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Skin and Mucosal Human Papillomavirus Seroprevalence in Persons with Fanconi Anemia

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Persons with Fanconi anemia (FA) are at risk for human papillomavirus (HPV)-associated cancers; however, their natural HPV exposure and infection rates are unknown as is the adequacy with which they mount antibodies to HPV vaccination. This study aimed to determine, in 62 persons with FA, the seroprevalence of skin and mucosal HPV types, the seroprevalence in individuals self-reporting a history of HPV vaccination, and the factors associated with HPV seropositivity. A bead Luminex assay was used to determine seropositivity for HPV1, -2, and -4 (low-risk skin), -6 and -11 (low-risk mucosal, included in one HPV vaccine), -16 and -18 (high-risk mucosal, included in both HPV vaccines), and -52 and -58 (high-risk mucosal). Health- and behavior-related questionnaires were completed. Type-specific seroprevalence estimates and participant characteristics associated with seroprevalence were calculated; 48% reported HPV vaccination. Type-specific seropositivity in unvaccinated persons ranged from 7 to 21% for skin HPV types and 7 to 38% for mucosal HPV types. Among the unvaccinated participants, adults versus children demonstrated increased HPV1, -6, -16, and -58 seroprevalence of 45% versus 6%, 64% versus 22%, 64% versus 17%, and 36% versus 0%, respectively (all $P < 0.05$). The vaccinated participants versus the nonvaccinated participants demonstrated increased seroprevalence of HPV6, -11, -16, and -18 of 92% versus 38%, 92% versus 24%, 96% versus 34%, and 75% versus 7%, respectively (all $P < 0.0001$). Our data demonstrate that the unvaccinated participants had serologic evidence of prior skin and mucosal HPV infections and that seroprevalence increased among adults; in self-reported vaccinees, seroprevalence of HPV vaccine types was 75 to 96%.

There are more than 100 human papillomaviruses (HPVs), and they are defined as distinct HPV types based on their genotypic differences. HPV types are also categorized based on their epidemiologic data of associated clinical diseases and on laboratory studies that determine their role in cancer development (1). All HPVs infect skin or mucosal epithelium, causing subclinical disease or warts, but only those in the high-risk clinical category lead to cancers (2–4). Genital mucosa HPV infections are the most common sexually transmitted infections worldwide (5, 6). They are acquired soon after sexual debut, and >75% of adults have evidence of a current or prior genital HPV infection (2, 7).

Several specific HPV genotypes frequently infect the skin or the genital tract. Types that are low risk for cancer cause common warts on the skin (HPV1, -2, and -4) and in the genital tract (HPV6 and -11). Types that are high risk for cancer are found in cervical cancer (HPV16, -18, -52, and -58) (4) and in other anogenital cancers in men and women (8–11), and types that are high-risk for skin cancer in immunocompromised patients include HPV5, -8, and -38. More recently, head and neck cancers have been linked to high-risk HPV infections (12–14). HPV-positive head and neck cancer incidence is on the rise in the United States and is projected to outstrip cervical cancer incidence by 2020 (15). The dynamics of all HPV-associated diseases and cancer are changing worldwide due to increased HPV screening and testing and HPV prevention through vaccination.

Most HPV infections are cleared by the immune system and lead to production of type-specific antibodies (16, 17) that potentially protect against future type-specific HPV infections (18). The presence of these antibodies marks prior infection in the general population; however, it is less clear whether persons with chronic immunodeficiency from bone marrow failure mount antibody responses to skin or mucosal low- and high-risk HPV infections. One specific population at great risk for HPV-associated cancers is patients with Fanconi anemia (FA).

FA is an autosomal recessive (and rarely X-linked) genetic disease involving the double-strand DNA repair pathway (19). FA increases the risk for multiple cancer types, including squamous cell carcinomas (SCC) (20–24). Additionally, when high-risk HPV oncogenes are expressed in FA patient-derived squamous cell lines, malignant phenotypes are augmented, and in FA mice with HPV oncogene coexpression, SCC development is accelerated (22, 25–27). Therefore, high-risk HPV infections in individuals with FA can further increase their risk for anogenital tract and perhaps head and neck SCCs.

