal cancer; 708 patients with rectal adenocarcinoma; and 915 population controls), the participation rate was 78 to 79 percent among female patients (cases and cancer controls) and 71 to 73 percent among male patients. Participation rates were 60 to 61 percent among population controls of both sexes. The sex, age, country, and smoking status of the study subjects are shown in Table 1.

Univariate Analyses

Women

Sexual behavior. There was a strong positive correlation between the lifetime number of sexual partners and the risk of anal cancer (P for trend <0.001 with either control group) (Table 2). The risk of anal cancer among women reporting 10 or more lifetime partners was nearly five times the risk among women with only 1 lifetime partner. Women who first had sexual intercourse at or before the age of 16 years were at moderately elevated risk as compared with women who first had sexual intercourse after the age of 20 years. The risk of anal cancer among women who had had four or more sexual partners before the age of 20 years was almost three times the risk among women who had not yet had sexual intercourse at that age (Table 2).

TABLE 1. CHARACTERISTICS OF 1505 PARTICIPANTS IN A CASE-CONTROL STUDY OF ANAL CANCER IN DENMARK AND SWEDEN, 1991-1994.*

CHARACTERISTIC	WOMEN				MEN		
	PATIENTS WITH ANAL CANCER		CONTROLS WITH RECTAL ADENOCARCINOMA (N=343)	POPULATION CONTROLS (N=349)	PATIENTS WITH ANAL CANCER (N=93)†	CONTROLS WITH RECTAL ADENOCARCINOMA (N=191)	POPULATION CONTROLS (N=205)
	<i>invasive</i> (n=262)	<i>in situ</i> (n=62)					
Country — no. (%)							
Denmark	109 (42)	48 (77)	160 (47)	174 (50)	51 (55)	99 (52)	114 (56)
Sweden	153 (58)	14 (23)	183 (53)	175 (50)	42 (45)	92 (48)	91 (44)
Age at diagnosis — no. (%)‡							
<30 yr	1 (0)	3 (5)	1 (0)	8 (2)	1 (1)	1 (1)	1 (0)
30-39 yr	12 (5)	13 (21)	12 (3)	38 (11)	5 (5)	4 (2)	16 (8)
40-49 yr	41 (16)	15 (24)	48 (14)	79 (23)	13 (14)	28 (15)	37 (18)
50-59 yr	56 (21)	13 (21)	91 (27)	46 (13)	25 (27)	44 (23)	38 (19)
60-69 yr	64 (24)	9 (15)	67 (20)	91 (26)	18 (19)	30 (16)	42 (20)
70-79 yr	62 (24)	6 (10)	92 (27)	69 (20)	26 (28)	67 (35)	53 (26)
≥80 yr	26 (10)	3 (5)	32 (9)	18 (5)	5 (5)	17 (9)	18 (9)
Age — yr							
Median	63	50	62	60	60	67	63
Range	26-94	23-97	21-87	22-84	29-86	28-87	29-86
Smoking status — no. (%)							
Current smoker	95 (36)	44 (71)	78 (23)	109 (31)	47 (51)	56 (29)	66 (32)
Former smoker	52 (20)	8 (13)	86 (25)	46 (13)	24 (26)	96 (50)	87 (42)
Nonsmoker	115 (44)	10 (16)	179 (52)	194 (56)	22 (24)	39 (20)	52 (25)

*Percentages may not sum to 100 because of rounding.

†Eighty-seven patients had invasive cancer, and six had in situ cancer.

‡A pseudo-year of diagnosis was assigned to each population control according to the distribution of year of diagnosis among patients of the same sex with anal cancer.

Approximately 1 of 10 women reported having had anal intercourse at least once. This practice was more common in the group of women with anal cancer than in either control group (odds ratio, 2.2). Having had anal intercourse for the first time at or after the age of 30 years was not associated with an increased risk of anal cancer. In contrast, women who had had anal intercourse before the age of 30 were clearly at increased risk of anal cancer (odds ratio, 4.4), and the lifetime number of anal-sex partners was positively correlated with the risk (Table 2).

Venereal diseases. Eighty-one women (8.0 percent), two thirds of whom had anal cancer, reported a history of anogenital warts (odds ratio, 6.0; 95 percent confidence interval, 3.6 to 10.2). The odds ratio for women with a history of genital warts was 5.4, but the association was even stronger when the analysis was restricted to anal warts (odds ratio, 9.8). Women with anal cancer were also significantly more likely than controls to have had gonorrhea (odds ratio, 4.4), trichomoniasis (odds ratio, 2.2), and prior cervical neoplasia (odds ratio, 2.6). No other venereal disease occurred significantly more frequently among women with anal cancer, but they had been tested for the human immunodeficiency virus (HIV) more often than controls (odds ratio, 2.0) (Table 2).

