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Longitudinal Incidence Study of  
Clinically-Relevant HPV and Vaccine-Type HPV in Young Online Female Daters

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**Abstract**

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The sexually transmitted human papillomavirus (HPV) causes cervical cancer, the fourth most common cancer in women world-wide; young women (typically 18- to 24-year-old) have highest levels of infections. Online dating is becoming increasingly prevalent, especially among young women. Better understanding of HPV infection risk in young female online daters is needed to inform public health initiatives and future research efforts. We performed a year-long longitudinal study (2010-2012) of a cohort of 18- to 24-year-old female online daters in the United States, examining their HPV incidence, HPV vaccination history, and sexual behaviors, using self-collected vaginal samples for HPV genotyping and behavioral questionnaires. Kaplan-Meier methods were used to estimate incidence of HPV infection and generalized estimating

equations logistic regression was used to identify risk factors for incident infections. The 164 young women that we studied reported a median lifetime number of 6 male sex partners (interquartile range: 3-12), with 58.4% having had at least one new male sex partner or multiple male sex partners in the past 6 months. The incidence rate of clinically-relevant HPV infection (high-risk types plus HPV-6 and HPV-11) was 36.9 (95% Confidence Interval (CI): 28.0-48.5) per 100 women-years. Our analysis did not find meeting sex partners online to be a significant factor in incidence of clinically-relevant HPV infections (odds ratio (OR) 0.91, 95% CI: 0.53-1.55, relative to those with at least one male sex partner, but none met online); however, other recent risky sexual behaviors—multiple sex partners, new sex partners, and/or casual sex partners in the prior 6 months (adjusted OR 5.77, 95% CI: 1.42-23.51, for those with all three risk factors versus not sexually active in the prior 6 months)—as well as higher number of total lifetime sex partners (adjusted OR 6.37, 95% CI: 1.56-26.05, for those with  $\geq 6$  relative to 1-2 lifetime sex partners) were significant factors. Nonetheless, in this high-risk cohort we found self-reported history of HPV vaccination to be protective against acquiring vaccine-type HPV (6/11/16/18) (OR 0.27, 95% CI: 0.07-1.03, adjusted for recent sexual behavior and lifetime number of sex partners). Consequently, despite HPV infection being common in young female online daters, vaccination appears protective against vaccine-types, which suggests that public health agencies should continue proactive actions to increasing vaccination uptake and education in order to limit the spread of HPV infection.

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## **DEDICATION**

To Paul Li and my mother, for all your love and support.

## Chapter 1. INTRODUCTION

Awarding of the 2008 Nobel Prize for Medicine to Harald zur Hausen for linking the sexually transmitted human papillomavirus (HPV) to cervical cancer was a seminal moment in the field of sexually transmitted diseases<sup>1</sup>. That the fourth most common cancer in women world-wide<sup>2</sup> was due to a sexually transmitted virus was a great discovery. In recent years, many research efforts have examined infection methods and patterns as well as prevention for HPV. The 2006 United States' Federal Drug Administration (FDA) approval of the Gardasil vaccine for the prevention of cervical cancer due to HPV types 6, 11, 16, and 18 was a major development, with the United States' Centers for Disease Control and Prevention (CDC) subsequently recommending vaccinations of preteen girls and boys at age 11 or 12 years with catch-up vaccination through age 26 years.<sup>3</sup> Today, we are starting to see the first wave of young women entering their twenties, historically, the age range with highest HPV infection prevalence.<sup>4</sup>

Another important recent development is the rise of online dating. A 2015 national survey indicates that 12% percent of all American adults have used an online dating site; online dating among 18- to 24-year-old Americans is even more prevalent at 27%, tripling from 2013 to 2015.<sup>5</sup> Furthermore, 29% of those surveyed indicated they know someone who has married or entered into a long-term partnership with someone they met via online dating. More knowledge about factors related to HPV infection among young women online daters is needed.

In our previous study, we examined HPV *prevalence* among 18- to 24-year-old female online daters in the United States, finding that the prevalence of oncogenic HPV infection was high (37%); having recent male sex partners in concurrent partnerships and not always using condoms were associated with an increased likelihood of infection; a history of at least 1 dose of

HPV vaccine was inversely related with likelihood of HPV-16 or HPV-18 infection.<sup>6</sup> In this paper, we report the longitudinal results—*incident infections*—from this cohort of 18- to 24-year-old female online daters, examining their HPV incidence, HPV vaccination history, and sexual behaviors. Women self-collected vaginal samples for HPV testing and completed detailed health and sexual behavior questionnaires for discernment of factors of interest.

Knowledge from our study provides insight into several areas. We provide information about the influence of various risk factors (e.g. sexual behaviors) on infection incidences among young women online daters. We also provide information about the progress in preventing HPV infections via vaccinations. These understandings may aid future development of treatment and public health messaging and prevention strategies for cancer-causing HPV infections

## Chapter 2. METHODS

For our one-year longitudinal study of 18- to 24-year-old online daters, we recruited participants from October 2010 to May 2012. Recruitment was primarily through the community-based free advertisements website: Craigslist.org. Recruitment advertisements (ads) were posted weekly in the “volunteer opportunities” section of cities in the United States, rotated through 37 major US cities. In addition, we also leveraged free research volunteer recruitment websites offered by the University of Washington and other national websites that offered free classified ads. We also recruited women who were previously enrolled from April 2010 to January 2011 (using identical recruitment methods) into a cross-sectional study designed to compare methods for transporting at-home self-collected samples for HPV testing.<sup>7</sup> Women who gave permission to be contacted for future studies were emailed an invitation to contact the research clinic to be screened for the new longitudinal study.

Recruitment ads specifically targeted 18- to 24-year-old women who reported using the internet to search for romantic partners within the previous 12 months. Women who responded to the recruitment ads or the email invitation were then screened by a study coordinator over the telephone to confirm eligibility; the coordinator specifically asked: “Have you used an internet dating Web site in the past year [e.g., posted or responded to an ad on an online dating Web site or social networking Web site]?” Women were excluded if they were pregnant, were breastfeeding, were planning a pregnancy 6 months from the time of enrollment, were hysterectomized, were immunocompromised, or reported no history of sex with men. Consent forms were mailed to interested women who passed phone screening. Eligible women who returned a signed consent form were enrolled into the study. The protocol for the study was reviewed and approved by the University of Washington Institutional Review Board.