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There are two FDA-approved vaccines that induce type-specific antibody responses either to HPV16 and -18 or to HPV6, -11, -16, and -18 (28–33). These vaccines have led to decreases in HPV infection, genital warts, and dysplasias internationally, but only one study to date has analyzed whether HPV vaccination in patients with bone marrow failure led to seroconversion (34). No study has specifically focused on the FA population to determine seroprevalence after vaccination, nor have any population-based studies estimated the proportion of FA individuals infected with low-risk or high-risk HPV types using seropositivity as a proxy for HPV infection (34–39).

We collected sera from individuals with FA to determine serologic evidence of prior skin and mucosal infection with types commonly found in the general population that are linked to clinical disease (skin warts, genital warts, and cervical cancer): HPV1, -2, -4, -6, -11, -16, -18, -52, and -58. Additionally, seroprevalence of vaccine types was determined in persons who reported a history of HPV vaccination. Finally, we conducted a survey to determine the risk and protective factors associated with HPV seropositivity in persons with FA.

MATERIALS AND METHODS

Participant recruitment. Participants in this cross-sectional study were recruited from an ongoing longitudinal study hosted by the Cincinnati Children's Hospital FA Comprehensive Care Center (CCFACCC). Patients visiting the CCFACCC were approached to participate from October 2011 through May 2013. Further, attendees of the 2012 Fanconi Anemia Research Fund (FARF) adult and family meetings were invited to participate. Individuals of all ages were eligible if they reported a diagnosis of FA and were willing to complete study-related questionnaires and provide serum samples. Informed consent was obtained by the Cincinnati Children's principal investigator (PI) or a trained study coordinator. Enrolled participants were invited to submit additional serum samples and questionnaires at subsequent visits to the CCFACCC or by mail. The institutional review board (IRB) protocol and the informed consent process were approved by the Cincinnati Children's Hospital Medical Center IRB and by the Seattle Children's Hospital IRB.

Survey administration. Study questionnaire data on demographics, health history, and sexual behavior were collected by paper or electronic survey and were managed using Research Electronic Data Capture (REDCap) tools hosted at Cincinnati Children's Hospital Medical Center and supported by the Center for Clinical and Translational Science and a training grant (NIH UL1-RR026314) (40). REDCap is a secure, web-based application designed to support data capture for research studies. Participants aged <12 years had their surveys completed by a consenting adult family member on their behalf. Participants aged 12 to 14 years had a consenting adult family member complete their surveys on their behalf; however, the sexual history section of the questionnaire was completed separately by a confidential participant interview. Participants aged 15 years and older were asked to complete a self-administered questionnaire. If there was a concern for a participant's competency to complete the survey, the questionnaire was completed by a consenting adult family member on his or her behalf. If a participant did not complete the questionnaire at the time of serum sample collection, the study coordinator contacted the participant up to three times by Email or mail to request that it be completed.

Serum sample collection and storage. Venous blood samples were collected by a trained phlebotomist. Samples collected in the CCFACCC were immediately placed in storage (at −80°C). Samples collected at the 2012 FARF family meeting were centrifuged within 8 h, stored on dry ice, shipped, and placed into storage. Samples collected at the 2012 FARF adult meeting were shipped and centrifuged within 24 h and then placed in storage. Aliquots of sera (0.5 to 1 ml typically) were sent via next-day FedEx to the Fred Hutchinson Cancer Research Center (FHCRC) for HPV testing.

