

Human Papillomavirus Genotypes From Vaginal and Vulvar Intraepithelial Neoplasia in Females 15–26 Years of Age

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OBJECTIVE: To estimate the proportion of vulvar and vaginal low-grade and high-grade squamous intraepithelial lesions (LSILs and HSILs) in females 15–26 years of age attributable to 14 human papillomavirus (HPV) genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59).

METHODS: A post hoc analysis of prospectively diagnosed vulvar and vaginal LSILs and HSILs among females 15–26 years of age enrolled in the placebo arms of two phase 3, randomized HPV vaccine trials assessed 14 prespecified HPV genotypes associated with cervical cancers or anogenital warts using a type-specific multiplex polymerase chain reaction

assay. The frequency of lesions associated with specific HPV genotypes was estimated by proportional and other attribution methods.

RESULTS: During approximately 4 years of follow-up in 8,798 females, 40 vulvar LSILs and 46 vulvar HSILs were diagnosed in 68 females, and 118 vaginal LSILs and 33 vaginal HSILs were diagnosed in 107 females. Females developing vulvar (41.2%) or vaginal (49.5%) lesions also had cervical lesions, whereas 6.5% of females with cervical lesions had vaginal or vulvar lesions. At least 1 of the 14 HPV genotypes was detected in females with vulvar LSIL (72.5%), vulvar HSIL (91.3%), vaginal LSIL (61.9%), and vaginal HSIL (72.7%). Considering only HPV-positive lesions, the nine most common genotypes causing cervical cancer and anogenital warts (6, 11, 16, 18, 31, 33, 45, 52, and 58) were

See related editorial on page 259.

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found in 89.4% of vulvar LSILs, 100% of vulvar HSILs, 56.0% of vaginal LSILs, and 78.3% of vaginal HSILs.

CONCLUSION: Most vulvar and vaginal lesions were attributable to at least 1 of the 14 HPV genotypes analyzed. Effective immunization programs could potentially prevent substantial numbers of HPV-related vulvar and vaginal LSILs and HSILs.

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Many common human papillomavirus (HPV) types have been detected in vulvar and vaginal lesions

typically diagnosed in women in the third to sixth decade of life.^{1–8} Human papillomavirus-related vulvar intraepithelial neoplasia (VIN) is divided into low-grade squamous intraepithelial lesions (LSIL, VIN 1) and potentially precancerous high-grade squamous intraepithelial lesions (HSIL, VIN 2/3).^{9,10} A substantial number of untreated vulvar HSILs (87.5%) progress to cancer.¹¹ Low-grade and high-grade vaginal intraepithelial neoplasia (VAIN) are also classified as LSIL (VAIN 1) and HSIL (VAIN 2/3), respectively; HSILs variably progress to invasive vaginal cancer.¹² Delayed diagnosis of vulvar and vaginal cancers is common and can adversely affect prognosis.

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Cervical cancer is predominately caused by 12 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), and most anogenital warts are caused by two low-risk HPV types (6 and 11).¹³ Three highly efficacious HPV vaccines are in widespread worldwide use against genital HPV infection and associated anogenital lesions: bivalent (HPV16/18 [Cervarix]),¹⁴ quadrivalent (HPV6/11/16/18 [GARDASIL]),^{15,16} and nonavalent vaccines (HPV6/11/16/18/31/33/45/52/58 [GARDASIL9]).¹⁷

Estimates for the frequencies of HPV types associated with vulvovaginal lesions remain imprecise.^{7,18–21} To delineate the association of HPV genotypes with incident vulvovaginal abnormalities, we estimated the proportion of vulvar or vaginal lesions attributable to the 14 HPV genotypes most frequently linked to cervical cancer and anogenital warts among females 15–26 year old prospectively followed in the placebo arms of two pivotal trials assessing the prophylactic efficacy of the quadrivalent vaccine.^{15,16}

MATERIALS AND METHODS

For this post hoc descriptive analysis, we used data to delineate the association of HPV genotypes from females 15–26 years of age in the placebo arms of two already published, randomized, double-blind clinical trials of the quadrivalent HPV vaccine (FUTURE I, NCT00092521,¹⁵ and FUTURE II, NCT00092534¹⁶) who developed vulvar or vaginal LSILs and HSILs during approximately 4 years of follow-up. Secondary objectives were to compare baseline characteristics of females with and without incident vulvar or vaginal lesions and to explore patterns of lesion development at multiple (vulvar, vaginal, and cervical) sites in the same female. An exploratory, but clinically informative, objective was to estimate the proportion of vulvar or vaginal LSILs or HSILs attributable to genotypes covered by current vaccines.

The study designs, protocols, and results for each study have been previously published.^{15,16} Both studies were sponsored by Merck & Co, Inc., conducted in accordance with principles of Good Clinical Practice, and approved by the appropriate institutional review boards and regulatory agencies governing each site. Females reporting a history of abnormal cervical Pap test results or multiple sexual partners (defined as four or greater lifetime partners by most but not all sites) were excluded. These trials enrolled a total of 17,622 healthy, nonpregnant females aged 15–26 years, 8,812 of whom were randomized to the placebo arms. Participants underwent

pelvic examinations for cytologic testing with visual inspection of the vulvovaginal and perianal areas and collection of cervical and anogenital swabs for HPV testing by polymerase chain reaction assay at randomization on day 1, at months 7 and 12, and then every 6–12 months for up to 48 months.