Partners' characteristics. Fewer patients with anal cancer (70 percent) than controls (82 percent) were

married or widowed, and patients with anal cancer reported more sexual promiscuity among their partners. The risk of anal cancer among women whose partners had had three or more other sexual partners was twice as high as the risk among women whose partners had had no other sexual partners. Among women who were unable or reluctant to provide an estimate of the number of their partners' other sexual partners, the risk was similarly elevated (odds ratio, 2.5). Patients with anal cancer were almost three times as likely as controls to report that their partners had had one or more sexually transmitted diseases (gonorrhea, syphilis, genital herpes, or genital warts) (Table 2).

Men

Sexual behavior. As with the women, indicators of sexual promiscuity were positively correlated with the risk of anal cancer among the men (Table 3). Among men who reported ever having had heterosexual intercourse, there was a statistically significant trend toward an association between the lifetime number of female sexual partners and the risk of anal cancer, with men reporting a total of two or three partners at lowest risk (P for trend, 0.004). Men with 10 or more female partners had 2.8 times the risk of those with 2 or 3 partners. Some 23 percent of patients with anal cancer, as compared with 15

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TABLE 2. UNIVARIATE ODDS RATIOS AND 95 PERCENT CONFIDENCE INTERVALS FOR THE RISK OF ANAL CANCER IN WOMEN.*