Serologic testing. Antibody testing at the FHCRC was performed using a Luminex-based assay described previously (16, 41, 42) with modifications. In brief, glutathione *S*-transferase (GST) and HPV L1 (HPV1, -2, -4, -6, -11, -16, -18, -52, and -58) plus BKPyV VP1 fusion proteins were expressed from a modified pGex4T vector (43), and bacterial lysates were prepared. Magnetic microspheres (beads) containing a unique combination of fluorescent dyes (Bio-Rad Laboratories, Hercules, CA) were covalently coupled with glutathione-linked casein (all chemicals supplied by Sigma Chemicals [St. Louis, MO], unless otherwise indicated). Each protein preparation was incubated with a different bead set. Unbound protein was removed by washing, and bead sets were combined. Human sera were diluted 1:100, blocked, and incubated with the combined bead sets in 96-well plates. Beads were then washed, and bound antibodies were detected by reaction with biotinylated anti-human IgG (KPL, Gaithersburg, MD) and streptavidin-phycoerythrin (Life Technologies Inc., Carlsbad, CA) on a BioPlex 200 instrument (Bio-Rad Laboratories). For each sample, the median fluorescent intensity (MFI) for GST was subtracted from the MFI of the other antigens. The HPV16 international standard serum from an HPV16 DNA-positive woman and serum from a quadrivalent vaccinated woman were run on each plate to calibrate a titration curve for each experiment and for each HPV type. The seropositivity cutoff for HPV16 was established at 500 MFI, and our seropositivity cutoffs for the positive-control BK virus (BKV) and all other HPV types tested were extrapolated to this MFI as well. An HPV16 MFI of 500 was approximately 3 IU/ml for HPV16.

Statistical methods. As participants in this study were recruited from a larger longitudinal study, flexibility was afforded in the timing of serum sample and questionnaire data collection. For this study, we selected one serum sample and one survey from each participant for analysis. To eliminate the possibility of error in interpretation of behavior and health characteristics associated with HPV seropositivity, we developed strict reliability criteria for inclusion of observations for the variables bone marrow transplant, sexual experience, HPV vaccination, genital or anal warts, and common warts when blood draw and survey collection dates occurred more than 30 days apart.

To include an observation for history of bone marrow transplant, sexual experience, or HPV vaccination >30 days apart from a blood draw, the survey must have been administered (i) after serology and the participant reported never having had a bone marrow transplant, sexual experience, or HPV vaccination or (ii) the participant reported having had a bone marrow transplant, sexual experience, or HPV vaccination at an age younger than the age reported at the time of the blood draw. Alternatively, an observation was included if the survey had been administered before serology, and the participant reported having had a bone marrow transplant, sexual experience, or HPV vaccination. For a history of sexual experience, the minimum age reported across all sexual activities was used to determine whether the first sexual experience occurred prior to the serology date. For the history of HPV vaccination, the year of vaccination was used to determine if the vaccine was administered prior to the serology date.

To include an observation for a history of genital, anal, or common warts more than 30 days apart from a blood draw, the survey must have been administered (i) after serology and the participant reported no history of genital, anal, or common warts or (ii) before serology and the participant reported a history of genital, anal, or common warts.

We evaluated type-specific HPV seroprevalence according to self-reported characteristics: age at time of blood draw (<18/≥18 years old), HPV vaccination status (ever received ≥1 dose of HPV vaccine [yes/no]), a history of bone marrow transplant (yes/no), sexual experience (defined as ever having had mouth to genital, finger to genital, or genital to genital contact with another person [yes/no]), genital or anal warts (yes/no), and common warts (yes/no).

Pearson's chi-square tests and Fisher's exact tests were used in the

TABLE 1 Baseline characteristics of 62 participants with Fanconi anemia[a]

Characteristic	Value
Age (mean ± SD) (yr)[b]	16.8 ± 8.9
Age at blood draw ($n = 62$)	
<18 yr	37 (59.7)
≥18 yr	25 (40.3)
Gender ($n = 62$)	
Female	35 (56.4)
Male	27 (43.6)
Individual who completed the survey ($n = 62$)	
Self	32 (51.6)
Mother	23 (37.1)
Father	6 (9.7)
Grandmother	1 (1.6)
Race ($n = 62$)[b]	
American Indian/Alaskan Native	2 (3.2)
Asian	0 (0.0)
Black/African American	3 (4.8)
Native Hawaiian/Pacific Islander	0 (0.0)
White	57 (91.9)
Other	2 (3.2)
Ethnicity ($n = 56$)[c]	
Hispanic/Latino	7 (12.5)
Not Hispanic/Latino	47 (83.9)
Do not know	2 (3.6)
Marital status (≥18 yr only) ($n = 25$)	
Unmarried	20 (80.0)
Married	4 (16.0)
Not asked	1 (4.0)
Ever had genital or anal warts ($n = 54$)[d]	
No	51 (94.4)
Yes	3 (5.6)
Ever had common warts ($n = 50$)[e]	
No	32 (64.0)
Yes	11 (22.0)
Not asked	7 (14.0)
Ever had HPV vaccine ($n = 56$)[f]	
No	32 (57.1)
Yes	24 (42.9)
FA complementation group ($n = 61$)[g]	
FancA	24 (39.3)
FancB	0 (0.0)
FancC	6 (9.8)
Other	10 (16.4)
Do not know	21 (34.4)
Ever had a bone marrow transplant ($n = 58$)[h]	
No	28 (48.3)
Yes	30 (51.7)
Ever had a sexual experience with another person ($n = 59$)[i]	
No	39 (66.1)
Yes	20 (33.9)