Enrolled women were mailed demographic, sexual behavior, and health history questionnaires (including HPV vaccination status), as well as self-collection kits for collection of vaginal samples for HPV DNA testing. Up to four kits and questionnaires were provided at 4-month intervals; each self-collection kit contained illustrated self-collection instructions, written instructions (e.g. refrain from douching, vaginal intercourse, and the use of vaginal medications or preparations for 48 hours before collecting the vaginal samples), 2 Dacron-tipped swabs, a capped tube containing 1.5 ml of Specimen Transport Medium (Qiagen, Germantown, MD), nitrile gloves, packing/shipping materials, and a prepaid standard overnight Federal Express mailing envelope addressed to the study coordinator.

Vaginal samples were tested for HPV DNA using polymerase chain reaction (PCR)-based methods described previously.<sup>8</sup> Human papillomavirus DNA and  $\beta$ -globin were amplified simultaneously using the HPV L1 consensus primers MY09, MY11, and HMB01 and  $\beta$ -globin primers PC04 and GH2. Specimens determined to be HPV positive by generic probe or that were  $\beta$ -globin negative by dot blot were typed using the Roche Linear Array HPV genotyping test (Roche Molecular Systems, Inc, Alameda, CA) for 37 HPV types, including 21 clinically-relevant types: HPV types 6 and 11 (due to their association with genital warts<sup>9,10</sup> and 19 “high-risk” types classified as carcinogenic, probably carcinogenic, or possibly carcinogenic: 16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/68/73/82/IS39.<sup>11,12</sup>

To analyze cumulative incidence of new HPV infections, we conducted Kaplan-Meier analyses. We conducted analyses for individual types and the following type groups: quadrivalent vaccine-type HPV (6, 11, 16 and 18), oncogenic types (the 19 high-risk types listed above), and clinically-relevant HPV types (types 6, 11, and the 19 high-risk types). HPV types first detected during follow-up were considered incident. Time at risk was calculated from

baseline to first detection of a specific HPV type or group of types (using the midpoint between the date of the incident positive and the previous sample), or the last sample.

To analyze risk factors for type-specific incident clinically-relevant HPV infections, we used generalized estimating equations (GEE) with an independence working correlation structure, robust standard errors, and logit link function to estimate odds ratios for associations. Each woman contributed an observation for each of the 21 HPV types she tested negative for at baseline (hereafter referred to as “women-types”). Correlation within women due to multiple outcome measurements was accounted for. A “visit number” indicator variable was included as a time surrogate. We first analyzed risk factors in univariate analyses. Potential risk factors evaluated included demographics, health history, and cumulative and recent sexual behaviors. Demographic variables included race (African American, Asian, White, Other), Hispanic ethnicity (yes, no), and the following time-varying variables: age (18-19, 20-21, 22-23, 24-25 years) and marital status (unmarried or separated, married or living with a partner). Time-varying health history variables included ever pregnant (yes, no), smoking history (never, former, current), current hormonal contraceptive use (yes, no), history of an abnormal Pap test (yes, no; restricted to women  $\geq 21$  years of age), and history of at least one dose of HPV vaccination (yes, no). Sexual behavior variables included age at 1<sup>st</sup> sexual intercourse ( $\leq 15$ , 16-17,  $\geq 18$  years) and the following time-varying variables: lifetime number of male sex partners (1-2, 3-5,  $\geq 6$ ), numbers of male sex partners in the past six months (0,  $\geq 1$ ) and other characteristics of recent male sex partners and partnerships. These partner/partnership characteristics were restricted to women reporting a male partner in the past six months and summarized as follows: number of male sex partners (1,  $\geq 2$ ), new male sex partners (0,  $\geq 1$ ), casual (versus regular) sex partners (0,  $\geq 1$ ), sex partners with other concurrent partners (0,  $\geq 1$ , unknown concurrent status), sex partners

met online ( $0, \geq 1$ ), and condom use with male sex partners (always with all partners, not always with  $\geq 1$  partner). Recent sexual behavior variables found to be statistically significant ( $p < 0.10$ ) in univariate analyses were used to construct a composite variable, to assess the overall impact of recent sexual behaviors. The variable ranged from not sexually active with male partners in the past 6 months to sexually active with male partners with 0, 1, 2, or 3 risk factors. The composite variable was entered into a final multivariate model with demographic, health history, and cumulative sexual behavior variables that were statistically significant at the  $p < 0.10$  level in univariate analyses.

Similar methods were used to evaluate the association between HPV vaccination history ( $\geq 1$  dose vs. 0 doses) and incident HPV infection with vaccine types (6, 11, 16 or 18). To construct a final multivariate model with vaccine-type HPV as the outcome, we included HPV vaccination history plus all variables selected for the multivariate analysis inclusive of all clinically-relevant HPV types.

## Chapter 3. RESULTS

In total, 195 age-eligible women were enrolled, including 75 out of 195 (38.5%) women invited from our prior cross-sectional study population<sup>7</sup> and 120 newly recruited. Of these, 164 (84.1%) returned  $\geq 1$  follow-up sample. Women who did not return  $\geq 1$  follow-up sample were similar to those that did with respect to age and lifetime number of male sex partners (data not shown). Our analyses were restricted to these 164 women.

### 3.1 DEMOGRAPHICS

At enrollment, the mean (standard deviation) age of the 164 subjects was 22.0 ( $\pm 1.7$ ) years. Subjects reported a median lifetime number of male sex partners of 6 (interquartile range: 3-12) and a mean age at first intercourse of 16.7 ( $\pm 2.6$ ) years. There were 35.8% with at least one dose of prophylactic HPV vaccine, and 58.4% had at least one new male sex partner or multiple sex partners in the past 6 months. Detailed demographic information is in Table 3.1 below.