The 8,798 placebo recipients who received one or greater study injections and had follow-up data were included in the current descriptive analysis. Individual participants could have more than one lesion of the same or different histologic type. Biopsies were obtained from all external anogenital lesions deemed possibly HPV-related on inspection. If multiple lesions were apparent, each suspicious lesion was sampled. All biopsy specimens were first read for clinical management by pathologists at a central laboratory (Diagnostic Cytology Laboratories, Indianapolis, Indiana) and then adjudicated by a panel of four gynecologic pathologists blinded to central laboratory and clinical diagnoses, treatment group, and HPV status. Specimens were tested for 14 prespecified HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) using a type-specific multiplex polymerase chain reaction assay developed by Merck Research Laboratories (Merck & Co, Inc.) to simultaneously amplify and detect the L1, E6, and E7 open reading frames of HPV6, 11, 16, 18, 31, 45, 52, and 58 or the E6 and E7 open reading frames of HPV33, 35, 39, 51, 56, and 59.^{22,23} The adjudicated diagnoses of vaginal and vulvar LSILs (not including condylomata) and HSILs were captured for these analyses.

Baseline characteristics of females who did and did not develop vulvar or vaginal lesions were analyzed by lesion location and grade. If a female developed greater than one lesion at a site, the highest grade lesion was used in the analysis. To calculate age-adjusted associations between baseline characteristics and lesion development, odds ratios (ORs) and 95% CIs were computed using a nested case-control approach. Five controls per case were randomly selected from females who did not develop cervical or anogenital lesions throughout the follow-up period. The comparison group was not matched by person-time at risk.

We explored patterns of lesion development at cervical, vulvar, and vaginal sites in individual females. The proportion of females with incident lesions at a specified site who also had lesions at other sites was calculated from females with histologically confirmed cervical, vulvar, or vaginal lesions during follow-up.



Human papillomavirus types were grouped into bivalent vaccine types (16/18), quadrivalent vaccine types (6/11/16/18), nonavalent vaccine types (6/11/16/18/31/33/45/52/58), the five additional types included in the nine-genotype vaccine, but not in the quadrivalent vaccine (31/33/45/52/58), and the most common nonvaccine high-risk types identified in the quadrivalent HPV vaccine trials (35/39/51/56/59).

Proportional attribution was the primary attribution approach.^{18–21,24} The relative proportion of co-infected lesions attributed to each HPV type detected in the lesion corresponded to the relative proportion of lesions from the same population in which that HPV type had been detected as a single infection. Four other approaches^{18–21,24} were used as sensitivity analyses (Appendix 1, available online at <http://links.lww.com/AOG/B118>). For additional interpretative context, we also present the type-specific analyses for only the subset of lesions in which any of the 14 HPV types being tested was identified.

RESULTS

There were 8,812 females aged 15–26 years at baseline randomized to the two placebo arms, but 14 fe-

males were excluded from this analysis because of missing data or cross-treatment with vaccine. During approximately 4 years of follow-up in the remaining 8,798 females, a total of 86 vulvar lesions (40 LSILs and 46 HSILs) were diagnosed in 68 females, and 151 vaginal lesions (118 LSILs and 33 HSILs) were diagnosed in 107 females (Appendix 2, available online at <http://links.lww.com/AOG/B118>).

Females who developed vulvar or vaginal lesions during follow-up tended to have higher numbers of lifetime and recent (last 6 months) sex partners and also tended to test positive for at least one measured HPV genotype at baseline than females who remained lesion-free. Females who developed vaginal lesions during follow-up were more likely to have a Pap test result abnormality at baseline compared with those with no lesions during follow-up (LSIL crude OR 3.1, 95% CI 1.7–5.7; HSIL crude OR 2.3, 95% CI 0.9–6.0); however, development of vulvar lesions during follow-up was not associated with abnormal Pap test results at baseline (Appendix 2, <http://links.lww.com/AOG/B118>).

By the end of the approximately 4 years of follow-up, 41.2% of females with vulvar lesions and 49.5% of females with vaginal lesions also had cervical lesions, whereas only 6.5% of females with cervical lesions had vaginal or vulvar lesions (Table 1). Overall,

Table 1. Proportion of Young Females Who Developed Lesions at Multiple Sites (Cervical, Vulvar, or Vaginal) During Follow-up