univariate analyses to compare type-specific HPV seroprevalence across categories of a variable. Logistic regression was used to compare type-specific HPV seroprevalence by vaccination status, adjusting for age (for all types) and sexual experience (for genital HPV types). *t* tests were used to compare mean antibody titers among type-specific seropositive individuals (for HPV6, -11, and -16 only) between those with and without prior sexual experience. A two-sided 0.05 test level determined statistical significance for all analyses. All analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Sixty-two individuals with FA who submitted sera for antibody testing and completed a survey were included in the analysis. Of the serum samples selected for analysis, 41 (66%) were collected within 30 days of the survey. Thirteen were collected >30 days prior to the survey (median, 95 days; range, 50 to 336 days), whereas eight were collected >30 days after the survey (median, 361 days; range, 31 to 484 days). Fifty-two percent of the surveys were completed by the participating individual, and 48% were completed by a parent or grandparent (Table 1).

At the time of serum collection, the mean age of participants was 16.8 years (standard deviation [SD], 8.9 years) (range, 3 to 42 years). Fifty-two percent reported a history of bone marrow transplant, 34% reported ever having had a sexual experience, and 6% reported a history of genital warts; 43% reported a history of HPV vaccination (Table 1).

Three of the 62 (5%) samples did not have antibodies to our serology positive control, BKV, and were thus excluded from further analyses. Among the 59 remaining participants, the seroprevalences of antibodies against all HPV types tested are shown in Table 2. The seroprevalences of antibodies against HPV6, -11, -16, and -18 among unvaccinated participants were 38%, 24%, 34%, and 7%, respectively. Among participants who reported HPV vaccination, the seroprevalences of HPV6, -11, -16, and -18 were 92%, 92%, 96%, and 75%, respectively. This increase in seroprevalence in vaccinated individuals was statistically significant ($P < 0.01$, adjusting for age and history of sexual experience) (Table 2).

TABLE 1 (Continued)

[a] Values are reported as no. (%) unless indicated otherwise.
[b] Two participants selected two categories (American Indian/Alaska Native and white).
[c] Six observations were excluded because of missing ethnicity data.
[d] Eight observations were excluded because serum was collected >30 days after survey completion and the participant reported no history of genital or anal warts.
[e] One observation was excluded because serum was collected >30 days after survey completion and the participant reported no history of common warts; 3 observations were excluded because serum was collected >30 days prior to survey completion and the participant reported a history of common warts. Eight observations were also excluded because of missing common wart data.
[f] Three observations were excluded because serum was collected >30 days prior to survey completion and the participant reported having had an HPV vaccine and the year of the last shot administered was not prior to the year of serum collection; 3 observations were excluded because serum was collected more than 30 days after survey completion and the participant reported having not had an HPV vaccine.
[g] One observation was excluded because of missing FA complementation group data.
[h] Four observations were excluded because serum was collected more than 30 days after survey completion and the participant reported no history of bone marrow transplant.
[i] One observation was excluded because serum was collected >30 days after survey completion and the participant reported no sexual experience; one observation was excluded because serum was collected >30 days prior to survey completion and the participant reported a history of sexual experience but the minimum age of any sexual activity was missing. One observation was also excluded because of missing sexual activity data.