Table 3.1. Demographic, health, and sexual history characteristics of 18- to 24-year-old female online daters (N=164)

Characteristic	n <sup>a</sup> (%)
Age at first visit, years	
18-19	16 (9.8)
20-21	43 (26.2)
22-23	67 (40.9)
24-25 <sup>b</sup>	38 (23.2)
Race	
African American	35 (21.9)
Asian	15 (9.4)
White	85 (53.1)
Other <sup>c</sup>	25 (15.6)
Hispanic ethnicity	
No	136 (87.7)
Yes	19 (12.2)

Geographic region <sup>d</sup>	
Midwest	42 (25.8)
Northeast	40 (24.5)
South	28 (17.2)
West	53 (32.5)
Highest level of education completed	
Some postsecondary education or less	110 (67.9)
Bachelor's degree or higher	52 (32.1)
Current marital status	
Unmarried / Separated	121 (75.6)
Married / Unmarried, living with a partner	39 (24.4)
Ever pregnant	
Yes	38 (23.5)
No	124 (76.5)
Smoking status	
Current	28 (17.2)
Former	18 (11.0)
Never	117 (71.8)
Currently using hormonal contraceptives	
Yes	50 (30.9)
No	112 (69.1)
History of an abnormal Pap test result <sup>e</sup>	
Yes	33 (27.3)
No	88 (72.7)
History of genital warts	
Yes	8 (4.9)
No	154 (95.1)
History of HPV vaccination	
No	104 (64.2)
Yes	58 (35.8)
Number of doses <sup>f</sup>	
1 dose	7 (13.2)
2 doses	11 (20.8)
3 doses	35 (66.0)
Age at first intercourse, years <sup>g</sup>	
≤15	50 (30.9)
16-17	54 (33.3)
≥18	58 (35.8)

Lifetime number of male sex partners <sup>h</sup>	
1-2	29 (18.0)
3-5	39 (24.2)
6-7	30 (18.6)
8-14	30 (18.6)
15+	33 (20.5)
Male sex partners in last 6 months	
0 male partners	11 (6.8)
1 male partner, non-new	56 (34.8)
new male partner(s) or multiple partners	94 (58.4)
High-risk HPV positive <sup>i</sup>	
Yes	53 (32.3)
No	111 (67.7)
Clinically-relevant HPV positive <sup>j</sup>	
Yes	53 (32.3)
No	111 (67.7)
Vaccine-type HPV positive <sup>k</sup>	
Yes	18 (11.0)
No	146 (89.0)

<sup>a</sup>164 (84.1% of 195) women returned a baseline self-collected sample and  $\geq 1$  follow-up sample that were sufficient for HPV DNA testing; numbers may not add up to totals due to missing data.

<sup>b</sup> 5 women turned 25 years-old between screening and the first visit.

<sup>c</sup> Includes individuals reporting other races (American Indian, Alaska Native, Native Hawaiian, other Pacific Islander) or multiple races.

<sup>d</sup> Northeast includes states: CT, MA, NJ, NY, PA, RI; Midwest includes states: IL, IN, KS, MI, MN, MO, ND, OH, WI; South includes states: AR, DC, FL, GA, MD, MS, OK, TN, TX; West includes states: AZ, CA, CO, OR, WA, WY

<sup>e</sup> Restricted to women 21 years or older at the first visit.

<sup>f</sup> 5 women who self-reported a history of HPV vaccination (8.6%) did not answer the question on number of doses received.

<sup>g</sup> Approximate tertiles.

<sup>h</sup> Approximate quintiles.

<sup>i</sup> Positive for any of the following 19 carcinogenic, probably carcinogenic, or possibly carcinogenic types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, IS39.

<sup>j</sup> Positive for any of the following clinically-relevant HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, IS39.

<sup>k</sup> Positive for any of the following quadrivalent vaccine HPV types: 6, 11, 16, 18.

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### 3.2 HPV INFECTION INCIDENCES

Overall, 100 women had incident clinically-relevant HPV detected during follow-up. The incidence rate of any new clinically-relevant HPV infection was 36.9 per 100 women-years. The most common individual HPV types were 56, 53, and 52. Ninety-four women had incident oncogenic HPV detected, with the incidence rate of any new oncogenic HPV at 34.4 per 100 women-years. There were 15 women with incident vaccine-type HPV detected (6 with type 6, 0 with type 11, 7 with type 16, and 2 with type 18). The incidence rate of detecting any new vaccine-type was 7.2 per 100 women years. Detailed incidence information is in Table 3.2 below.

Table 3.2. Type-specific incidence rates of newly detected HPV in 18 -to 24-year-old female online daters (N=164)

HPV Type(s)	No. of Women with Incident Detection	No. of Person-Years at Risk	Incidence Rate per 100 Person-Years (95% CI)
Any clinically-relevant HPV <sup>a</sup>	100	3456	36.9 (28.0-48.5)
Any vaccine-type HPV <sup>b</sup>	15	661	7.2 (4.1-12.8)
Any oncogenic HPV <sup>c</sup>	94	3210	34.4 (25.9-45.6)
HPV 6	6	163	3.7 (1.7-8.2)
HPV 11	0	172	0
HPV 16	7	158	4.4 (2.1-9.3)
HPV 18	2	168	1.2 (0.3-4.8)
HPV 26	0	172	0
HPV 31	5	156	3.2 (1.3-7.7)
HPV 33	1	172	0.6 (0.1-4.1)
HPV 35	4	168	2.4 (0.9-6.4)
HPV 39	8	159	5.0 (2.5-10.1)
HPV 45	1	171	0.6 (0.1-4.1)
HPV 51	6	160	3.8 (1.7-8.4)
HPV 52	9	163	5.5 (2.9-10.6)
HPV 53	9	161	5.6 (2.9-10.8)
HPV 56	9	158	5.7 (3.0-11.0)
HPV 58	6	164	3.7 (1.6-8.1)
HPV 59	6	161	3.7 (1.7-8.3)
HPV 66	7	157	4.5 (2.1-9.4)
HPV 68	5	163	3.1 (1.3-7.4)

HPV 73	3	169	1.8 (0.6-5.5)
HPV 82	5	170	3.0 (1.2-7.1)
HPV IS39	1	172	0.6 (0.1-4.1)

<sup>a</sup> Positive for any of the following clinically-relevant HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, IS39.

