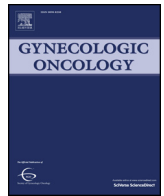




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## Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: Data from the ATHENA trial

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### HIGHLIGHTS

- HPV 16 was the most common genotype in all populations and conferred the highest risk for high grade disease.
- We found that 50% of the adenocarcinoma in situ and 50% of the invasive cancer cases were attributable to HPV18 infection.
- We confirmed the utility of 16/18 genotyping in cervical cancer screening strategies, while pooled detection of non-16/18 genotypes is sufficient.

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### ABSTRACT

**Objective.** We assessed the age-related prevalence of high risk human papillomavirus (HR-HPV) genotypes and the genotype-associated risk for high-grade cervical intraepithelial neoplasia (CIN) in a large U.S. screening population.

**Methods.** A total of 40,901 women aged  $\geq 25$  years were screened with liquid-based cytology and HPV testing in the ATHENA (Addressing the Need for Advanced HPV Diagnostics) trial. Genotyping was performed using the LINEAR ARRAY HPV Genotyping Test.

**Results.** HPV16 was the most prevalent genotype in all age groups, ranging from 3.5% to 0.8% in women aged 25–29 and  $\geq 50$  years, respectively. The next most prevalent genotypes were HPV52, HPV31 and HPV18. In the overall population, HPV16 conferred the greatest absolute risk of  $\geq$  CIN3 both in women aged 25–29 and  $\geq 30$  years (14.2% and 15.1%, respectively) followed by HPV31 (8.0% and 7.9%), HPV52 (6.7% and 4.4%) and HPV18 (2.7% and 9.0%). Similar trends were seen in women with negative cytology. The percent positivity increased markedly with disease progression for HPV16 and HPV18 which were responsible for 45.6% and 8.4% of  $\geq$  CIN3, respectively. Of note, HPV 18 was responsible for 50% of adenocarcinoma in situ (AIS) and 50% of invasive cancer cases.

**Conclusions.** HPV16 played a major role in the development of  $\geq$  CIN3 irrespective of age, supporting the identification of HPV16 in primary screening for all women. Identification of HPV18 is also warranted, given its significant contribution to AIS and cancer. Identification of non-16/18 genotypes as a pool should provide sufficient information for screening.

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**Abbreviations:** ADC, adenocarcinoma of the cervix; AIS, adenocarcinoma in situ; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; GT, genotype; LA-HPV, LINEAR ARRAY HPV Genotyping Test; NILM, negative for intraepithelial lesion or malignancy; NPV, negative predictive value; PCR, polymerase chain reaction; SCC, squamous cell carcinoma (SCC).

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## Introduction

In the past several decades, the implementation of screening programs has substantially decreased the incidence of cervical cancer. Because cervical cancer is caused by persistent infection of the cervix with high-risk human papillomavirus (HR-HPV) genotypes, adding HR-HPV testing to screening strategies can improve the effectiveness of screening. Although HPV testing in cervical samples typically detects a pool of 14 HR-HPV genotypes, it has been established that 2 HR-HPV types, HPV16 and HPV18, are responsible for  $\approx 70\%$  of cervical cancers [1].

The current standards for the prevention of cervical cancer are based on HPV vaccination in young women before exposure to HPV, followed by cervical cancer screening in all women starting at 21 years of age [2–4]. The 2012 U.S. cervical cancer screening guidelines [5–7] recommend that women aged  $\geq 21$  years be screened every 3 years by cervical cytology alone. For women aged  $\geq 30$  years, the preferred option is to screen every 5 years with a combination of cervical cytology and HR-HPV testing (“cotesting”).

Two of the 4 HPV tests approved for cotesting by the Food and Drug Administration (FDA) allow testing specifically for HPV16 and HPV18; 1 additional test was approved for testing for HPV16 and HPV18/45. The argument for incorporating HPV16/18 genotyping into screening strategies is supported by the finding that women with negative cytology who test positive for HPV16/18 have a much greater short- and long-term risks of cervical precancer and cancer ( $\geq$  CIN3) than women who test positive for any of the 12 other HR-HPV types [8–10]. Based on these data, the 2012 screening guidelines for cotesting endorsed the option of triaging women positive for HPV 16 or 18 to colposcopy and deferral of women positive for the other 12 pooled HR-HPV (non-HPV16/18) genotypes to repeat cotesting in 12 months [6]. In a single screening round, cotesting with genotyping for HPV16/18 has been shown to be 1.32 times more sensitive than cytology alone for women aged  $\geq 30$  years [11].