	Subset With Cervical Lesions*			Subset With Vulvar Lesions*			Subset With Vaginal Lesions*			With Lesions at Any Other Site*	With Lesions at Both Other Sites*
	Any Cervical	LSIL	HSIL	Any Vulvar	LSIL	HSIL	Any Vaginal	LSIL	HSIL		
Cervical lesions											
Any (N=1,201) [†]	—	—	—	28 (2.3)	13 (1.1)	15 (1.3)	53 (4.4)	41 (3.4)	12 (1.0)	78 (6.5)	3 (0.3)
LSIL (n=677) [†]	—	—	—	16 (2.4)	8 (1.2)	8 (1.2)	36 (5.3)	28 (4.1)	8 (1.2)	50 (7.4)	2 (0.3)
HSIL (n=524) [†]	—	—	—	12 (2.3)	5 (1.0)	7 (1.3)	17 (3.2)	13 (2.5)	4 (0.8)	28 (5.3)	1 (0.2)
Vulvar lesions											
Any (N=68) [†]	28 (41.2)	16 (23.5)	12 (17.7)	—	—	—	7 (10.3)	4 (5.9)	3 (4.4)	32 (47.1)	3 (4.4)
LSIL (n=32) [†]	13 (40.6)	8 (25.0)	5 (15.6)	—	—	—	3 (9.4)	1 (3.1)	2 (6.3)	16 (50.0)	—
HSIL (n=36) [†]	15 (41.7)	8 (22.2)	7 (19.4)	—	—	—	4 (11.1)	3 (8.3)	1 (2.8)	16 (44.4)	3 (8.3)
Vaginal lesions											
Any (N=107) [†]	53 (49.5)	36 (33.6)	17 (15.9)	7 (6.5)	3 (2.8)	4 (3.7)	—	—	—	57 (53.3)	3 (2.8)
LSIL (n=81) [†]	41 (50.6)	28 (34.6)	13 (16.1)	4 (4.9)	1 (1.2)	3 (3.7)	—	—	—	43 (53.1)	2 (2.5)
HSIL (n=26) [†]	12 (46.2)	8 (30.8)	4 (15.4)	3 (11.5)	2 (7.7)	1 (3.9)	—	—	—	14 (53.9)	1 (3.9)

LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

Data are n (%).

* Number of females with a specified lesion type in each row who also developed the lesion type indicated in the column.

[†] Total number of females who developed the lesion type specified in the row during the follow-up.



10.3% of females with vulvar lesions had vaginal lesions, whereas 6.5% of females with vaginal lesions also had vulvar lesions. In 47.1% of females who developed vulvar lesions and 53.3% of females who developed vaginal lesions, abnormalities involving cervical and other sites were also present. Females who developed vulvar HSILs had the highest likelihood of having lesions at all three genital sites (8.33%). For females with lesions at multiple sites, no consistent pattern in the relative timing of diagnosis was observed.

In total, 72.5% of vulvar LSILs and 91.3% of vulvar HSILs tested positive for one or greater of the 14 HPV types assayed (Table 2 and Fig. 1A), and co-infections multiple HPV types were present in 24.1% and 45.2% of the HPV-positive LSILs and HSILs, respectively. Using proportional attribution, 87.7% and 89.4% of all HPV-positive vulvar LSILs were attributable to HPV types in the quadrivalent and nonavalent vaccines, respectively (Fig. 1B). Specifically, 52.0% of all vulvar LSILs and 71.7% of HPV-positive vulvar LSILs were associated with HPV6,