<sup>b</sup> Positive for any of the following vaccine-type HPV: 6, 11, 16, 18.

<sup>c</sup> Positive for any of the following 19 carcinogenic, probably carcinogenic, or possibly carcinogenic HPV types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, IS39.

Kaplan-Meier analysis (Figure 3.1) indicates a 6-month cumulative incidence of infection of 18.7% (13.4%-25.6%) and a 12-month cumulative incidence of infection of 32.9% (26.0%-41.0%) for any clinically-relevant HPV. For oncogenic HPV, the 6- and 12-month cumulative incidences were 18.0% (12.9%-24.9%) and 30.9% (24.1%-38.9%) respectively. For vaccine-type HPV, the 6-month cumulative incidence of infection was 3.1% (1.3%-7.4%) and the 12-month incidence was 8.1% (4.7%-13.9%).

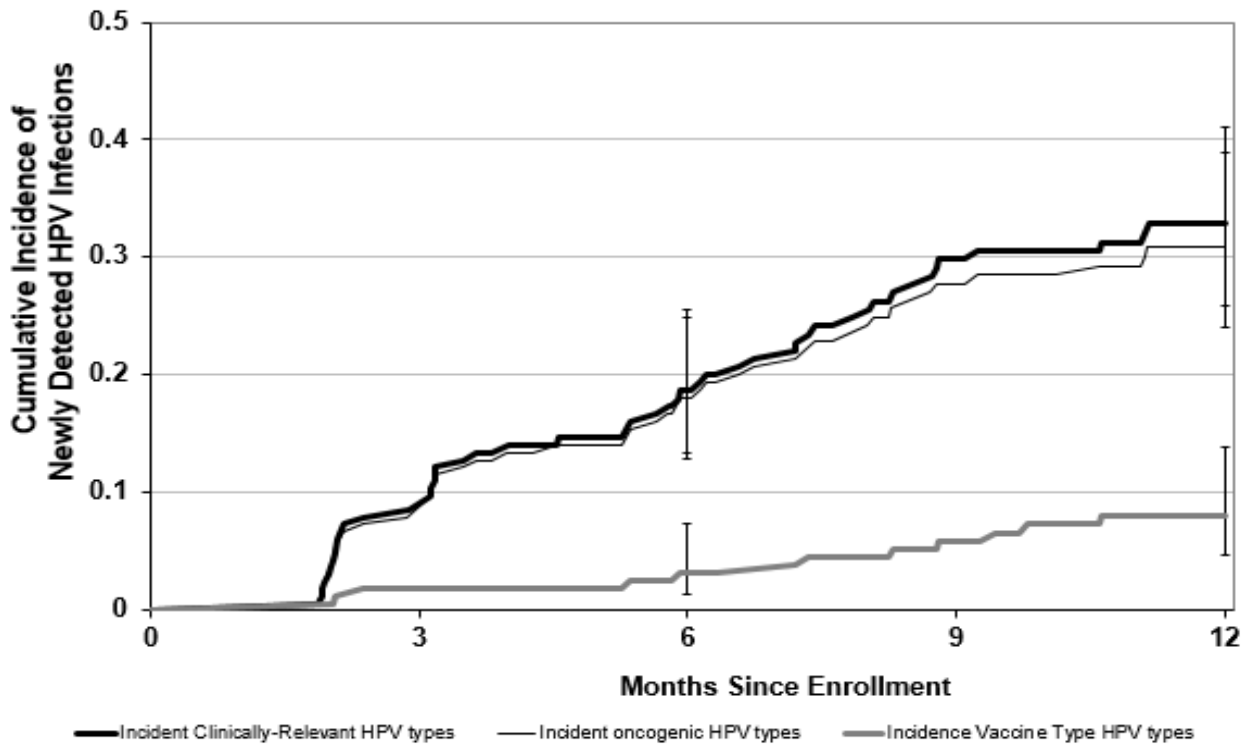


Figure 3.1. Kaplan-Meier analysis of cumulative incidence of detecting new HPV infections, not present at baseline. Data are for 164 women aged 18-24 years. Error bars represent 95% confidence intervals.

### 3.3 FACTORS FOR INCIDENT HPV INFECTION

In univariate analyses of risk factors related to incident clinically-relevant HPV infection, higher lifetime number of male sex partners and reporting sex with a male partner in the past 6 months were each associated with higher odds of infection. Subsequently, in analyses restricted to women reporting male partners in the past 6 months, multiple sex partners, new sex partners, and casual male partners were each associated with an increased likelihood of infection (see Table 3.3). We then created a composite variable capturing the risk factors associated with having new, casual, and/or multiple male sex partners; women with one, two, or all three of the risk factors had progressively higher likelihoods of infection.

In multivariate analysis, we included the composite variable and the lifetime number of partners into a multivariate model. Lifetime number of partners remained significantly associated with incident HPV after adjusting for the composite recent sexual behavior variable. Relative to those with 1-2 partners, having 3-5 partners was associated with an OR of 4.29 (95% CI: 0.90-20.28), while having 6 or more partners was associated with an OR of 6.37 (95% CI: 1.56-26.05). Relative to those that did not have any sex partners in the past 6 months, those having only 1 male sex partner who was not new or casual had a non-statistically-significantly increased likelihood of incident HPV (OR 2.64, 95% CI: 0.64-10.91). Women with one or two risk factors (multiple sex partners, new sex partners, or casual sex partners) had ORs of 4.48 (95% CI: 1.03-19.56) and 4.43 (95% CI: 0.89-22.03), respectively. Women with all three risk factors had an OR of 5.77 (95% CI: 1.42-23.51) relative to those with no recent partners. For details see Table 3.3.