The objective of the present study was to evaluate the prevalence of the various HR-HPV genotypes in a routine U.S. cervical screening population of women aged  $\geq 25$  years, to determine the risk for  $\geq$  CIN3 (and  $\geq$  CIN2) associated with each genotype and to provide data to assess the potential impact of genotyping on screening strategies. To this end, we used data from the baseline phase of the ATHENA (Addressing the Need for Advanced HPV Diagnostics) trial, the largest cervical cancer screening study to date in the United States (U.S.) [12].

## Methods

### Study population

The ATHENA study enrolled 47,208 women aged  $\geq 21$  years attending obstetric/gynecologic clinics (61 sites) for routine cervical cancer screening in 23 states in the U.S. between May 2008 and August 2009 [12]. This post-hoc analysis was confined to women aged  $\geq 25$  years because this age group has the most potential to benefit from HR-HPV screening. Women were excluded if they were pregnant, had undergone hysterectomy, had had ablative or excisional therapy to the cervix within the previous 12 months, or were participating in an HPV treatment trial. The study protocol was approved by institutional review boards at all participating centers. All participants provided informed consent before enrollment. The study was registered with ClinicalTrials.gov (NCT00709891).

### Study procedures

The ATHENA protocol was previously described in detail [12,13]. In brief, 2 cervical samples were collected in PreservCyt medium (Hologic, Bedford, MA) and used for both liquid-based cytology (ThinPrep; Hologic, Bedford, MA) and testing with multiple HPV tests, including

genotyping using the LINEAR ARRAY HPV Genotyping Test (LA-HPV; Roche Molecular Systems Inc., Pleasanton, CA).

Women aged  $\geq 25$  years with abnormal cervical cytology (atypical squamous cells of undetermined significance or worse [ $\geq$  ASC-US]) or with negative cervical cytology (negative for intraepithelial lesion or malignancy [NILM]) and a positive HPV test result were referred to colposcopy. Colposcopic biopsies were performed according to a standardized protocol, and a random biopsy was required in all women with adequate colposcopy in whom no lesion was seen [14]; patients and colposcopists were blinded to the cytology and HPV results.

### Cytology and histology

Cytology was conducted without knowledge of HPV status at 4 accredited clinical laboratories and reported according to the Bethesda 2001 nomenclature [15]. A threshold of  $\geq$  ASC-US was used to define abnormal cytology. Histology results were determined by a panel of 3 pathologists blinded to all participants' demographic and laboratory data [12]. Standard CIN terminology was used to report results.

### HPV genotyping

The LA-HPV assay is a polymerase chain reaction (PCR)-based assay that qualitatively determines the presence of 37 individual HPV DNA genotypes (21 types considered to be either high risk or potentially high risk, and 16 low-risk types not associated with carcinogenesis). For this study, a modified LA-HPV assay was used, which detected only the 16 HR-HPV genotypes considered to have the best evidence for association with cancer: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, and 82 [16]. Cervical specimens were tested with the modified LA-HPV assay according to the manufacturer's instructions at 4 accredited clinical laboratories where blinding to other HPV results was maintained. Of note, the presence of HPV52 is determined with a probe that cross-hybridizes with HPV 33, 35, and 58-specific probes and can only be confirmed if no hybridization with HPV 33, 35, or 58-type specific probes occurred. As a result, concurrent infection with HPV52 and the 3 other types cannot be detected. The LA-HPV assay has been shown to have good clinical performance for the detection of high-grade cervical lesions in clinical specimens [17,18].

### Statistical analysis

The HPV infection status was defined using 2 categories: [1] single infection, in which only a single HR-HPV genotype was present and [2] hierarchical ranking of multiple infections. The hierarchical ranking, as described by Schiffman et al. [19], was determined by iterative exclusion based on the calculated absolute risk for  $\geq$  CIN3 for each genotype; each preceding genotype was excluded when calculating the risk of the subsequent genotype. The prevalence of single and multiple HPV genotypes was calculated for the overall, negative cytology and abnormal cytology populations and stratified by different age categories. The distribution of specific HR-HPV genotypes by hierarchy across cervical disease was calculated as the proportion of the specific genotype present within each disease category and stratified by age (25–29 years or  $\geq 30$  years) for the overall, negative cytology and abnormal cytology populations. The risk of specific HR-HPV genotypes by hierarchical ranking was calculated for the overall population and for each specific genotype as the proportion of women with valid results for high-grade cervical disease ( $\geq$  CIN2 or  $\geq$  CIN3) stratified by age groups and cytology status.