TABLE 2 Serologic status according to vaccination status[a]

HPV type	No. (%) seropositive			*P*	
	All participants ($n = 59$)	Never had HPV vaccine[b] ($n = 29$)	Had HPV vaccine[b] ($n = 24$)	Unadjusted chi-square	Logistic regression
Low risk, skin					
HPV1	25 (42)	6 (21)	17 (71)	0.0002	0.0009[c]
HPV2	16 (27)	2 (7)	13 (54)	0.0001	0.0012[c]
HPV4	14 (24)	4 (14)	9 (38)	0.0459	0.0475[c]
Vaccine					
HPV6	36 (61)	11 (38)	22 (92)	<0.0001	0.0017[d]
HPV11	32 (54)	7 (24)	22 (92)	<0.0001	0.0003[d]
HPV16	36 (61)	10 (34)	23 (96)	<0.0001	0.0012[d]
HPV18	21 (36)	2 (7)	18 (75)	<0.0001	<0.0001[d]
High risk, mucosal					
HPV52	16 (27)	3 (10)	12 (50)	0.0014	0.0047[d]
HPV58	21 (36)	4 (14)	16 (67)	<0.0001	0.0005[d]

[a] Excludes 3 samples that were BKV seronegative.
[b] Restricted to observations with reliable questionnaire data on HPV vaccination history, based on prespecified criteria.
[c] Adjusted for age.
[d] Adjusted for age and sexual experience.

Positive serology against nonvaccine HPV1, -2, and -4 (low-risk skin types) and -52 and -58 (high-risk genital types) was also analyzed according to self-reported vaccination status (Table 2). For those who reported no vaccination, seroprevalences were 21%, 7%, 14%, 10%, and 14%, respectively. For those reporting a history of vaccination, seroprevalences were 71%, 54%, 38%, 50%, and 67%, respectively. The differences in seroprevalences between vaccinated and unvaccinated individuals were statistically significant for each type ($P < 0.05$, adjusting for age [HPV1, -2, -4, -52, and -58] and sexual experience [HPV52 and -58 only]) (Table 2).

Among unvaccinated individuals, no statistically significant associations were observed between type-specific seroprevalence and history of bone marrow transplant, sexual experience, or warts (common, genital, or anal). However, participants 18 years of age and older were more likely to be seropositive for each type, and this difference was statistically significant for HPV1, -6, -16, and -58 (Table 3). Among vaccinated individuals, those reporting a history of bone marrow transplant were less likely to be seropositive for HPV2 and -58 than those reporting no history of bone marrow transplant (89% versus 33% and 100% versus 47%, respectively) (Table 4).

Eight (42%) of 19 participants who reported no history of vaccination and no prior sexual experience (including 16 participants who were excluded from Table 3 because they were <15 years of age) were seropositive for at least one of the six genital HPV types,

TABLE 3 Serologic status among unvaccinated participants, according to selected characteristics[a]

HPV type	No. (%) seropositive having:							
	Bone marrow transplant[b]		Sexual experience[b,c]		Warts[b,d]		Age	
	Never ($n = 16$)	Ever ($n = 11$)	Never ($n = 3$)	Ever ($n = 9$)	Never ($n = 19/26$)	Ever ($n = 5/1$)	<18 yr ($n = 18$)	≥18 yr ($n = 11$)
Low risk, skin								
HPV1	3 (19)	2 (18)			4 (21)	1 (20)	1 (6)	5 (45)[e]
HPV2	1 (6)	1 (9)			1 (5)	1 (20)	0 (0)	2 (18)
HPV4	1 (6)	3 (27)			3 (16)	1 (20)	1 (6)	3 (27)
Vaccine								
HPV6	7 (44)	3 (27)	2 (67)	5 (56)	10 (38)	0 (0)	4 (22)	7 (64)[e]
HPV11	4 (25)	2 (18)	2 (67)	2 (22)	6 (23)	0 (0)	3 (17)	4 (36)
HPV16	4 (25)	5 (45)	1 (33)	6 (67)			3 (17)	7 (64)[e]
HPV18	1 (6)	1 (9)	1 (33)	1 (11)			0 (0)	2 (18)
High risk, mucosal								
HPV52	1 (6)	2 (18)	1 (33)	1 (11)			1 (6)	2 (18)
HPV58	2 (13)	2 (18)	1 (33)	3 (33)			0 (0)	4 (36)[e]

[a] Excludes 3 samples that were BKV seronegative.
[b] Restricted to observations with reliable questionnaire data on history of bone marrow transplant, sexual experience, or warts, based on prespecified criteria.
[c] Restricted to participants ≥15 years of age.
[d] For HPV1, -2, and -4, only common warts were considered (sample size, 19 versus 5). For HPV6 and -11, only genital or anal warts were considered (sample size, 26 versus 1).
[e] $P < 0.05$.