Table 3.3. Odds Ratios (ORs) for associations between risk factors and incidence of type-specific clinically-relevant HPV detection in 18- to 24-year-old female online daters (N=3,347 women-types)

	No. of Incident Detections <sup>a</sup>	n <sup>b</sup>	Univariate OR (95% CI)	Multivariate OR (95% CI) <sup>c</sup>
<b>Age, years</b>				
18-19	8	246	Ref (1.0)	
20-21	18	890	0.51 (0.23-1.10)	
22-23	32	1,422	0.55 (0.21-1.45)	
24-26	42	1,513	0.58 (0.22-1.54)	
<b>Race</b>				
African American	18	733	Ref (1.0)	
Asian	12	303	1.70 (0.56-5.21)	
White	52	1,739	1.23 (0.55-2.76)	
Other <sup>d</sup>	15	489	1.25 (0.51-3.01)	
<b>Hispanic ethnicity</b>				
No	87	2,801	Ref (1.0)	
Yes	6	361	0.54 (0.25-1.17)	
<b>Age at first intercourse, years</b>				
≤15	30	1,015	Ref (1.0)	
16-17	39	1,088	1.16 (0.60-2.21)	
≥18	31	1,202	0.82 (0.39-1.71)	
<b>Marital status</b>				
Unmarried / Separated	70	2,733	Ref (1.0)	
Married / Unmarried, living with a partner	30	1,257	1.11 (0.62-1.99)	
<b>Lifetime number of male sex partners</b>				
1-2	2	515	Ref (1.0)	Ref (1.0)
3-5	16	806	5.71 (1.19-27.38)	4.29 (0.90-20.38)
≥6	82	2,256	9.59 (2.36-38.91)	6.37 (1.56-26.05)
<b>Ever pregnant</b>				
No	74	2,437	Ref (1.0)	
Yes	26	1,012	0.97 (0.54-1.76)	
<b>Currently using hormonal contraceptives</b>				
No	74	2,569	Ref (1.0)	
Yes	26	1,075	1.00 (0.53-1.88)	
<b>Smoking status</b>				

Never	62	2,240	Ref (1.0)
Former	24	789	1.37 (0.69-2.73)
Current	14	678	1.07 (0.43-2.68)
Ever had an abnormal Pap test <sup>e</sup>			
No	49	1,823	Ref (1.0)
Yes	26	860	1.18 (0.65-2.12)
History of HPV vaccinations <sup>f</sup>			
No	65	1,977	Ref (1.0)
Yes	35	1,467	0.75 (0.42-1.34)
Number of male sex partners in the past 6 months			
0 male partner	2	430	Ref (1.0)
≥1 male sex partner	98	3,139	5.61 (1.40-22.43)

*For women with ≥1 male sex partner in the past 6 months <sup>g</sup>*

Number of male sex partners			
1 male sex partner	39	2,280	Ref (1.0)
≥2 male sex partner	59	1,880	2.09 (1.23-3.56)
New sex partners			
0 new male partner	28	2,090	Ref (1.0)
≥1 new male partner	70	2,174	2.32 (1.32-4.05)
Casual male sex partners			
0 casual male sex partners	45	2,275	Ref (1.0)
≥1 casual male sex partners	53	1,640	1.77 (0.99-3.16)
Male sex partners w/concurrent partnership			
0 male sex partners w/concurrent partnership	40	1,704	Ref (1.0)
≥1 male sex partners w/concurrent partnership	47	1,472	1.46 (0.83-2.59)
Unknown concurrent partnership status	11	1,017	0.60 (0.23-1.56)
Male sex partners met online			
0 male sex partners met online	58	2,082	Ref (1.0)
≥1 male sex partners met online	40	1,663	0.91 (0.53-1.55)
Condom use <sup>h</sup>			
Always	17	1,043	Ref (1.0)
Not always	81	2,520	1.73 (0.83-3.58)

*Composite variable for women with sexual behavior in the past 6 months for multivariate analysis*

Sexual behavior with male partners				
0 sex partners	2	430	Ref (1.0)	Ref (1.0)
1 partner (not new or casual)	22	1,746	3.20 (0.75-13.62)	2.64 (0.64-10.91)
1 risk factor (new, casual, or multiple partners)	16	976	5.53 (1.24-24.56)	4.48 (1.03-19.56)
2 risk factors (new, casual or multiple partners)	14	806	5.89 (1.15-30.17)	4.43 (0.89-22.03)
All three risk factors	46	1,436	8.86 (2.16-36.39)	5.77 (1.42-23.51)

<sup>a</sup> Clinically-relevant HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, IS39).

<sup>b</sup> The 164 women contributed an observation for each type for which they tested negative at baseline, or N=3,347 women-types. However, women-types could change categories throughout follow-up for time-dependent variables, therefore the number of women-types at risk (n) may exceed total N for time-dependent variables.

<sup>c</sup> Multivariate analysis with all women included, with a composite variable replacing individual risky sexual behaviors.

<sup>d</sup> Includes individuals reporting other race or multiple races.

<sup>e</sup> Restricted to women 21 years or older at the first visit.

<sup>f</sup> Coded “no” if self-reported no history of HPV vaccination and “yes” if self-reported at least 1 dose HPV vaccine.

<sup>g</sup> Restricted to women reporting a male sex partner in the past 6 months

<sup>h</sup> The variable was coded as “always” if the subject reported always using condoms with all male partners during the past 6 months. If a subject reported not always using condoms with at least 1 male partner, the variable was coded as “not always.” Otherwise, if condom use data were missing for at least 1 partner, the variable was set to missing.

For analyses restricted to vaccine-type HPV, history of HPV vaccination was inversely associated with incident infection. Compared to women self-reporting no history of HPV vaccination, those reporting at least 1 dose had an OR of 0.21 (95% CI: 0.05-0.86) in univariate analysis. HPV vaccination remained borderline statistically significant in multivariate analysis after adjusting for lifetime number of male sex partners and the composite recent sexual behavior variable: OR 0.27 (95% CI: 0.07-1.03). The two cases of incident vaccine-type HPV detected in women reporting a history of HPV vaccination were both HPV type 16. One of these women reported receiving 1 dose of the HPV vaccine, and the other reported 2 doses. For details see Table 3.4.