## Results

### Demographic and baseline characteristics

Of the 41,955 women aged  $\geq 25$  years eligible for enrollment, 40,901 were evaluable with valid results for LA-HPV, cytology, and histology.

Demographic and baseline characteristics are presented in Table 1. The mean (SD) age was 41.8 (11.3) years. Most of the women (83.4%) enrolled were white. The mean interval of screening was 1.4 years (range, 1 month to 6 years) and most women (90.9%) had had cytology performed in the past 5 years, indicating a well-screened population. Only 1.2% of the women were vaccinated.

#### Prevalence of HPV genotypes in the overall population and by cytology status

##### Overall population

In the overall population, the prevalence of pooled single HR-HPV types was 10.3% (4220 of 40,901) and decreased with age, with a plateauing of prevalence noted between 50–60 years; a range from 17.8% in women aged 25–29 years to 6.5% in women aged  $\geq 50$  years was observed (Table 2). Among the HR-HPV types, HPV16 was the most prevalent genotype, occurring in 1.6% of women as a single infection for all age groups and in 3.5%, 1.8%, 1.1%, and 0.8% in women aged 25–29, 30–39, 40–49, and  $\geq 50$  years, respectively (Fig. 1A and Table 2). The second most prevalent type as a single infection was HPV52, occurring in 1.0% (Table 2). Infection with multiple genotypes was common and followed the same trends as single infection (Supplemental Table 1). The prevalence for multiple type infections by hierarchical ranking for pooled HR-HPV was 13.4% (5464 of 40,901) and was highest with HPV16 (2.5% [1030 of 40,901]) (Supplemental Table 1).

##### By cytology status

**Negative cytology (NILM).** The prevalence of HR-HPV types in women with NILM cytology was 9.0% and followed the same trends as in the overall population, with slightly lower prevalence rates (Fig. 1B). NILM/HR-HPV-positive results were most common in women aged 25–29 years and decreased in each subsequent decade, with a leveling off of prevalence beginning in the sixth decade. HPV16 was the most prevalent single HR-HPV type in all age groups: 2.7%, 1.5%, 0.9%, and 0.7% in women aged 25–29, 30–39, 40–49, and  $\geq 50$  years, respectively (Fig. 1B and Table 2). The second most prevalent HPV type was HPV52

(0.9%). HPV16 also had the highest prevalence in hierarchical ranking of multiple infections, regardless of age (Supplemental Table 1).

**Abnormal cytology.** In women with abnormal cytology, the prevalence of the different HR-HPV types was 29.7%, approximately 3 times greater than that in women with NILM cytology but followed the same trend (Fig. 1C and Table 2).

##### Prevalence of HPV genotypes by histology

In women with  $< \text{CIN}2$  histology, HPV genotypes other than HPV16 or HPV18 were much more common; this occurred independent of cytology status and was true in women aged 25–29 and  $\geq 30$  years (Table 3). The prevalence and percent positivity for HPV16 and HPV18 increased with the severity of disease (Table 3 and Fig. 2), and an increase in HPV16/18 prevalence between CIN2 and  $\geq \text{CIN}3$  occurred in both the 25–29 and  $\geq 30$  year populations. Overall, HPV16 was associated with 45.6% of  $\geq \text{CIN}3$  cases (125 of 274), whereas HPV18 was associated with 8.4% (23 of 274). HPV31 was responsible for 12.0% (33 of 274) but the percent positivity decreased with progression from CIN2 to  $\geq \text{CIN}3$ . Interestingly, the prevalence of HPV45 in  $\geq \text{CIN}3$  was only 2.6% (7 of 274), and there were no cancers. The 6 cancers detected at baseline extrapolate to 14.7/100,000 women which is consistent with the higher U.S. cervical cancer prevalence when corrected for hysterectomy (18.6/100,000) [20], a population more similar to the ATHENA cohort where women who had had hysterectomies were excluded from enrollment. All cancers occurred in women aged  $\geq 30$  years, with HPV16 responsible for 16.7% (1 of 6), HPV18 for 50% (3 of 6), and HPV31 and HPV39 each for 16.7% (1 of 6) (Table 3). HPV16 prevalence in CIN2 and  $\geq \text{CIN}3$  was significantly greater than that of HPV18, particularly in women aged 25–29 years (Table 3). However, HPV18 was responsible for 50% of AIS cases (8 of 16) as well as 50% of the invasive cancers.