RISK FACTOR	PATIENTS WITH ANAL CANCER	CONTROLS WITH RECTAL ADENOCARCINOMA	POPULATION CONTROLS	Odds RATIO (95% CI)		
				ANAL CANCER VS. CANCER CONTROLS	ANAL CANCER VS. POPULATION CONTROLS	ANAL CANCER VS. ALL CONTROLS
	no. of participants (%)					
Lifetime no. of male sexual partners						
0	1 (0)	2 (1)	2 (1)	0.7 (0.1-8.1)	0.9 (0.1-10.7)	0.9 (0.1-8.1)
1	62 (20)	123 (38)	125 (37)	1.0 (reference)	1.0 (reference)	1.0 (reference)
2 or 3	90 (30)	114 (35)	94 (28)	1.5 (0.99-2.3)	2.1 (1.4-3.2)	1.8 (1.2-2.6)
4-9	93 (30)	65 (20)	80 (24)	2.8 (1.8-4.5)	3.2 (2.0-5.1)	3.0 (2.0-4.5)
≥10	59 (19)	24 (7)	36 (11)	4.5 (2.5-8.2)†	4.9 (2.8-8.6)†	4.8 (2.9-7.9)†
Age at first sexual intercourse						
≤16 yr	68 (21)	47 (14)	67 (19)	1.8 (1.01-3.0)	1.8 (1.1-3.1)‡	1.8 (1.1-2.9)‡
17 or 18 yr	108 (34)	111 (33)	108 (31)	1.3 (0.9-2.1)	1.6 (0.99-2.5)	1.4 (0.95-2.1)
19 or 20 yr	77 (24)	86 (26)	88 (26)	1.3 (0.8-2.0)	1.2 (0.8-2.0)	1.2 (0.8-1.8)
>20 yr	66 (21)	92 (27)	82 (24)	1.0 (reference)	1.0 (reference)	1.0 (reference)
No. of sexual partners before the age of 20 yr						
0	113 (36)	143 (43)	127 (37)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	104 (33)	118 (36)	121 (36)	1.1 (0.8-1.7)	1.1 (0.8-1.7)	1.1 (0.8-1.6)
2 or 3	60 (19)	52 (16)	73 (21)	1.4 (0.9-2.2)	1.3 (0.8-2.1)	1.3 (0.9-2.0)
≥4	38 (12)	19 (6)	19 (6)	2.1 (1.1-4.2)‡	3.7 (1.9-7.4)‡	2.9 (1.7-5.0)‡
Anal intercourse						
No	273 (85)	319 (94)	307 (90)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	47 (15)	19 (6)	33 (10)	2.5 (1.4-4.5)	2.0 (1.2-3.3)	2.2 (1.4-3.4)
Age at first anal intercourse						
Never	273 (85)	319 (94)	307 (91)	1.0 (reference)	1.0 (reference)	1.0 (reference)
<30 yr	32 (10)	4 (1)	15 (4)	7.6 (2.6-22.5)	3.4 (1.7-6.7)	4.4 (2.4-8.2)
≥30 yr	15 (5)	15 (4)	16 (5)	1.2 (0.6-2.5)	1.2 (0.6-2.4)	1.2 (0.6-2.2)
No. of anal-intercourse partners						
0	273 (85)	319 (94)	307 (90)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	35 (11)	18 (5)	27 (8)	2.0 (1.1-3.7)	1.8 (1.04-3.2)	1.9 (1.2-3.1)
≥2	12 (4)	1 (0)	6 (2)	12.3 (1.6-96.8)‡	2.7 (0.95-7.4)‡	4.1 (1.6-10.7)‡
History of venereal disease§						
Genital warts	39 (12)	6 (2)	14 (4)	6.9 (2.8-17.0)	4.6 (2.3-9.0)	5.4 (3.0-9.8)
Anal warts	32 (10)	6 (2)	3 (1)	5.4 (2.2-13.5)	19.4 (5.7-66.5)	9.8 (4.5-21.3)
Gonorrhoea	33 (10)	9 (3)	9 (3)	4.1 (1.9-8.7)	4.9 (2.3-10.5)	4.4 (2.4-8.0)
Syphilis	3 (1)	2 (1)	1 (0)	2.2 (0.4-13.5)	2.8 (0.3-28.4)	2.3 (0.5-11.5)
Chlamydia	13 (4)	7 (2)	12 (3)	1.7 (0.7-4.6)	1.4 (0.6-3.1)	1.5 (0.7-3.1)
Trichomoniasis	19 (6)	10 (3)	10 (3)	2.0 (0.9-4.4)	2.3 (1.02-5.1)	2.2 (1.1-4.1)
Labial herpes	109 (34)	120 (35)	123 (35)	1.0 (0.7-1.4)	0.9 (0.7-1.3)	0.9 (0.7-1.3)
Genital herpes	12 (4)	6 (2)	8 (2)	2.0 (0.7-5.6)	1.9 (0.7-4.8)	1.9 (0.9-4.3)
Hepatitis	15 (5)	21 (6)	15 (4)	0.8 (0.4-1.7)	1.0 (0.5-2.1)	0.8 (0.5-1.6)
HIV test	49 (15)	30 (9)	29 (8)	1.7 (0.98-2.8)	2.4 (1.4-4.1)	2.0 (1.3-3.1)
Cervical neoplasia	31 (10)	15 (4)	13 (4)	2.3 (1.2-4.4)	2.7 (1.4-5.4)	2.6 (1.5-4.4)
Marital status						
Unmarried without current male partner	17 (5)	13 (4)	13 (4)	1.9 (0.9-4.1)	1.7 (0.8-3.7)	1.8 (0.9-3.5)
Unmarried with current male partner	19 (6)	6 (2)	18 (5)	3.1 (1.2-8.4)	2.0 (0.9-4.1)	2.4 (1.2-4.7)
Separated or divorced without current male partner	40 (12)	23 (7)	23 (7)	2.6 (1.5-4.7)	2.3 (1.3-4.1)	2.4 (1.5-3.9)
Separated or divorced with current male partner	22 (7)	12 (3)	19 (5)	2.3 (1.1-4.9)	1.7 (0.9-3.3)	2.0 (1.1-3.5)
Widowed	88 (27)	94 (27)	83 (24)	1.5 (0.99-2.3)	1.2 (0.8-1.9)	1.4 (0.98-2.1)
Married	138 (43)	195 (57)	193 (55)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Partner's lifetime no. of other sexual partners¶						
0	23 (7)	50 (15)	50 (15)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1 or 2	51 (16)	80 (24)	80 (23)	1.2 (0.7-2.3)	1.4 (0.8-2.7)	1.4 (0.8-2.5)
≥3	73 (23)	64 (19)	74 (22)	1.9 (0.98-3.5)‡	2.5 (1.4-4.7)‡	2.3 (1.3-4.0)‡
Unknown	115 (36)	108 (32)	104 (30)	2.3 (1.3-4.2)	2.5 (1.4-4.4)	2.5 (1.5-4.2)
No current male partner	57 (18)	36 (11)	36 (10)	3.2 (1.7-6.3)	3.6 (1.9-7.0)	3.5 (2.0-6.3)
History of venereal disease in partner						
No	298 (92)	333 (97)	335 (97)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	25 (8)	9 (3)	12 (3)	3.2 (1.4-7.0)	2.6 (1.3-5.4)	2.8 (1.6-5.2)

*Odds ratios are adjusted for age (<40, 40 to 49, 50 to 59, 60 to 69, 70 to 79, or ≥80 years), year of diagnosis (with the use of a pseudo-year for population controls), and country (Denmark or Sweden). Subjects with missing data were excluded in the calculation of percentages and odds ratios. Percentages may not sum to 100 because of rounding. CI denotes confidence interval.

†P value for trend <0.001.

‡P value for trend <0.05.

§The numbers are the numbers and percentages of respondents who reported that they had had the venereal disease in question (or HIV test or cervical neoplasia). In the calculation of odds ratios for these variables, respondents reporting that they had never had the venereal disease (or HIV test or cervical neoplasia) served as the reference group.