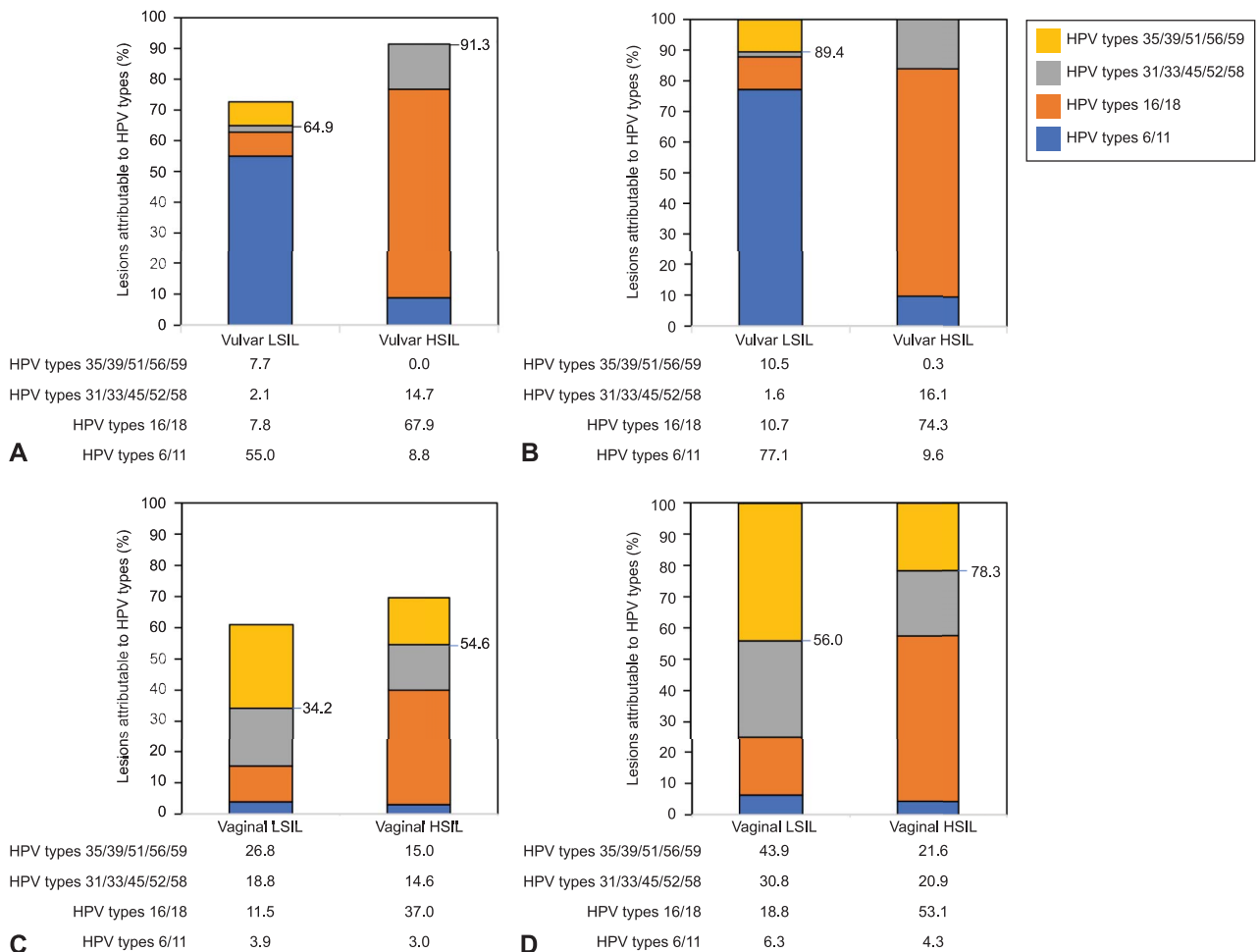


Fig. 1. Vulvar or vaginal lesion attribution in females aged 15–26 years to nonavalent vaccine types (6/11, 16/18, and 31/33/45/52/58) and high-risk nonvaccine types (35/39/51/56/59). **A.** Based on the proportional attribution method, percentage of all human papillomavirus (HPV)-positive vulvar low-grade squamous intraepithelial lesions (LSILs) or high-grade squamous intraepithelial lesions (HSILs) attributable to quadrivalent and nonavalent vaccine types were measured, respectively; denominator includes all lesions (n=40 for vulvar LSILs and n=46 for vulvar HSILs). **B.** Using multitype-adjusted attribution methods; percentage of all vulvar LSIL and HSILs attributable to all HPV vaccine types; denominator includes HPV-positive lesions (n=29 for vulvar LSILs and n=42 for vulvar HSILs). **C.** Based on the proportional attribution method; denominator includes all lesions (n=118 for vaginal LSILs and n=33 for vaginal HSILs). **D.** Using multitype-adjusted attribution methods, percentage of all vaginal LSILs and HSILs attributable to HPV genotypes; denominator includes HPV-positive lesions only (n=72 for vaginal LSILs and n=23 for vaginal HSILs).

Garland. Human Papillomavirus-Related Vulvar and Vaginal Lesions. *Obstet Gynecol* 2018.



Table 2. Type-specific Prevalence and Proportional Attribution of Human Papillomavirus Genotypes in Vulvar Lesions Among Females 15–26 Years of Age

HPV Type(s) Tested	Vulvar LSIL					
	All Lesions in Denominator (n=40)			Only HPV-Positive Lesions in Denominator (n=29)		
	Any*	Single [†]	Proportion [‡]	Any*	Single [†]	Proportion [‡]
6	21 (53)	17 (43)	20.8 (52)	21 (72)	17 (59)	20.8 (72)
11	3 (8)	1 (3)	1.6 (4)	3 (10)	1 (3)	1.6 (5)
16	4 (10)	2 (5)	3.1 (8)	4 (14)	2 (7)	3.1 (11)
18	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
31	1 (3)	0 (0)	0.5 (1)	1 (3)	0 (0)	0.5 (2)
33	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
45	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
52	2 (5)	0 (0)	0 (0)	2 (7)	0 (0)	0 (0)
58	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
35	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
39	3 (8)	1 (3)	1.6 (4)	3 (10)	1 (3)	1.6 (5)
51	1 (3)	0 (0)	0.5 (1)	1 (3)	0 (0)	0.5 (2)
56	1 (3)	1 (3)	1.0 (3)	1 (3)	1 (3)	1.0 (3)
59	1 (3)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
6/11/16/18	—	—	25.4 (63.6)	—	—	25.4 (87.7)
31/33/45/52/58	—	—	0.5 (1.3)	—	—	0.5 (1.7)
6/11/16/18/31/33/45/52/58	—	—	25.9 (64.9)	—	—	25.9 (89.4)
35/39/51/56/59	—	—	3.1 (7.7)	—	—	3.1 (10.6)

HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

Data are number of females with indicated lesion type (%).

* n (%) of all lesions testing positive for the respective HPV type, regardless of co-infections.