##### Absolute risk of CIN2 and $\geq \text{CIN}3$ by HPV genotype for multiple infections (hierarchical ranking)

The risk of CIN2 and  $\geq \text{CIN}3$  was estimated by a hierarchical ranking of multiple infections, as described in the statistical methodology. The absolute risk for HR-HPV infection for CIN2 by LA-HPV (pooled for all HR-HPV genotypes) for ages 25–29 and  $\geq 30$  years, respectively was as follows: overall, 4.5% and 2.5%; NILM, 2.8% and 1.7%; and abnormal cytology, 9.3% and 5.5%. For  $\geq \text{CIN}3$ , the risk for ages 25–29 and  $\geq 30$  years, respectively was: overall, 6.1% and 5.4%; NILM, 4.7% and 3.0%; and abnormal cytology, 10.1% and 14.9% (Table 4a).

##### Overall population by genotype

In the overall population, the absolute risk of  $\geq \text{CIN}3$  attributed to HPV16 in women aged 25–29 years was 14.2% (46 of 324) and for HPV 33 it was 15.0%, although this risk was based on only 3 cases [3 of 20]. The next highest risk observed was for HPV 31 (8.0% [11 of 138]) and 2.7% for HPV18 (3 of 111) (Tables 3 and 4a). In women aged  $\geq 30$  years, HPV16 had the highest risk for  $\geq \text{CIN}3$  (15.1% [79 of 524]) followed by HPV18 (9.0% [20 of 223]; and HPV 31 (7.9% [22/277]) (Tables 3 and 4a).

The presence of HPV16 conferred a similar risk of  $\geq \text{CIN}3$  in both age groups; in contrast, the risk of  $\geq \text{CIN}3$  with HPV18 more than tripled after 30 years (from 2.7% to 9.0%; Table 4a). By comparison, for HPV 33 and HPV52 a decrease in risk for  $\geq \text{CIN}3$  was observed for women aged  $\geq 30$  years compared to women aged 25–29 years (15.0% to 5.4% and 6.7% to 4.4% for HPV33 and HPV52, respectively). For CIN2, the risk decreased in women aged  $\geq 30$  years compared to 25–29 years for HPV16 (7.1% to 3.2%), HPV18 (2.7% to 0.9%), HPV33 (5.0% to 2.2%) and HPV52 (4.0% to 2.2%) (Table 4a).

Although absolute risks for the remaining genotypes have also been calculated, it is difficult to draw meaningful conclusions about risk because of either relatively low prevalence or the limited number of

**Table 1**  
Demographics for overall population (age  $\geq 25$  years).

Characteristics	Evaluable women (N = 40,901)
Age (years)	
Mean (SD)	41.8 (11.3)
25–29, n (%)	6647 (16.3)
30–39, n (%)	12,248 (29.9)
40–49, n (%)	11,689 (28.6)
$\geq 50$ , n (%)	10,317 (25.2)
Race, n (%)	
White	34,128 (83.4)
Black/African American	5590 (13.7)
Asian	639 (1.6)
American Indian or Alaskan Native	226 (0.6)
Native Hawaiian or other Pacific Islander	98 (0.2)
Any combination <sup>a</sup> or missing	220 (0.5)
Ethnicity, n (%)	
Hispanic/Latino	7364 (18.0)
Postmenopausal, n (%)	13,033 (31.9)
HPV vaccine, n (%)	487 (1.2)
Screening interval, year (SD)	1.4 ( $\pm 0.7$ )
Pap test result in the past 5 years, n (%)	37,180 (90.9)
NILM	34,823 (85.1)
ASC-US	1494 (3.7)
$>$ ASC-US	863 (2.1)
Colposcopy in the past 5 years, n (%)	2879 (7.0)

ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy.

<sup>a</sup> Any combination refers to subjects who selected more than one race.