¶The trend analysis excluded women in the last two categories.

||Venereal diseases included gonorrhoea, syphilis, genital herpes, and genital warts.

TABLE 3. UNIVARIATE ODDS RATIOS AND 95 PERCENT CONFIDENCE INTERVALS FOR THE RISK OF ANAL CANCER IN MEN.*

RISK FACTOR	PATIENTS WITH ANAL CANCER	CONTROLS WITH RECTAL ADENOCARCINOMA	POPULATION CONTROLS	Odds Ratio (95% CI)		
				ANAL CANCER VS. CANCER CONTROLS	ANAL CANCER VS. POPULATION CONTROLS	ANAL CANCER VS. ALL CONTROLS
no. of participants (%)						
Lifetime no. of female sexual partners						
0	4 (5)	0	3 (2)	∞	7.3 (1.3–41.9)	12.7 (2.3–70.8)
1	12 (15)	31 (18)	37 (19)	1.7 (0.6–4.6)	1.3 (0.5–3.4)	1.4 (0.6–3.5)
2 or 3	10 (12)	40 (23)	41 (21)	1.0 (reference)	1.0 (reference)	1.0 (reference)
4–9	19 (23)	49 (28)	58 (29)	1.5 (0.6–3.6)	1.3 (0.6–3.2)	1.4 (0.6–3.2)
≥10	37 (45)	53 (31)	58 (29)	2.6 (1.1–6.1)†	3.0 (1.3–7.0)†	2.8 (1.3–6.1)†
Age at first sexual intercourse						
≤16 yr	26 (28)	35 (19)	37 (18)	1.0 (0.5–2.3)	2.2 (1.01–4.8)	1.5 (0.8–3.0)
17 or 18 yr	23 (25)	61 (33)	54 (27)	0.6 (0.3–1.2)	1.0 (0.5–2.0)	0.8 (0.4–1.6)
19 or 20 yr	18 (20)	46 (25)	48 (24)	0.7 (0.3–1.4)	0.9 (0.4–1.9)	0.8 (0.4–1.6)
>20 yr	25 (27)	45 (24)	61 (30)	1.0 (reference)	1.0 (reference)	1.0 (reference)
No. of sexual partners before the age of 20 yr						
0	34 (40)	72 (40)	89 (44)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1	9 (11)	32 (18)	26 (13)	0.6 (0.3–1.4)	0.8 (0.4–2.0)	0.7 (0.3–1.6)
2 or 3	24 (28)	45 (25)	41 (20)	1.0 (0.5–2.0)	1.5 (0.8–3.0)	1.3 (0.7–2.4)
≥4	18 (21)	31 (17)	46 (23)	1.1 (0.5–2.3)	1.1 (0.5–2.2)	1.1 (0.6–2.1)
Visit to a prostitute						
No	69 (77)	160 (85)	172 (85)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	21 (23)	29 (15)	30 (15)	2.0 (1.01–3.9)	1.8 (0.96–3.5)	1.8 (0.99–3.2)
Homosexual contact						
No	78 (85)	190 (100)	202 (100)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	14 (15)	0	0	∞	∞	∞
History of venereal disease‡						
Genital warts	2 (2)	3 (2)	6 (3)	1.0 (0.2–6.5)	0.8 (0.2–4.4)	0.9 (0.2–4.3)
Anal warts	7 (8)	2 (1)	1 (0)	9.1 (1.8–45.8)	18.3 (2.1–157)	11.7 (2.9–47.3)
Gonorrhea	17 (18)	17 (9)	20 (10)	2.2 (1.04–4.7)	2.2 (1.1–4.4)	2.2 (1.2–4.2)
Syphilis	8 (9)	0	1 (0)	∞	18.6 (2.2–155)	38.8 (4.7–319)
Chlamydia	2 (2)	0	2 (1)	∞	2.5 (0.3–18.7)	4.0 (0.5–29.4)
Labial herpes	37 (40)	54 (28)	61 (30)	1.6 (0.9–2.9)	1.7 (0.98–2.9)	1.7 (1.04–2.8)
Genital herpes	2 (2)	6 (3)	4 (2)	0.7 (0.1–3.8)	1.3 (0.2–7.4)	0.9 (0.2–4.1)
Hepatitis	11 (12)	10 (5)	8 (4)	2.6 (1.02–6.6)	3.5 (1.3–9.2)	3.0 (1.4–6.8)
HIV test	23 (25)	20 (10)	30 (15)	2.9 (1.4–6.0)	2.5 (1.2–5.1)	2.6 (1.4–4.8)
Marital status						
Unmarried without current female partner	12 (13)	8 (4)	14 (7)	4.9 (1.7–13.7)	2.7 (1.1–6.3)	3.3 (1.5–7.3)
Unmarried with current female partner	10 (11)	4 (2)	5 (2)	7.9 (2.2–29.0)	8.6 (2.5–29.6)	8.7 (3.1–25.0)
Separated or divorced without current female partner	8 (9)	10 (5)	13 (6)	2.3 (0.8–6.4)	1.5 (0.6–4.0)	1.8 (0.7–4.3)
Separated or divorced with current female partner	4 (4)	9 (5)	10 (5)	1.7 (0.5–6.2)	1.4 (0.4–4.8)	1.4 (0.5–4.5)
Widowed	8 (9)	14 (7)	21 (10)	2.7 (0.95–7.6)	1.1 (0.4–2.7)	1.5 (0.6–3.7)
Married	51 (55)	146 (76)	142 (69)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Partner's lifetime no. of other sexual partners						
0	12 (14)	42 (22)	50 (26)	1.0 (reference)	1.0 (reference)	1.0 (reference)
1 or 2	14 (16)	48 (25)	41 (21)	1.0 (0.4–2.5)	1.6 (0.6–3.8)	1.2 (0.5–2.8)
≥3	16 (18)	30 (16)	44 (22)	1.7 (0.7–4.3)	1.8 (0.7–4.3)	1.7 (0.7–3.9)
Unknown	26 (30)	52 (27)	34 (17)	1.8 (0.8–4.0)	3.5 (1.5–8.1)	2.4 (1.1–5.1)
No current female partner	19 (22)	18 (9)	27 (14)	3.4 (1.3–8.9)	3.2 (1.3–7.7)	3.2 (1.4–7.2)
History of venereal disease in partner§						
No	91 (98)	186 (97)	200 (98)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes	2 (2)	5 (3)	4 (2)	0.7 (0.1–4.1)	1.4 (0.2–8.9)	1.0 (0.2–5.2)