† n (%) of lesions testing positive for the respective HPV type only, excluding co-infections.

‡ n (%) of lesions attributable to the respective HPV type, as determined applying the proportional attribution method.

predominantly as single-type infections. Overall, 60.0–67.5% of vulvar LSILs were linked to nonavalent vaccine types, most (60.0–65.0%) of which were quadrivalent vaccine types (Appendix 3, available online at <http://links.lww.com/AOG/B118>).

In vulvar HSILs, HPV16 was the predominant type detected with proportional attribution rates of 67.9% among all lesions and 74.3% among HPV-positive lesions (Table 2). Using multitype adjusted attribution methods, 76.1–91.3% of all vulvar HSILs were attributable to nonavalent vaccine types and a smaller proportion (65.2–78.3%) to quadrivalent vaccine types, indicating a nontrivial contribution of types 31, 33, 45, 52, and 58 (10.9–14.7%) (Appendix 3, <http://links.lww.com/AOG/B118>). All HPV-positive vulvar HSILs were attributable to nonavalent vaccine types (Table 2 and Fig. 1B).

In vaginal LSILs (Table 3), HPV31, 56, and 16 were the most prevalent types and only HPV33 went undetected. Overall, 61.9% of vaginal LSILs and 72.7% of vaginal HSILs tested positive for one or greater of the 14 HPV types assayed with co-infections present in 41.1% and 33.3% of the HPV-positive LSILs and HSILs, respectively. By

the proportional method, 3.9% of all vaginal LSILs (6.3% of HPV-positive vaginal LSILs) were attributable to the low-risk types (HPV6 [2.2%] and HPV11 [1.7%]) (Fig. 1C, D). Of all HPV-positive vaginal LSILs, 56.0% were attributable to nonavalent vaccine types by the proportional method (Fig. 1D). Adjusting for co-infections, 27.1–43.2% of all vaginal LSILs were attributable to the nonavalent vaccine types and 15.3–21.2% to quadrivalent vaccine types, revealing a substantial contribution of types 31, 33, 45, 52, and 58 (11.9–22.0%; Appendix 4, available online at <http://links.lww.com/AOG/B118>). An estimated 18.6–26.8% of vaginal LSILs were attributable to the nonvaccine types 35, 39, 51, 56, and 59.

The most common HPV type in vaginal HSILs was HPV16, accounting for 32.5% of all lesions and 46.6% of HPV-positive lesions using the proportional method (Table 3). Most other HPV types tested were also detected (except 11, 45, and 39), but usually as co-infections with HPV16. For all vaginal HSILs, multitype-adjusted attribution estimates ranged from 30.3–48.5% for quadrivalent vaccine types; from 12.1–21.2% for types 31, 33, 45, 52, and 58 (Appendix



Vulvar HSIL

All Lesions in Denominator (n=46)			Only HPV-Positive Lesions in Denominator (n=42)		
Any*	Single [†]	Proportion [‡]	Any*	Single [†]	Proportion [‡]
8 (17)	3 (7)	4.03 (9)	8 (19)	3 (7)	4.03 (10)
2 (4)	0 (0)	0.0 (0)	2 (5)	0 (0)	0 (0)
32 (70)	16 (35)	31.2 (68)	32 (76)	16 (38)	31.2 (74)
1 (2)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
8 (17)	4 (9)	6.8 (15)	8 (19)	4 (10)	6.8 (16)
2 (4)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2 (4)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
2 (4)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 (2)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
6 (13)	0 (0)	0 (0)	6 (14)	0 (0)	0 (0)
2 (4)	0 (0)	0 (0)	2 (5)	0 (0)	0 (0)
—	—	35.2 (76.6)	—	—	35.2 (83.9)
—	—	6.8 (14.7)	—	—	6.8 (16.1)
—	—	42.0 (91.3)	—	—	42.0 (100)
—	—	0 (0)	—	—	0 (0)

4, <http://links.lww.com/AOG/B118>); and from 42.4–60.6% for all nonavalent vaccine types. Although nonvaccine HPV types were detected in 30.3% of vaginal HSILs, 60% of these lesions were co-infected with quadrivalent HPV types; therefore, only 12.1–15.1% of vaginal HSILs were exclusively attributable to this group (Table 3; Appendix 4 [<http://links.lww.com/AOG/B118>]). Of all HPV-positive vaginal HSILs, 78.3% were attributable to nonavalent vaccine types (Table 3 and Fig. 1D).

The majority of HPV-positive lesions were attributable to types targeted by the nonavalent vaccine using the proportional method, including 89.4% and 100% of vulvar LSILs and HSILs and 56.0% and 78.3% of vaginal LSILs and HSILs, respectively (Appendix 5, available online at <http://links.lww.com/AOG/B118>). These estimates compare with 87.7% and 83.9% of vulvar LSILs and HSILs and 25.2% and 57.4% of vaginal LSILs and HSILs, respectively, attributable to the four genotypes (6, 11, 16, and 18) in the quadrivalent vaccine. The nonvaccine high-risk types (35, 39, 51, 56, and 59) measured in the quadrivalent vaccine trials were detected in 10.6% of vulvar LSILs, no vulvar HSILs, 44.0% of vaginal LSILs, and 21.7% of vaginal HSILs.