**Table 2**  
Prevalence of Linear Array genotypes by age group and cytology for single infection.

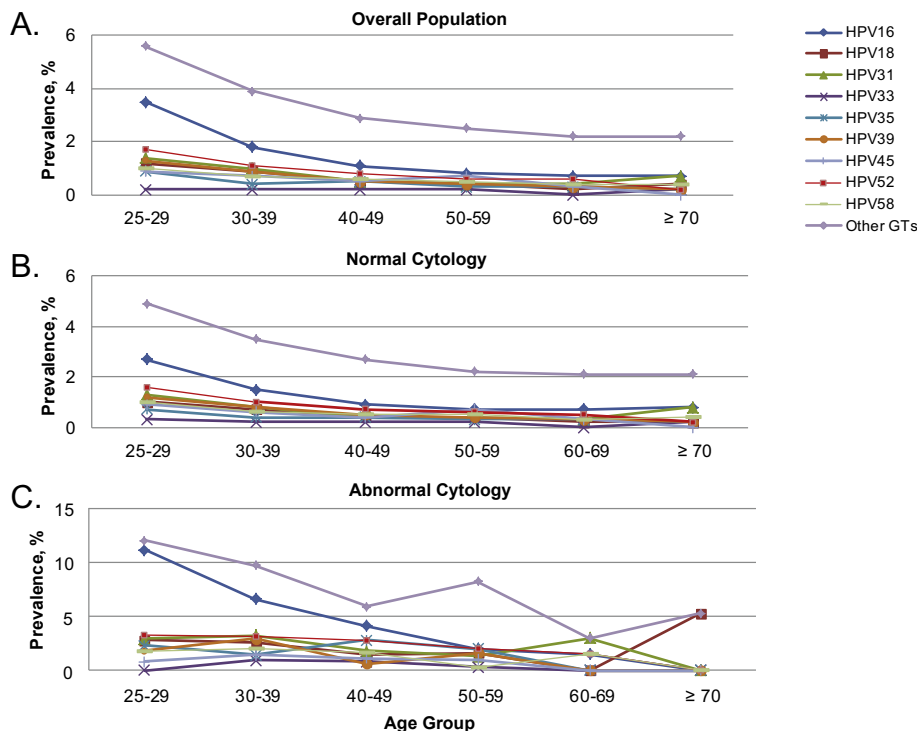
Age group (years)	N	All GTs % (n)	HPV16 % (n)	HPV18 % (n)	HPV31 % (n)	HPV33 % (n)	HPV35 % (n)	HPV39 % (n)	HPV45 % (n)	HPV52 <sup>a</sup> % (n)	HPV58 % (n)	Other GTs <sup>b</sup> % (n)
<b>Overall population</b>												
25–29	6647	17.8% (1181)	3.5% (233)	1.2% (79)	1.4% (95)	0.2% (15)	0.9%(58)	1.3% (85)	0.9% (60)	1.7% (115)	1.0% (69)	5.6% (372)
30–39	12,248	11.6% (1426)	1.8% (225)	0.9% (107)	1.0% (119)	0.2% (26)	0.4%(53)	0.9% (114)	0.7% (80)	1.1% (136)	0.7% (88)	3.9% (478)
40–49	11,689	8.1% (945)	1.1% (124)	0.5% (54)	0.5% (59)	0.2% (29)	0.5%(63)	0.5% (59)	0.5% (54)	0.8% (95)	0.6% (67)	2.9% (341)
≥50	10,317	6.5% (668)	0.8% (80)	0.3% (36)	0.5% (47)	0.2% (20)	0.3%(34)	0.4% (37)	0.6% (60)	0.6% (61)	0.4% (45)	2.4% (248)
50–59	7419	6.9% (514)	0.8% (59)	0.4% (30)	0.4% (33)	0.2% (18)	0.3%(25)	0.4% (30)	0.7% (53)	0.6% (47)	0.5% (34)	2.5% (185)
60–69	2353	5.4% (126)	0.7% (17)	0.2% (4)	0.4% (10)	0.0% (1)	0.3%(8)	0.3% (6)	0.3% (7)	0.6% (13)	0.4% (9)	2.2% (51)
≥70	545	5.1% (28)	0.7% (4)	0.4% (2)	0.7% (4)	0.2% (1)	0.2%(1)	0.2% (1)	0.0% (0)	0.2% (1)	0.4% (2)	2.2% (12)
All ages	40,901	10.3% (4220)	1.6% (662)	0.7% (276)	0.8% (320)	0.2% (90)	0.5%(208)	0.7% (295)	0.6% (254)	1.0% (407)	0.7% (269)	3.5% (1439)
<b>Normal cytology</b>												
25–29	5996	15.5% (929)	2.7% (161)	1.0%(61)	1.3% (76)	0.3% (15)	0.7% (43)	1.2% (73)	0.9% (55)	1.6% (94)	1.0% (57)	4.9% (294)
30–39	11,399	10.0% (1139)	1.5% (169)	0.7%(85)	0.8% (92)	0.2% (18)	0.4% (41)	0.8% (89)	0.6% (68)	1.0% (110)	0.6% (71)	3.5% (396)