*Odds ratios are adjusted for age (<40, 40 to 49, 50 to 59, 60 to 69, 70 to 79, or ≥80 years), year of diagnosis (with the use of a pseudo-year for population controls), and country (Denmark or Sweden). Subjects with missing data were excluded in the calculation of percentages and odds ratios. Percentages may not sum to 100 because of rounding. CI denotes confidence interval.

†P value for trend <0.05 for respondents with one or more female sexual partners.

‡The numbers are the numbers and percentages of respondents who reported that they had had the venereal disease in question (or HIV test). In the calculation of odds ratios for these variables, respondents reporting that they had never had the venereal disease (or HIV test) served as the reference group.

§Venereal diseases included gonorrhea, syphilis, genital herpes, and genital warts.

percent of controls, reported having visited a prostitute (odds ratio, 1.8). Unmarried men were at high risk for anal cancer: among those who did not report a current heterosexual relationship, the odds ratio was 3.3, and among those who lived with a female partner, the odds ratio was 8.7. The few men ($n=7$) who reported never having had heterosexual intercourse were at particularly high risk (odds ratio, 12.7). Homosexual contact was reported by 14 men (2.9 percent), of whom 7 had engaged in receptive anal intercourse. All the homosexual and bisexual men in our study had anal cancer (odds ratio, ∞ ; $P<0.001$, by Fisher's exact test) (Table 3).

Venereal diseases. Patients with anal cancer were more likely than controls to report a history of venereal disease. An increased risk of anal cancer was associated with a history of gonorrhea (odds ratio, 2.2) and a history of anogenital warts (odds ratio, 3.3; 95 percent confidence interval, 1.3 to 8.2). The latter association was due exclusively to anal warts (odds ratio, 11.7); penile condylomata were not associated with an increased risk of anal cancer (odds ratio, 0.9). Eight patients with anal cancer (9 percent) but only one control (0.3 percent) had had syphilis. Other diseases that may have a sexual route of transmission were also significantly more frequent among patients with anal cancer, including labial herpes (odds ratio, 1.7) and hepatitis (odds ratio, 3.0). Men with anal cancer, like their female counterparts, had been tested for HIV more often than controls (odds ratio, 2.6) (Table 3).

Multivariate Analyses

Women

The significant trend toward an association between the number of male partners and the risk of anal cancer persisted after adjustment for differences in smoking status and education (Table 4). Likewise, anal intercourse remained associated with an elevated risk, but the odds ratio was reduced from 2.2 to 1.6 after adjustment for differences in the lifetime number of male partners, smoking status, and education. However, the risk associated with a young age (<30 years) at the time of first anal intercourse remained elevated (odds ratio, 3.4), and the trend toward an association between the number of anal-sex partners and the risk of anal cancer remained significant ($P=0.03$). Subjects who had a history of anogenital warts, gonorrhea, or cervical neoplasia, those who had been tested for HIV, and those whose partners had had sexually transmitted infections remained at significantly increased risk (Table 4).