Table 3.4. Odds ratios (ORs) for associations between self-reported HPV vaccination and incidence of type-specific vaccine-type HPV in 18- to 24-year-old female online daters (N=635 women-types)

	<b>No. of Incident Detections<sup>a</sup></b>	<b>n<sup>b</sup></b>	<b>Univariate OR (95% CI)</b>	<b>Multivariate OR (95% CI)<sup>d</sup></b>
History of HPV vaccination <sup>c</sup>				
No	13	373	Ref (1.0)	Ref (1.0)
Yes	2	279	0.21 (0.05-0.86)	0.27 (0.07-1.03)

<sup>a</sup> Vaccine-type HPV (6, 11, 16, and 18)

<sup>b</sup> Women-types could change categories throughout follow-up for time-dependent variables, therefore the number of women-types at risk (n) may exceed total N for time-dependent variable.

<sup>c</sup> Coded “no” if self-reported no history of HPV vaccination and “yes” if self-reported at least 1 dose HPV vaccine.

<sup>d</sup> The multivariate model was adjusted for lifetime number of male sex partners and a composite variable coded as 0 sex partners, 1 partner (not new or casual or multiple), 1 risk factor (new, casual, or multiple partners), 2 risk factors (new, casual, and/or multiple partners) or all three risk factors.

## Chapter 4. DISCUSSION AND CONCLUSION

In this study, we followed 164 young (18- to 24-year-old) female online daters for a year-long longitudinal study to study their incident HPV infections, taking into consideration their HPV vaccination history and their sexual behaviors. Overall, 100 of the 164 young women had newly detected clinically-relevant HPV during the study (36.9 infections per 100 person-years at risk). Restricting to oncogenic HPV and comparing to our previous study of mid-adult (25- to 65-year-old) female online daters, we found that young female online daters had a slightly higher incidence of oncogenic HPV infection (34.4 per 100 person-years, 95% CI: 25.9-45.6) compared to mid-adult women female online daters (29.5 per 100 person-years, 95% CI: 24.4-35.6).<sup>13</sup> For our young female online daters, the most common individual HPV types were 56, 53, and 52, while for mid-adult female online daters, the most common types were 53, 16, 52, and 51.<sup>13</sup> While the lower incidence of type 16 HPV in younger versus older women is likely due to the benefits of vaccination (discussed later), generally, we observed higher incidence rates of HPV among the younger women. This aligns with a previous national survey of 14-59 years-old women, which reported that HPV prevalence is highest in 20- to 24-year-old women<sup>4</sup>; we see similar patterns, though both of our studies are high-risk online daters. These results suggest that public health efforts to combat HPV infections—vaccination, education, etc.—needs to occur *early*, so that young women are prepared and protected when they enter the high-risk time-period of their lives.

As for online dating, we did not find evidence that online dating, per se, increases the risk of HPV infection for young women. Among our group of young women who date online, some characteristics of their male sex partners—multiple, new, or casual—appeared to be factors in HPV infection, but whether or not a partner was met online was not a factor. Univariate analysis

suggests that, for incident clinically-relevant HPV infection, online dating was unassociated (OR 0.91, 95% CI: 0.53-1.55). This may be due to online dating becoming mainstream among Americans, especially among 18- to 24-year-olds<sup>5</sup>; consequently, meeting partners online may no longer be indicative of increased risk of infection. Instead, other risky sexual behavior—having multiple sex partners, new sex partners, and/or casual sex partners—may be more indicative of increased risk of HPV infection. Those with all three risk factors, even after adjusting for the total lifetime number of sex partners, had a nearly 6-fold increased risk of infection relative to women with no recent partners (5.77 OR, 95% CI: 1.42-23.51). Similarly, in our previous analysis of the prevalence data from this cohort, meeting partners online was not associated with HPV, but recent sexual behavior (specifically reporting partners with other concurrent partners) was associated with increased likelihood of infection.<sup>6</sup> Furthermore, in our previous study of 25- to 65-year-old women, online dating was associated with prevalent but not incident HPV infections.<sup>8,14</sup> While there is some evidence that online dating is associated with an increased likelihood of high risk sexual behaviors for heterosexual women (such as higher rates of casual sex partners<sup>15</sup> and higher rates of being drunk/high during sex in young women<sup>16</sup>) the relationship to HPV infection may be a second-order effect (i.e. those who date online have more sexual partners<sup>16,17</sup>, which in turn, leads to HPV infections).

As for other risk factors, we note that there was a positive association between inconsistent condom use and incident HPV infection. While it did not reach statistical significance in univariate analysis, the direction and magnitude of effect aligns with the results of our prevalence paper of 18- to 24-year-old online daters, which found a significant association between inconsistent condom use and prevalent HPV infection.<sup>6</sup>

Overall, having more sexual contact appears to be a dominant factor for HPV infections. After adjusting for the number of lifetime partners in our multivariate analysis, those with more than 6 lifetime sex partners had the highest risk of infection (OR 6.37, 95% CI: 1.56-26.05). This is an interesting finding because lifetime partners are *past* partners, not current partners, and, in theory, should not contribute to new infections. A possible explanation may be the intermittent nature of HPV detections, especially among young women. Multiple studies of incidence of HPV infection among young women have found that they commonly have infection reappearance following a period of non-detection (likely due to a return to detectable levels of a previously acquired infection).<sup>18,19</sup> We have also previously found similar intermittent HPV detection patterns among mid-adult women.<sup>14</sup> Since HPV infections may be intermittently detected, apparent “new” infections can represent either new acquisition, reactivation, or intermittent persistent detection of previously acquired infections. Therefore, we might expect to see an association with lifetime number of partners if women with higher lifetime numbers of partners were more likely to have been infected in the past, and thus more likely to have prior infections reactivate or be re-detected, relative to women with fewer lifetime partners. Needing additional studies to understand detection patterns of type-specific HPV infections aside, the public health implications of sexual contact are clear. Increased sexual contact (i.e. higher number of lifetime sexual partners) increases the risk of HPV infections.