TABLE 4 Serologic status among vaccinated participants, according to selected characteristics[a]

HPV type	No. (%) seropositive having:							
	Bone marrow transplant[b]		Sexual experience[b,c]		Warts[b,d]		Age	
	Never ($n = 9$)	Ever ($n = 15$)	Never ($n = 8$)	Ever ($n = 10$)	Never ($n = 9/20$)	Ever ($n = 6/2$)	<18 ($n = 11$)	≥18 ($n = 13$)
Low risk, skin								
HPV1	6 (67)	11 (73)			6 (67)	4 (67)	7 (64)	10 (77)
HPV2	8 (89)	5 (33)[e]			3 (33)	4 (67)	6 (55)	7 (54)
HPV4	4 (44)	5 (33)			4 (44)	2 (33)	3 (27)	6 (46)
Vaccine								
HPV6	8 (89)	14 (93)	7 (88)	9 (90)	18 (90)	2 (100)	10 (91)	12 (92)
HPV11	8 (89)	14 (93)	7 (88)	9 (90)	18 (90)	2 (100)	10 (91)	12 (92)
HPV16	9 (100)	14 (93)	7 (88)	10 (100)			10 (91)	13 (100)
HPV18	8 (89)	10 (67)	7 (88)	6 (60)			9 (82)	9 (69)
High risk, mucosal								
HPV52	6 (67)	6 (40)	4 (50)	5 (50)			5 (45)	7 (54)
HPV58	9 (100)	7 (47)[e]	5 (63)	7 (70)			7 (64)	9 (69)

[a] Excludes 3 samples that were BKV seronegative.
[b] Restricted to observations with reliable questionnaire data on history of bone marrow transplant, sexual experience, or warts, based on prespecified criteria.
[c] Restricted to participants ≥15 years of age.
[d] For HPV1, -2, and -4, only common warts were considered (sample size, 9 versus 6). For HPV6 and -11, only genital or anal warts were considered (sample size, 20 versus 2).
[e] $P < 0.05$.

with type-specific seroprevalence ranging from 5% to 32% (Table 5). Five of the eight seropositive individuals were seropositive for more than one type, including two positive for HPV6 and -11, two positive for HPV6, -11, and -16, and one positive for all 6 types. Among type-specific seropositive individuals, a comparison of antibody titers between those with and without prior sexual experience did not reveal any appreciable differences in titers for HPV6, -11, or -16 (data not shown).

DISCUSSION

These findings are among the first to document the type-specific serologic status of persons with FA for skin and mucosal HPV infections and indicate that a significant minority of persons with FA have evidence of prior infections with HPV, be they low-risk or high-risk skin or mucosal types. Antibodies to low-risk skin HPV1, -2, and -4 were commonly detected in participants as were antibodies to low-risk and high-risk HPV6, -11, -16, -18, -52, and -58 among unvaccinated, sexually experienced participants. Therefore, persons with FA can mount detectable antibodies to an HPV infection. Additionally, seroprevalence estimates increased with age; adults with FA had greater seropositivity to skin and mucosal HPV types than children with FA.

TABLE 5 Serologic status among unvaccinated participants with no sexual experience,[a] genital types

Vaccine type	No. (%) seropositive
Vaccine	
HPV6	6 (32)
HPV11	5 (26)
HPV16	4 (21)
HPV18	1 (5)
High risk, mucosal	
HPV52	2 (11)
HPV58	1 (5)
HPV6, -11, -16, -18, -52, or -58	8 (42)

[a] Restricted to observations with reliable questionnaire data on HPV vaccination history and history of sexual experience, based on prespecified criteria. $n = 19$.