Men

Among the men who reported ever having had heterosexual intercourse, the significant trend toward an association between the number of female sexual

TABLE 4. MULTIVARIATE ODDS RATIOS AND 95 PERCENT CONFIDENCE INTERVALS FOR THE RISK OF ANAL CANCER.*

RISK FACTOR	ODDS RATIO (95% CI)	
	WOMEN	MEN
Lifetime no. of sexual partners†		
0	1.0 (0.1–9.0)	15.7 (2.7–90.9)
1	1.0 (reference)	1.4 (0.6–3.7)
2 or 3	1.6 (1.1–2.4)	1.0 (reference)
4–9	2.6 (1.7–4.0)	1.4 (0.6–3.2)
≥ 10	4.5 (2.7–7.4)‡	2.5 (1.1–5.5)§
Anal intercourse		
No	1.0 (reference)	NA
Yes	1.6 (1.00–2.6)	
Age at first anal intercourse		
Never	1.0 (reference)	NA
<30 yr	3.4 (1.7–6.6)	
≥ 30 yr	0.9 (0.4–1.7)	
Lifetime no. of anal-intercourse partners		
0	1.0 (reference)	NA
1	1.5 (0.9–2.5)	
≥ 2	2.5 (0.9–7.3)§	
Visit to a prostitute		
No	NA	1.0 (reference)
Yes		1.4 (0.7–2.9)
History of venereal disease¶		
Genital warts	4.6 (2.5–8.7)	0.8 (0.1–4.1)
Anal warts	11.7 (4.7–28.9)	8.0 (1.8–35.9)
Gonorrhea	3.3 (1.7–6.6)	1.9 (0.9–4.0)
Syphilis or hepatitis	0.9 (0.4–1.6)	6.5 (3.0–14.1)
Chlamydia	1.3 (0.6–2.8)	1.9 (0.2–23.3)
Trichomoniasis	1.9 (0.96–3.9)	NA
Labial herpes	1.0 (0.7–1.3)	1.6 (0.9–2.8)
Genital herpes	1.9 (0.8–4.5)	0.9 (0.2–4.6)
HIV test	1.7 (1.1–2.8)	3.1 (1.6–6.1)
Cervical neoplasia	2.3 (1.3–4.1)	NA
Marital status		
Unmarried without current partner	1.8 (0.9–3.8)	3.8 (1.6–8.9)
Unmarried with current partner	1.4 (0.6–2.9)	6.9 (2.0–23.8)
Separated or divorced without current partner	1.4 (0.8–2.3)	1.4 (0.6–3.6)
Separated or divorced with current partner	1.2 (0.6–2.2)	1.2 (0.3–4.5)
Widowed	1.4 (0.9–2.0)	1.7 (0.7–4.2)
Married	1.0 (reference)	1.0 (reference)
Partner's lifetime no. of other sexual partners		
0	1.0 (reference)	1.0 (reference)
1 or 2	1.3 (0.7–2.3)	1.1 (0.5–2.6)
≥ 3	1.5 (0.8–2.8)	1.2 (0.5–3.1)
Unknown	1.8 (1.1–3.2)	1.7 (0.7–4.0)
No current partner	2.0 (1.1–3.8)	2.4 (0.98–5.8)
History of venereal disease in partner		
No	1.0 (reference)	1.0 (reference)
Yes	2.4 (1.3–4.5)	1.1 (0.2–6.1)

*Odds ratios are adjusted for age (<40 , 40 to 49, 50 to 59, 60 to 69, 70 to 79, or ≥ 80 years), year of diagnosis (1991, 1992, 1993, or 1994–1995, with the use of a pseudo-year for population controls), and country (Denmark or Sweden), as well as for lifetime number of sexual partners of the opposite sex (0, 1, 2 or 3, 4 to 9, or ≥ 10), smoking status (current smoker, former smoker, or nonsmoker), years of school (<10 or ≥ 10), and years of postgraduate education (none, ≤ 3 , or >3). "Partners" refers to partners of the opposite sex. CI denotes confidence interval, and NA not analyzed.

†The analysis of trend for lifetime number of female sexual partners among the men was restricted to those with one or more female partners.

‡P value for trend <0.001 .

§P value for trend <0.05 .

¶In the calculation of odds ratios for venereal diseases (or HIV test or cervical neoplasia), respondents reporting that they had never had the venereal disease (or HIV test or cervical neoplasia) served as the reference group.

TABLE 5. HUMAN PAPILLOMAVIRUSES (HPV) IN TUMORS FROM 388 PATIENTS WITH INVASIVE OR IN SITU ANAL CANCER, AS DETERMINED BY THE POLYMERASE CHAIN REACTION.