Finally, our results indicate that HPV vaccination appears protective against acquiring vaccine-type HPV in sexually active young women. In our analysis of vaccine-type HPV we found that a history of vaccination significantly reduced the risk of vaccine-type HPV infection, even adjusted for the lifetime number of partners and recent sexual behaviors, with those having at least one dose of the HPV vaccine having a 0.27 OR (95% CI: 0.07-1.03) of HPV infection

compared to those with no doses. Our findings align with published findings on the efficacy of HPV vaccinations. In one double-blind trial of a HPV vaccine (bivalent HPV 16 and 18) in 15- to 25-year-old women in Brazil and North America, vaccine efficacy was found to be 67.6% (95% CI: 48.9%-79.4%) for incident infections in the intention-to-treat population after 18 months of follow-up.<sup>20</sup> In another double blind trial of the HPV vaccine (quadrivalent HPV 6, 11, 16 and 18) in 16- to 23-year-old women in Brazil, Europe, and the USA, vaccine efficacy was found to be 89% (95% CI: 73%-96%) for persistent infection in the intent-to-treat population over 36 months of follow-up.<sup>21</sup> A recent meta-analysis (research papers from 2007 to 2016) about the effectiveness of HPV vaccinations world-wide<sup>22</sup> indicates that HPV vaccination maximally reduces infections by approximately 90%. A study of HPV infection prevalence data by the National Health and Nutrition Examination Survey (for the United States) indicate an overall reduction of 34% for vaccine types 6, 11, 16 and 18 for those aged 20- to 24 year-old females, between the pre-vaccine era (2003-2006) and 4 years of the vaccine era (2009-2012); for those reporting at least one dose of the HPV vaccine (compared to those unvaccinated), the adjusted prevalence ratio for vaccine types was 0.11, a reduction of 89% (95% CI: 0.05-0.24).<sup>23</sup> Our previous prevalence analysis in our young online dater cohort indicated a statistically significant reduction in vaccine-type HPV prevalence for  $\geq 1$  versus no doses of HPV vaccine (61%, 95% CI: 6%-84%).<sup>6</sup> We note that although intent to treat in controlled trials ( $\geq 1$  dose) are similar to our  $\geq 1$  dose results, there are several important differences. Unlike controlled trials, we did not restrict entry based on the lifetime number of sex partners (vaccine controlled trials were restricted to young women with fewer than 4 or 6 lifetime sex partners<sup>24,21</sup>); we were not able to regulate the number of vaccine doses in our study (in both of our studies, many women had less than the 3 recommended vaccine doses); vaccine trials also excluded women with evidence of

prior-vaccine type infection (via positive antibodies), whereas we did not have similar information. Overall, we have higher risk subjects; nevertheless, in our study, we were able to determine a significant reduction in the risk of infection in this high-risk cohort. This encouraging result reinforces the need to continue public health initiatives world-wide to vaccinate against HPV; public health agencies in the United States, in particular, can take proactive actions to increasing vaccination uptake in the United States and around the world.<sup>25</sup>

While HPV vaccination was protective against incidence of vaccine types, as expected, we did not find HPV vaccination to reduce the likelihood of infection when we considered infection with clinically-relevant HPV overall. Other types of HPV – types 52, 53, and 56 – were the most common types detected. There are two important implications. First, these findings reaffirm the need for continued funding and research into HPV vaccines. For example, the recently approved Gardasil 9 further protects against HPV types 31, 33, 45, 52, and 58.<sup>26</sup> Second, public health education and messaging may also need to be improved to clarify public understanding about the breadth of protection provided by current HPV vaccines; while they are important, they do not protect against all types of oncogenic HPV. Compared to a 2010 analysis of 16- to 23-year-old women enrolled in the placebo arm of the quadrivalent vaccine trial, while the incidence rates of vaccine-type HPV (16 and 18) were lower in our study, the incidences of non-vaccine type HPV were as high or higher for five out of the seven types studied (types 31, 35, 52, 58, and 59).<sup>18</sup> Since 58% of the young women in our study had at least one dose of the HPV vaccine, lower incidence rates of HPV type 16 and 18 infections were expected; higher incidence rates of non-vaccine type HPV infections were also expected since the subjects in our study were higher risk (e.g. the median lifetime number of sex partners was 6). Compared to incidence rates of two vaccine-type HPV (type 16 and type 18) in our previous study of 25- to

65-year-old female online daters (4.5 per 100 person-years, 95% CI: 2.9–7.2, and 2.5 per 100 person-years, 95% CI: 1.3–4.6, respectively)<sup>14</sup>, placebo arms of vaccine trials of 16- to 23- year-old young women (5.0 per 100 person-years, 95% CI: 5.2-6.6, and 2.2 per 100 person-years, 95% CI: 1.8-2.6, respectively)<sup>18</sup>, and low-risk young women (18- to 22-year-old newly sexually active women: 7.6 per 100 person-years at risk and 1.9 per 100 person-years at risk, respectively<sup>27</sup>), the incidence rates in our study of 18- to 24-year-old female online daters were *both low* (4.4 per 100 person-years, 95% CI: 2.1-9.3, and 1.2 per 100 person-years, 95% CI: 0.3-4.8, respectively). These results reinforce the effectiveness of the HPV vaccine, since women in control arms of vaccine trials are not vaccinated. However, while the incidence rate for vaccine-type HPV was 7.2 per 100 women-years, the overall risk of oncogenic HPV remained high at 34.4 per 100 women-years. Oncogenic HPV remains a major health risk; the current available vaccines are not panaceas.

There are several threats to the validity in our study. Our study focused on 18- to 24-year-old female online daters in the United States. Findings may not generalize (i.e. have threats to external validity) to other age groups, non-online-daters, or women in other countries; furthermore, the participants are self-selected and may not reflect all young female online daters. Also, our study was conducted in 2010-2012; the proportion of 18- to 24-year-olds that has used online dating in 2013 was lower at 10% compared to 27% in 2015. While the growth in online dating increases the relevance of our results, “online dating” may have changed over time. For example, though our study defined “online dating” rather broadly—any use of the internet to search for romantic partners—it may not cover all kinds, e.g. mobile apps. The interpretation of terms and the ability to discern types of relationships, e.g. casual and concurrent, may differ between women in our study (i.e. threats of construct validity), as well as women in other

studies. Furthermore, the behaviors of women online daters (e.g. the subsequent sexual contact) likely differ in different age groups, which may lead to differences in risks of infection. Finally, our study was limited in size and in duration, and we had incomplete information in various areas (e.g. the relation between timing of vaccination and sexual debut); these limitations may have impacted our ability to detect relationships.