These data are also among the first to quantify seroprevalence of low-risk and high-risk HPV6, -11, -16, and -18 based on vaccination history in individuals with FA (34). Although HPV vaccination history in this study was dichotomized as yes or no and did not require completion of the series, all vaccinated participants reported receiving the quadrivalent vaccine, and seroprevalence in participants was high, ranging from 75 to 96%, with the lowest estimate being observed for HPV18. Despite 100% seropositivity not being achieved against all vaccine types, these findings strongly support the immunoprotection that might be gained against HPV in individuals with FA.

Among the seven participants who self-reported vaccination or had family-reported vaccination but did not seroconvert to all four HPV types, three reported completing the three-shot series, while those remaining reported not having completed the series or did not report how many shots they had received. In the general population, the HPV18 virus-like particles (VLP) mount the lowest titers compared to those for HPV6, -11, and -16 (44); those titers wane rapidly, and children and adolescents who have HIV have lower seroconversion rates for HPV18 than for HPV6, -11, or -16 (45). Finally, vaccination history recall can be inaccurate (46). Therefore, the pattern of seropositivity after vaccination in individuals with FA can be explained by not yet having completed the vaccination series, by having completed the vaccination series several months in the past, by having relative immunosuppression, or by inaccurate recall of vaccination status by the participant or a family member.

Although the seroprevalence of HPV1, -2, -4, -52, and -58 was higher in participants who reported HPV vaccination (38 to 71% versus 7 to 21% in unvaccinated individuals), the increased seropositivity was most likely due to a robust antibody response to the vaccine, leading to cross-reactivity in the Luminex assay with

HPV6, -11, -16, or -18 antibodies. Even with more stringent assays such as virus-like particle assays, cross-reactivity can be detected due to titer, reactivity, and avidity of the sera in vaccinated individuals (47). The titers of sera from nine participants were evaluated for potentially cross-reactive antibodies, and their average titer of antibodies to vaccine types was almost 10-fold higher than that to other types (median titer for HPV6, -11, -16, and -18 of 2,214; median titer for HPV1, -2, -4, -52, and -58 of 291) (data not shown). A weak correlation between titers for vaccine and nonvaccine types was found but did not approach statistical significance ($P = 0.38$). The typical seroprevalences of HPV1, -2, -4, -52, and -58 range broadly from 1.2% to 59.3%, affected not only by the population studied but also by the methodology used for detection (48–50). Our data on the seropositivity of unvaccinated individuals fall well within that range. No behavioral or exposure-based histories were related to an increased risk of seropositivity among vaccinated individuals, except for never having had a bone marrow transplant.

In nonvaccinated participants, the seroprevalences of mucosal HPV6, -11, -16, -18, -52, and -58 ranged from 7 to 38% (Table 2). These seroprevalence rates are similar to those in the unvaccinated general population for males and females (51–53). Seropositivity among these individuals was most likely due to exposure from sexual experience; however, due to our study size, we were unable to detect significant differences among participants 15 and older who reported never or ever having had a sexual experience as a behavioral risk (Table 3).

Eight of 19 participants who reported no history of vaccination and no sexual experience tested seropositive for one or more genital types (Table 5). Seropositivity in these individuals is potentially attributable to several factors. Three of these individuals were seropositive for three or all four HPV types included in the quadrivalent HPV vaccine; these results likely reflect misreporting, as the chance of this occurring in the unvaccinated general population is <1% (46, 54). Next, young participants may have misreported sexual experiences during their direct interview or because their survey was completed by a family member on their behalf. This underreporting has been found in other studies as well (55). Finally, unmeasured behaviors and exposures are important to consider as etiologies for HPV seropositivity. Vertical transmission, auto/heteroinoculation, fomites, and the receipt of blood products, including immunoglobulins, are additional ways in which HPV exposure and seroconversion can occur, and these were not assessed in this survey as possible sources of seropositivity.

In summary, individuals with FA are at risk for SCC and for HPV-associated cancers. Our data indicate that persons with FA have antibodies to both skin and genital mucosa HPV infections and when vaccinated against HPV are seropositive for HPV vaccine types in proportions typical of the general child and adult populations. Among unvaccinated participants, there is greater seropositivity of adults for low-risk and high-risk skin and mucosal HPV types. As in the general population, these results suggest that exposure to HPV increases over time and that vaccination against high-risk types is important to prevent HPV-related cancers.

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