40–49	10,962	7.1% (779)	0.9% (94)	0.4%(44)	0.4% (46)	0.2% (23)	0.4% (43)	0.5% (55)	0.4% (46)	0.7% (75)	0.5% (55)	2.7% (298)
≥50	9927	6.0% (597)	0.7% (73)	0.3%(30)	0.4% (41)	0.2% (19)	0.3% (28)	0.3% (32)	0.6% (57)	0.5% (54)	0.4% (43)	2.2% (220)
50–59	7114	6.4% (452)	0.7% (53)	0.4%(25)	0.4% (29)	0.2% (17)	0.3% (19)	0.4% (25)	0.7% (50)	0.6% (41)	0.5% (33)	2.2% (160)
60–69	2287	5.2% (119)	0.7% (16)	0.2%(4)	0.3% (8)	0.0% (1)	0.3% (8)	0.3% (6)	0.3% (7)	0.5% (12)	0.3% (8)	2.1% (49)
≥70	526	4.9% (26)	0.8% (4)	0.2%(1)	0.8% (4)	0.2% (1)	0.2% (1)	0.2% (1)	0.0% (0)	0.2% (1)	0.4% (2)	2.1% (11)
All ages	38,284	9.0% (3444)	1.3% (497)	0.6%(220)	0.7% (255)	0.2% (75)	0.4% (155)	0.7% (249)	0.6% (226)	0.9% (333)	0.6% (226)	3.2% (1208)
<b>Abnormal cytology</b>												
25–29	651	38.7% (252)	11.1% (72)	2.8% (18)	2.9% (19)	0.0% (0)	2.3% (15)	1.8% (12)	0.8% (5)	3.2% (21)	1.8% (12)	12.0% (78)
30–39	849	33.8% (287)	6.6% (56)	2.6% (22)	3.2% (27)	0.9% (8)	1.4% (12)	2.9% (25)	1.4% (12)	3.1% (26)	2.0% (17)	9.7% (82)
40–49	727	22.8% (166)	4.1% (30)	1.4% (10)	1.8% (13)	0.8% (6)	2.8% (20)	0.6% (4)	1.1% (8)	2.8% (20)	1.7% (12)	5.9% (43)
≥50	390	18.2% (71)	1.8% (7)	1.5% (6)	1.5% (6)	0.3% (1)	1.5% (6)	1.3% (5)	0.8% (3)	1.8% (7)	0.5% (2)	7.2% (28)
50–59	305	20.3% (62)	2.0% (6)	1.6% (5)	1.3% (4)	0.3% (1)	2.0% (6)	1.6% (5)	1.0% (3)	2.0% (6)	0.3% (1)	8.2% (25)
60–69	66	10.6% (7)	1.5% (1)	0.0% (0)	3.0% (2)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	1.5% (1)	1.5% (1)	3.0% (2)
≥70	19	10.5% (2)	0.0% (0)	5.3% (1)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)	5.3% (1)
All ages	2617	29.7% (776)	6.3% (165)	2.1% (56)	2.5% (65)	0.6% (15)	2.0% (53)	1.8% (46)	1.1% (28)	2.8% (74)	1.6% (43)	8.8% (231)