In summary, our findings indicate that, HPV infections were common and associated with cumulative and recent high-risk sexual behavior (though not online dating per se), and that vaccination was protective against acquiring vaccine-type HPV.

## BIBLIOGRAPHY

1. Lever A, Berhout B. 2008 Nobel Prize in Medicine for Discoverers of HPV. *Retrovirology*. 2008;5(1):1.
2. WHO. Human Papillomavirus (HPV) and Cervical Cancer. Fact Sheets. <http://www.who.int/mediacentre/factsheets/fs380/en/>. Published 2016. Accessed July 2, 2016.
3. CDC. HPV Vaccines: Vaccinating Your Preteen or Teen. <http://www.cdc.gov/hpv/parents/vaccine.html>. Published 2015. Accessed July 18, 2016.
4. Markowitz LE, Hariri S, Lin C, et al. Reduction in Human Papillomavirus (HPV) Prevalence among Young Women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003-2010. *J Infect Dis*. 2013;208(3):385-393.
5. Smith A. *15% of American Adults Have Used Online Dating Sites or Mobile Dating Apps.*; 2016. <http://www.pewinternet.org/2016/02/11/15-percent-of-american-adults-have-used-online-dating-sites-or-mobile-dating-apps/>.
6. Barrere A, Stern JE, Feng Q, Hughes JP, Winer RL. Oncogenic Human Papillomavirus Infections in 18- to 24-Year-Old Female Online Daters. *Sex Transm Dis*. 2015;42(9):492-497.
7. Wolfrum SG, Koutsky LA, Hughes JP, et al. Evaluation of Dry and Wet Transport of At-Home Self-Collected Vaginal Swabs for Human Papillomavirus testing. *J Med Microbiol*. 2012;61(PART 11):1538-1545.
8. Winer RL, Hughes JP, Feng Q, et al. Prevalence and Risk Factors for Oncogenic Human Papillomavirus Infections in High-Risk Mid-Adult Women. *Sex Transm Dis*. 2012;39(11):848-856.
9. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. *J Clin Microbiol*. 1995;33(8):2058-2063.
10. Brown DR, Schroeder JM, Bryan JT, Stoler MH, Fife KH. Detection of Multiple Human Papillomavirus Types in Condylomata Acuminata Lesions from Otherwise Healthy and Immunosuppressed Patients. *J Clin Microbiol*. 1999;37(10):3316-3322.
11. Munoz N, Bosch X, Sanjosé S, Herrero R, Castellsagué X. Epidemiologic Classification

- of Human Papillomavirus Types Associated with Cervical Cancer — NEJM. *N Engl J Med*. 2003;348:518-527.
12. Bouvard, Veronique et al. A Review of Human Carcinogens - Part B Biological Agents. *Lancet Oncol*. 2009;10(4):321-322.
  13. Winer RL, Hughes JP, Feng Q, Stern JE, Xi LF, Koutsky LA. Incident Detection of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Women Aged 25-65 Years. *J Infect Dis*. 2016;(jiw074):1-11.
  14. Winer RL, Hughes JP, Feng Q, Stern JE, Xi LF, Koutsky LA. Incident Detection of High-Risk Human Papillomavirus Infections in a Cohort of High-Risk Mid-Adult Women. 2016;jiw(074).
  15. Bolding, Graham et al. Heterosexual Men and Women Who Seek Sex Through the Internet. *Int J STD AIDS*. 2006;17(8):530-534.
  16. McFarlane M, Bull SS, Rietmeijer C a. Young Adults on the Internet: Risk Behaviors for Sexually Transmitted Diseases and HIV. *J Adolesc Heal*. 2002;31(1):11-16.
  17. Mcfarlane, Mary et al. Women, the Internet, and Sexually Transmitted Infections. *J Women's Heal*. 2004;13(6):689-694.
  18. Insinga RP, Perez G, Wheeler CM, et al. Incidence, Duration, and Reappearance of Type-Specific Cervical Human Papillomavirus Infections in Young Women. *Cancer Epidemiol Biomarkers Prev*. 2010;19(6):1585-1594.
  19. Winer RL, Hughes JP, Feng Q, et al. Early Natural History of Incident , Type-Specific Human Papillomavirus Infections in Newly Sexually Active Young Women. *Cancer Epidemiol Biomarkers Prev*. 2011;20(4):699-707.
  20. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a Bivalent L1 Virus-Like Particle Vaccine in Prevention of Infection with Human Papillomavirus Types 16 and 18 in Young Women: A Randomised Controlled Trial. *Lancet (London, England)*. 2004;364(9447):1757-1765.
  21. Villa LL. Prophylactic Quadrivalent Human Papillomavirus (Types 6, 11, 16, and 18) L1 Wirus-like Particle Vaccine in Young Women: a Randomised Double-Blind Placebo-Controlled Multicentre Phase II Efficacy Trial. *Lancet Oncol*. 2005;6(5):271-278.
  22. Garland SM, Kjaer SK, Muñoz N, et al. Impact and Effectiveness Of the Quadrivalent Human Papillomavirus Vaccine: A Systematic Review of Ten Years of Real-World

- Experience. *Clin Infect Dis*. 2016;1-9.
23. Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV After Introduction of the Vaccination Program in the United States. *Pediatrics*. 2016;137(3):1-9.
  24. Koutsky LA, Harper DM. Current Findings from Prophylactic HPV Vaccine Trials. *Vaccine*. 2006;24(3).
  25. Rimer BK, Harper H, Witte ON. Accelerating HPV Vaccine Uptake: Urgency for Action to Prevent Cancer. 2014.
  26. FDA. FDA Approves Gardasil 9 for Prevention of Certain Cancers Caused by Five Additional Types of HPV. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm426485.htm>. Published 2014. Accessed July 18, 2016.
  27. Winer RL, Hughes JP, Feng Q, et al. Condom Use and the Risk of Genital Human Papillomavirus Infection in Young Women. *N Engl J Med*. 2010;354(25):2645-2654. doi:10.1056/NEJMoa1514204.