DISCUSSION

These analyses show 14 high-risk HPV types are detectable more often than not in both vaginal and

vulvar LSIL and HSIL. Most HPV-positive lesions were attributable to HPV types targeted by the nonavalent vaccine using the proportional attribution method, including 89.4% and 100% of vulvar LSILs and HSILs and 56.0% and 78.3% of vaginal LSILs and HSILs, respectively. The five additional HPV types in the nonavalent vaccine, but not in the quadrivalent vaccine, were detected in 1.7% of vulvar LSILs, 16.1% of vulvar HSILs, 30.8% of vaginal LSILs, and 20.9% of vaginal HSILs. Nonvaccine HPV types contributed more frequently to vaginal LSIL but less to vaginal HSIL, which is also seen in cervical intraepithelial neoplasia.²⁴

Of the 40 vulvar LSILs, 72.5% were positive for one or greater of the 14 HPV types tested. These findings are consistent with several meta-analyses.^{3,4} Applying the proportional method to HPV-positive vulvar LSILs, 89.4% were attributed to nonavalent vaccine types with only 1.7% attributed to the five additional HPV types not in the quadrivalent vaccine. The most common type in these lesions was HPV6, identified in 71.7% of HPV-positive vulvar LSILs and targeted by the quadrivalent and nonavalent vaccine.

All HPV-positive vulvar HSILs were attributable to genotypes covered by the nonavalent vaccine, including HPV16 found in 74.3% of lesions by the proportional method. Nonvaccine types were unusual and only identified in co-infections with vaccine types. In close agreement with our findings, a multinational,



Table 3. Type-specific Prevalence and Proportional Attribution of Human Papillomavirus Genotypes in Vaginal Lesions Among Females 15–26 Years of Age

HPV Type(s) Tested	Vaginal LSIL					
	All Lesions in Denominator (n=118)			Only HPV-Positive Lesions in Denominator (n=73*)		
	Any [†]	Single [‡]	Proportion [§]	Any [†]	Single [‡]	Proportion [§]
6	4 (3)	2 (2)	2.6 (2)	4 (5)	2 (3)	2.6 (4)
11	4 (3)	1 (1)	2.0 (2)	4 (5)	1 (1)	2.0 (3)
16	14 (12)	6 (5)	9.5 (8)	14 (19)	6 (8)	9.5 (13)
18	7 (6)	2 (2)	4.0 (3)	7 (10)	2 (3)	4.0 (6)
31	18 (15)	9 (8)	14.7 (12)	18 (25)	9 (12)	14.7 (20)
33	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
45	3 (3)	2 (2)	2.5 (2)	3 (4)	2 (3)	2.5 (3)
52	13 (11)	1 (1)	3.3 (3)	13 (18)	1 (1)	3.3 (5)
58	5 (4)	1 (1)	1.7 (1)	5 (7)	1 (1)	1.7 (2)
35	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
39	8 (7)	5 (4)	6.4 (5)	8 (11)	5 (7)	6.4 (9)
51	12 (10)	2 (2)	5.3 (4)	12 (16)	2 (3)	5.3 (7)
56	15 (13)	7 (6)	12.0 (10)	15 (21)	7 (10)	12.0 (17)
59	11 (9)	5 (4)	7.9 (6.7)	11 (15)	5 (7)	7.9 (11)
6/11/16/18	—	—	18.1 (15.4)	—	—	18.1 (25.2)
31/33/45/52/58	—	—	22.2 (18.8)	—	—	22.2 (30.8)
6/11/16/18/31/33/45/52/58	—	—	40.4 (34.2)	—	—	40.4 (56.0)
35/39/51/56/59	—	—	31.7 (26.8)	—	—	31.7 (44.0)

HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

Data are number of females with indicated lesion type (%).

* As a result of incomplete data for two lesions (one LSIL and one HSIL), these two cases were excluded from the proportional attribution analyses; therefore, the denominator is 72 for LSILs and 23 for HSILs.

[†] n (%) of all lesions testing positive for the respective HPV type, regardless of co-infections.

[‡] n (%) of lesions testing positive for the respective HPV type only, excluding co-infections.