GT, genotype.  
<sup>a</sup> HPV52 is considered positive if present in the absence of genotypes 33, 35 and 58.  
<sup>b</sup> Other GTs includes genotypes 51, 56, 59, 66, 68, 73, and 82.

disease cases. General trends of increasing or decreasing risks can be seen for these genotypes (Table 4b), but significant differences are difficult to assess based on the low number of disease cases for individual genotypes.

*By cytology status*

*Negative cytology.* In women with NILM cytology, HPV16 conferred the highest risk of ≥CIN3 in women aged ≥30 years (31 of 380 [8.2%];



**Fig. 1.** Prevalence of HPV genotypes stratified by age for (A) the overall population, (B) women with negative cytology, and (C) women with abnormal cytology.

**Table 3**  
Prevalence of Linear Array genotypes (hierarchical ranking) stratified by disease status.

	25–29 years (N = 6647)					≥ 30 years (N = 34,254)					Total n
	Negative % (n)	CIN1 % (n)	CIN2 % (n)	CIN3+AIS % (n)	CA % (n)	Negative % (n)	CIN1 % (n)	CIN2 % (n)	CIN3+AIS % (n)	CA % (n)	
<i>Overall population</i>											
All GTs	68.9 (1084)	84.5 (164)	95.5 (63)	100.0 (85)	51.0 (2666)	64.9 (257)	85.7 (78)	90.7 (166)	100.0 (6)		4569
HPV 16	14.2 (224)	16.0 (31)	34.8 (23)	54.1 (46)	7.4 (389)	9.8 (39)	18.7 (17)	42.6 (78)	16.7 (1)		848
HPV 31	6.2 (97)	10.8 (21)	13.6 (9)	12.9 (11)	3.9 (203)	7.8 (31)	23.1 (21)	11.5 (21)	16.7 (1)		415
HPV 18	5.9 (93)	6.2 (12)	4.5 (3)	3.5 (3)	3.5 (183)	4.5 (18)	2.2 (2)	9.3 (17)	50.0 (3)		334
HPV 33	0.8 (13)	1.5 (3)	1.5 (1)	3.5 (3)	1.5 (78)	2.0 (8)	2.2 (2)	2.7 (5)			113
HPV 52 <sup>a</sup>	7.1 (111)	11.3 (22)	9.1 (6)	11.8 (10)	5.2 (273)	6.1 (24)	7.7 (7)	7.7 (14)			467
HPV 35	3.4 (54)	3.6 (7)	7.6 (5)	3.5 (3)	2.8 (144)	4.0 (16)	6.6 (6)	3.3 (6)			241
HPV 39	4.9 (77)	5.2 (10)	1.5 (1)	3.5 (3)	3.7 (193)	6.3 (25)	3.3 (3)	3.8 (7)	16.7 (1)		320
HPV 45	3.4 (54)	3.1 (6)	1.5 (1)	0.0 (0)	2.7 (141)	3.0 (12)	1.1 (1)	3.8 (7)			222
HPV 58	3.2 (50)	3.6 (7)	9.1 (6)	1.2 (1)	3.1 (160)	3.0 (12)	5.5 (5)	1.6 (3)			244
Other GTs	19.8 (311)	23.2 (45)	12.1 (8)	5.9 (5)	17.2 (902)	18.2 (72)	15.4 (14)	4.4 (8)			1,365
Total	1573	194	66	85	5229	396	91	183	6		7823
<i>Normal cytology</i>											
All GTs	72.0 (862)	90.1 (91)	96.7 (29)	100.0 (48)	57.5 (2244)	70.3 (161)	82.4 (42)	93.7 (74)	100.0 (1)		3552
HPV 16	13.6 (163)	13.9 (14)	33.3 (10)	50.0 (24)	8.2 (321)	10.0 (23)	9.8 (5)	39.2 (31)			591
HPV 31	6.2 (74)	15.8 (16)	10.0 (3)	12.5 (6)	4.3 (166)	7.9 (18)	25.5 (13)	12.7 (10)			306
HPV 18	5.5 (66)	6.9 (7)	6.7 (2)	4.2 (2)	3.8 (149)	4.4 (10)	3.9 (2)	8.9 (7)	100.0 (1)		246
HPV 33	0.8 (10)	2.0 (2)	0.0 (0)	4.2 (2)	1.6 (64)	1.7 (4)	2.0 (1)	1.3 (1)			84
HPV 52 <sup>a</sup>	7.9 (94)	8.9 (9)	6.7 (2)	16.7 (8)	5.9 (229)	6.6 (15)	13.7 (7)	8.9 (7)			371
HPV 35	3.6 (43)	4.0 (4)	10.0 (3)	4.2 (2)	2.9 (112)	4.4 (10)	2.0 (1)	1.3 (1)			176
HPV 39	5.6 (67)	6.9 (7)	3.3 (1)	4.2 (2)	4.4 (173)	6.1 (14)	2.0 (1)	7.6 (6)			271
HPV 45	4.0 (48)	4.0 (4)	3.3 (1)	0.0 (0)	3.1 (120)	3.9 (9)	2.0 (1)	6.3 (5)			188
HPV 58	3.8 (46)	2.0 (2)	10.0 (3)	2.1 (1)	3.6 (142)	3.5 (8)	5.9 (3)	1.3 (1)			206
Other GTs	21.0 (251)	25.7 (26)	13.3 (4)	2.1 (1)	19.7 (768)	21.8 (50)	15.7 (8)	6.3 (5)			1,113
Total	1197	101	30	48	3901	229	51	79	1		5637
<i>Abnormal cytology</i>											
All GTs	59.0 (222)	78.5 (73)	94.4 (34)	100.0 (37)	31.8 (422)	57.5 (96)	90.0 (36)	88.5 (92)	100.0 (5)		1017
HPV 16	16.2 (61)	18.3 (17)	36.1 (13)	59.5 (22)	5.1 (68)	9.6 (16)	30.0 (12)	45.2 (47)	20.0 (1)		257
HPV 31	6.1 (23)	5.4 (5)	16.7 (6)	13.5 (5)	2.8 (37)	7.8 (13)	20.0 (8)	10.6 (11)	20.0 (1)		109
HPV 18	7.2 (27)	5.4 (5)	2.8 (1)	2.7 (1)	2.6 (34)	4.8 (8)	0.0 (0)	9.6 (10)	40.0 (2)		88
HPV 33	0.8 (3)	1.1 (1)	2.8 (1)	2.7 (1)	1.1 (14)	2.4 (4)	2.5 (1)	3.8 (4)			29
HPV 52 <sup>a</sup>	4.5 (17)	14.0 (13)	11.1 (4)	5.4 (2)	3.3 (44)	5.4 (9)	0.0 (0)	6.7 (7)			96
HPV 35	2.9 (11)	3.2 (3)	5.6 (2)	2.7 (1)	2.4 (32)	3.6 (6)	12.5 (5)	4.8 (5)			65
HPV 39	2.7 (10)	3.2 (3)	0.0 (0)	2.7 (1)	1.5 (20)	6.6 (11)	5.0 (2)	1.0 (1)	20.0 (1)		49
HPV 45	1.6 (6)	2.2 (2)	0.0 (0)	0.0 (0)	1.6 (21)	1.8 (3)	0.0 (0)	1.9 (2)			34
HPV 58	1.1 (4)	5.4 (5)	8.3 (3)	0.0 (0)	1.4 (18)	2.4 (4)	5.0 (2)	1.9 (2)			38
Other GTs	16.0 (60)	20.4 (19)	11.1 (4)	10.8 (4)	10.1 (134)	13.2 (22)	15.0 (6)	2.9 (3)			252
Total	376	93	36	37	1328	167	40	104	5		2186