VARIABLE	WOMEN (N=304)	MEN (N=84)
	no. of patients (%)	
HPV detected*	282 (93)	58 (69)
High-risk type		
Any†	272 (89)	55 (65)
16	235 (77)	48 (57)
18	18 (6)	4 (5)
31	3 (1)	0
33	20 (7)	3 (4)
Low-risk type		
Any‡	11 (4)	5 (6)
6	3 (1)	2 (2)
11	0	0
HPV not detected	22 (7)	26 (31)

*Some patients had more than one type of HPV.

†The high-risk types included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

‡The low-risk types included 6, 11, 40, 42, 43, and 44.

partners and the risk of anal cancer persisted in the multivariate analysis (P for trend, 0.03), whereas the association between anal cancer and visits to prostitutes became statistically insignificant (Table 4). Anal warts, syphilis or hepatitis, and being tested for HIV remained significantly associated with the risk of anal cancer. When the 14 men with homosexual experience (all of whom had anal cancer) were excluded from the analysis, the multivariate odds ratios for anal warts and for syphilis or hepatitis were reduced from 8.0 to 4.9 and from 6.5 to 4.0, respectively. Unmarried men remained at elevated risk, and among men without a current female partner, the risk of anal cancer was more than three times the risk among married men. Also, men in current heterosexual relationships who were unmarried remained at high risk (odds ratio, 6.9) after adjustment for the lifetime number of female partners (Table 4).

For both women and men, we added a term for effect modification by country to the multivariate model for each variable that was significantly associated with an elevated risk of anal cancer ($P < 0.05$). In only 1 of 17 such tests for effect modification was the term statistically significant. All other significant variables had similar associations with the risk of anal cancer in Denmark and Sweden, but among women, the overall effect of ever having had anal intercourse was higher in Sweden ($P = 0.04$). However, there was no significant difference between the countries in the strong associations between the risk of anal cancer and age at first anal intercourse ($P = 0.76$ for effect modification by country) or number of partners with whom anal intercourse was performed ($P = 0.39$).

Tissue Analyses

We obtained tumor tissue from 394 of the 417 patients with anal cancer whom we interviewed (94 percent) and from 20 patients with rectal adenocarcinoma. Successful PCR analyses were performed in tumors from 388 patients with anal cancer (304 women and 84 men) (Table 5) and in all 20 patients with rectal adenocarcinoma. A total of 340 patients with anal cancer (88 percent) had tissue specimens that were positive for HPV, and 73 percent of the 388 specimens were positive for HPV-16. Anal cancers were more likely to be HPV-positive in women (93 percent) than in men (69 percent, $P < 0.001$), because a higher proportion of anal cancers in women were positive for high-risk HPV types, notably HPV-16. All rectal adenocarcinomas tested were negative for HPV.

DISCUSSION

In the 1940s and 1950s, the annual incidence of anal cancer in Denmark was approximately 2 per 1 million person-years, world standardized, for both men and women. There has been a significant increase in cases of anal cancer since then, particularly among women. Now the incidence of anal cancer among women (7 per 1 million person-years, world standardized) is almost twice that among men (4 per 1 million person-years).² Similar increases have been reported in Sweden¹ and the United States.³

Changes in sexual practices, including increasing promiscuity among young people,¹⁹ may be responsible in part for the increased incidence of anal cancer.² A number of clinical observations⁴⁻⁶ and registry-linkage studies²⁰⁻²² have suggested that sexual practices and venereal diseases are associated with the risk of anal cancer, but there have been only three case-control studies of the risk factors for anal cancer.⁷⁻⁹ In their study of 148 patients with anal cancer and 166 controls with colon cancer, Daling et al. found that the risk of anal cancer was strongly associated with homosexual contact, genital warts, and gonorrhea among men and with genital warts, infection with herpes simplex virus type 2, and chlamydia trachomatis infection among women.⁷ In a study of 56 women with anal cancer and 56 population controls, Holmes et al. failed to find an association between anal cancer and genital warts. However, a history of a positive or questionable cervical Pap smear, a positive serologic test for herpes simplex virus type 2, and a large number of sexual partners were more common among patients with anal cancer than among controls.⁸ Holly et al., in their study of 126 patients with anal cancer and 372 population controls, substantiated the association between male homosexual contact and anal cancer. Furthermore, for both sexes, genital warts, syphilis, and gonorrhea were more common among patients with anal cancer than among controls.⁹

In our study, the group of patients with anal cancer was larger than the combined groups of patients with anal cancer in the three previous case-control studies.⁷⁻⁹ A number of our findings substantiate the previously recognized associations between anal cancer and male homosexual contact. The fact that no controls but 15 percent of male patients with anal cancer reported homosexual contact and the strong statistical influence of being an unmarried man without a current female partner, never having had heterosexual intercourse, and having a history of syphilis, hepatitis, or anal (but not penile) warts²³ strongly support the idea that male homosexual contact is a risk factor for anal cancer. Also in line with two of the three previous case-control studies^{7,9} is our finding that genital warts are a risk factor in women.