[§] n (%) of lesions attributable to the respective HPV type, as determined applying the proportional attribution method.

retrospective analysis reported that 88.7% (509/587) of vulvar HSILs were HPV-positive, 77.3% of the positive lesions were attributable to HPV16, and 94.2% to nonavalent vaccine types.¹ Both HPV positivity exceeding 80% and the predominance of HPV16 in vulvar HSILs have been observed previously.^{3,4} Human papillomavirus-31, closely related to type 16,²⁵ appeared to play a relatively modest role in vulvar HSILs (14.7% in our study using proportional attribution), whereas HPV33 was infrequently detected. In contrast, prior analyses identified HPV33 (10.6%) in vulvar HSILs, whereas type 31 (1.2%) was rare.¹

Nonavalent HPV types were found in 56.0% of vaginal LSILs in this study, as also observed in cervical intraepithelial neoplasia.^{24,26} Other investigators have observed mainly HPV16 associated with vaginal LSILs.^{3,4,12} Non-HPV causes of vaginal LSILs may reflect past in utero exposure to diethylstilbestrol in women 40 years of age and older.^{27,28}

Of the 33 vaginal HSILs in our study, 72.7% tested HPV-positive, of which 78.3% were attributable to nonavalent vaccine types by the proportional

attribution method. A nearly identical attribution of 79.0% was found in 181 HPV-positive vaginal HSILs from a recent multinational, retrospective survey.⁵ In contrast to our findings, most previous reports have identified greater than 90% of vaginal lesions as HPV-positive.²⁹ Differences in assays to detect HPV, variations in the prevalent genotypes, sample storage and handling, age distribution of study participants, sample size, and intraobserver variability in the cytologic or histologic diagnosis may explain some differences across studies.

Approximately 41% and 50% of females with vulvar and vaginal lesions, respectively, also developed cervical lesions in our study, confirming the multifocal nature of anogenital infection and subsequent disease, particularly the association of cervical disease with HPV-related vaginal and vulvar lesions.^{30,31} Although the coexistence of HPV-related cervical, vulvar, and vaginal cancers and precancerous lesions has not been well studied, a history of cervical cancer or cervical intraepithelial neoplasia 2/3 has been associated with an increased risk of both vulvar and vaginal cancers.



Vaginal HSIL

All Lesions in Denominator (n=33)			Only HPV-Positive Lesions in Denominator (n=24*)		
Any [†]	Single [‡]	Proportion [§]	Any [†]	Single [‡]	Proportion [§]
1 (3)	1 (3)	1.0 (3)	1 (4)	1 (4)	1.0 (4)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
13 (39)	6 (18)	10.7 (32)	13 (54)	6 (25)	10.7 (46)
2 (6)	1 (3)	1.5 (5)	2 (8)	1 (4)	1.5 (7)
2 (6)	1 (3)	1.5 (5)	2 (8)	1 (4)	1.5 (7)
3 (9)	1 (3)	1.3 (4)	3 (13)	1 (4)	1.3 (6)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 (3)	1 (3)	1.0 (3)	1 (4)	1 (4)	1.0 (4)
1 (3)	1 (3)	1.0 (3)	1 (4)	1 (4)	1.0 (4)
2 (6)	1 (3)	1.2 (4)	2 (8)	1 (4)	1.2 (5)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2 (6)	1 (3)	1.2 (4)	2 (8)	1 (4)	1.2 (5)
5 (15)	1 (3)	1.6 (5)	5 (21)	1 (4)	1.6 (7)
1 (3)	1 (3)	1.0 (3)	1 (4)	1 (4)	1.0 (4)
—	—	13.2 (40.0)	—	—	13.2 (57.4)
—	—	4.8 (14.6)	—	—	4.8 (20.9)
—	—	18.0 (54.6)	—	—	18.0 (78.3)
—	—	5.0 (15.1)	—	—	5.0 (21.7)

Approximately 30% of patients with vaginal cancer had previous premalignant or cancerous cervical lesions.

Our study has several important limitations. This study was a post hoc, descriptive analysis of clinical trial data; the relatively small number of events can affect the precision of the estimated attribution fractions. Applying five attribution approaches may be a better measure of biological variability as opposed to statistical variability. The ideal approach for measuring attribution is laser-capture microdissection. Because our study was limited to females 15–26 years of age, it is possible that older women could differ in HPV type attribution or positivity. Nonetheless, our results were consistent with those reported from recent large multinational studies of vulvar and vaginal HSILs conducted in women over a wider age range with mean (\pm SD) ages of 50 (\pm 15) years for vulvar HSILs and 50 (\pm 14) years for vaginal HSILs.^{1,2}

Vulvar and vaginal lesions are rare but increasing findings in young women.⁷ Human papillomavirus infections, including vaccine genotypes, cause the majority of genital abnormalities. Widespread adoption of an efficacious prophylactic vaccine has the potential to prevent a substantial fraction of HPV-related vulvar and vaginal lesions in addition to preventing the majority of cervical lesions.^{15–17,24,32}