Unlike previous investigators, we found strong associations between anal cancer and measures of heterosexual promiscuity. For both men and women, there was a statistically significant trend toward an association between the lifetime number of opposite-sex partners and the risk of anal cancer. Furthermore, divorce and nonmarital heterosexual relationships were more common among patients with anal cancer, and for women, a young age at the time of first sexual intercourse and a nonmonogamous partner or a partner with a history of a sexually transmitted disease were associated with the risk of anal cancer. Each of these observations supports the view that anal cancer is linked to heterosexual promiscuity and that a sexually transmitted agent plays a role in anal carcinogenesis.

There is some evidence that the types of HPV that are causally linked to cervical cancer may also be linked to anal cancer.²⁴⁻³¹ Using general-primer-based PCR to detect a broad spectrum of mucosotropic HPV types, if not all of them,¹⁶ we found the highest prevalence of HPV (88 percent) reported in an unselected, population-based sample of patients with anal cancer. This finding was due mainly to a very high prevalence of HPV, particularly oncogenic HPV-16, in the tumors in women. Hitherto the largest such study of HPV in invasive anal cancers involved samples from 99 patients in Norway³¹; HPV-16 was detected by *in situ* hybridization and PCR in 84 percent and 52 percent of anal epidermoid carcinomas in women and men, respectively, which is in close agreement with our results. In an analysis of 129 invasive and *in situ* anal cancers by PCR, one or more of HPV types 6, 11, 16, and 18 were found in 70 percent and 67 percent of cancer specimens among women and men, respectively.²⁹

Heterosexual anal intercourse has long been regarded as a plausible mode of transmission of an infectious oncogenic agent, but unlike the findings in men, the association of anal cancer with receptive anal intercourse in women has not been statistically significant in previous studies. We found a strong as-

sociation between the age at which receptive anal intercourse was first practiced and the risk of anal cancer. Indeed, women who had anal intercourse only after the age of 30 years were not at any greater risk than those who reported no anal intercourse. These observations suggest that anal intercourse itself does not carry an increased risk. Rather, anal sex at a young age and with multiple partners is likely to increase the risk of acquiring the presumably relevant infection. However, most men and women with anal cancer in our study did not engage in anal sex. Thus, if HPV is truly a causative agent in anal cancer, other modes of transmission to the anal area should be considered. A detailed analysis of sexual and other behavior among patients with HPV-positive tumors and those with HPV-negative tumors may add to our understanding of the factors leading to the development of anal cancer.

Our interview data are subject to misclassification, and external validation is obviously not possible at the individual level. However, in 1990, a self-administered, mailed questionnaire survey of sexual practices was carried out in a random sample of 4680 men and women (<60 years of age) in Denmark,¹⁹ permitting an evaluation of the validity of the Danish part of the present study. In the present study, the median number of lifetime partners reported by Danish population controls was similar to or even higher than that reported by subjects in similar age groups in the 1990 survey. Moreover, in various age groups, the percentages of women who had ever practiced anal intercourse and of men who had ever visited a prostitute were similar in the two studies (unpublished data). Overall, our telephone interviews resulted in prevalence estimates for measures of sexual promiscuity that were similar to or even higher than those based on entirely anonymous survey data.

We designed the study with two control groups. Patients with rectal adenocarcinoma were chosen as controls to minimize recall and reporting bias, because patients with anal cancer and controls with rectal adenocarcinoma should interpret associations with sexual factors similarly. Since there is no evidence of an association between sexual factors or HPV and adenocarcinoma of the rectum,^{13,32} the sexual behavior reported by these controls is likely to reflect sexual practices in the general population. The fact that there were similarly high participation rates among patients with anal cancer and controls with rectal adenocarcinoma also minimizes any selection bias. Moreover, by including two control groups, we increased the likelihood that the interviewers were unaware of an individual patient's diagnosis.

We conclude that a large number of partners, a young age at the time of first sexual intercourse, receptive anal intercourse, an unmarried status, divorce, a variety of sexually transmitted diseases, and in wom-

en, sexual promiscuity of the partner are all linked to the risk of anal cancer. A few decades ago, cancer of the uterine cervix was hypothesized to be caused by a sexually transmitted infection on similar epidemiologic grounds.^{33,34} The consistency of our findings with two independent control groups and the detection of oncogenic HPV types in most of the anal cancers we studied strongly suggest that in the majority of cases, anal cancer is a sexually transmitted and thus potentially preventable disease.

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