REFERENCES

- de Sanjosé S, Alemany L, Ordi J, Tous S, Alejo M, Bigby SM, et al. Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. *Eur J Cancer* 2013;49:3450–61.
- Alemany L, Saunier M, Tinoco L, Quirós B, Alvarado-Cabrero I, Alejo M, et al. Large contribution of human papillomavirus in vaginal neoplastic lesions: a worldwide study in 597 samples. *Eur J Cancer* 2014;50:2846–54.
- De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer* 2009;124:1626–36.
- Smith JS, Backes DM, Hoots BE, Kurman RJ, Pimenta JM. Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. *Obstet Gynecol* 2009; 113:917–24.
- Chao A, Chen TC, Hsueh C, Huang CC, Yang JE, Hsueh S, et al. Human papillomavirus in vaginal intraepithelial neoplasia. *Int J Cancer* 2012;131:E259–68.
- Srodon M, Stoler MH, Baber GB, Kurman RJ. The distribution of low and high-risk HPV types in vulvar and vaginal intraepithelial neoplasia (VIN and VaIN). *Am J Surg Pathol* 2006;30:1513–8.
- Kurdgelashvili G, Dores GM, Srouf SA, Chaturvedi AK, Huycke MM, Devesa SS. Incidence of potentially human papillomavirus-related neoplasms in the United States, 1978 to 2007. *Cancer* 2013;119:2291–9.
- Joura EA, Lösch A, Haider-Angeler MG, Breitenacker G, Leodolter S. Trends in vulvar neoplasia. Increasing incidence of vulvar intraepithelial neoplasia and squamous cell carcinoma of the vulva in young women. *J Reprod Med* 2000;45:613–5.
- Darragh TM, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background



- and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol* 2013;32:76–115.
10. Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon (France): International Agency for Research on Cancer (IARC); 2014.
 11. Jones RW, Rowan DM. Vulvar intraepithelial neoplasia III: a clinical study of the outcome in 113 cases with relation to the later development of invasive vulvar carcinoma. *Obstet Gynecol* 1994;84:741–5.
 12. Lamos C, Mihaljevic C, Aulmann S, Bruckner T, Domschke C, Wallwiener M, et al. Detection of human papillomavirus infection in patients with vaginal intraepithelial neoplasia. *PLoS One* 2016;11:e0167386.
 13. Human papillomavirus vaccines: WHO position paper–May 2017. Available at: www.who.int/wer/2017/wer9227/en/. Retrieved July 12, 2017.
 14. Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet* 2009;374:301–14.
 15. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007;356:1928–43.
 16. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007;356:1915–27.
 17. Huh WK, Joura EA, Giuliano AR, Iversen OE, de Andrade RP, Ault KA, et al. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16–26 years: a randomised, double-blind trial. *Lancet* 2017;390:2143–59.
 18. Insinga RP, Liaw KL, Johnson LG, Madeleine MM. A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev* 2008;17:1611–22.
 19. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;11:1048–56.
 20. Insinga RP, Perez G, Wheeler CM, Koutsky LA, Garland SM, Leodolter S, et al. Incident cervical HPV infections in young women: transition probabilities for CIN and infection clearance. *Cancer Epidemiol Biomarkers Prev* 2011;20:287–96.
 21. Sahasrabudhe VV, Castle PE, Follansbee S, Borgonovo S, Tokugawa D, Schwartz LM, et al. Human papillomavirus genotype attribution and estimation of preventable fraction of anal intraepithelial neoplasia cases among HIV-infected men who have sex with men. *J Infect Dis* 2013;207:392–401.
 22. Else EA, Swoyer R, Zhang Y, Taddeo FJ, Bryan JT, Lawson J, et al. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with INNO-LiPA HPV genotyping extra assay. *J Clin Microbiol* 2011;49:1907–12.
 23. Roberts CC, Swoyer R, Bryan JT, Taddeo FJ. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with the linear array HPV genotyping PCR assay and influence of DNA extraction method on HPV detection. *J Clin Microbiol* 2011;49:1899–906.
 24. Joura EA, Ault KA, Bosch FX, Brown D, Cuzick J, Ferris D, et al. Attribution of 12 high-risk human papillomavirus genotypes to infection and cervical disease. *Cancer Epidemiol Biomarkers Prev* 2014;23:1997–2008.
 25. Bernard HU. Taxonomy and phylogeny of papillomaviruses: an overview and recent developments. *Infect Genet Evol* 2013;18:357–61.
 26. Fu Xi L, Schiffman M, Ke Y, Hughes JP, Galloway DA, He Z, et al. Type-dependent association between risk of cervical intraepithelial neoplasia and viral load of oncogenic human papillomavirus types other than types 16 and 18. *Int J Cancer* 2017;140:1747–56.
 27. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878–81.
 28. Mittendorf R. Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. *Teratology* 1995;51:435–45.
 29. Bruni L, Barrionuevo-Rosas L, Albero G, Serrano B, Mena M, Gómez D, et al; ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human papillomavirus and related diseases in USA. Summary report 27 July 2017. Available at: <http://www.hpvcentre.net/statistics/reports/USA.pdf>. Retrieved July 11, 2017.
 30. Edgren G, Sparén P. Risk of anogenital cancer after diagnosis of cervical intraepithelial neoplasia: a prospective population-based study. *Lancet Oncol* 2007;8:311–6.
 31. Gaudet M, Hamm J, Aquino-Parsons C. Incidence of anogenital and head and neck malignancies in women with a previous diagnosis of cervical intraepithelial neoplasia. *Gynecol Oncol* 2014;134:523–6.
 32. Pitisuttithum P, Velicer C, Luxembourg A. 9-valent vaccine for cancers, pre-cancers and genitals warts related to HPV. *Expert Rev Vaccines* 2015;14:1405–14.