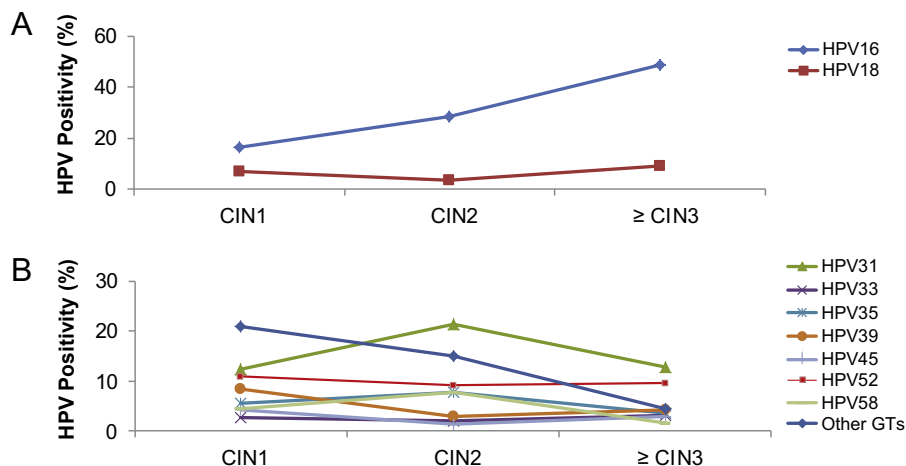
GT, genotype.

Other includes genotypes 51, 56, 59, 66, 68, 73, and 82.

<sup>a</sup> HPV52 is considered positive if present in the absence of genotype 33, 35 and 58.

Tables 3 and 4a). In women aged <30 years, the risk of ≥CIN3 for HPV16 was 11.4% (24 of 211); although the risk was higher for HPV33 (2 of 14 [14.3%]), the low number of women with this specific genotype

precludes any conclusive interpretation. The risk of ≥CIN3 associated with other HPV types in the younger group was much lower than that for HPV16: 7.1% for HPV52, 6.1% for HPV31, and 2.6% for HPV18. There



**Fig. 2.** Percent positivity of HPV16 and HPV 18 (A) and the non-16/18 genotypes (B) as a function of disease severity.

**Table 4a**  
Absolute risk by HPV genotype (hierarchical ranking) and age group.

	All GTs, % (95% CI)		HPV16, % (95% CI)		HPV31, % (95% CI)		HPV18, % (95% CI)		HPV33, % (95% CI)		HPV52, % (95% CI) <sup>a</sup>	
	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year
<b>Overall population</b>												
CIN2	4.5 (3.5, 5.7)	2.5 (2.0, 3.1)	7.1 (4.8, 10.4)	3.2 (2.0, 5.1)	6.5 (3.5, 11.9)	7.6 (5.0, 11.3)	2.7 (0.9, 7.6)	0.9 (0.2, 3.2)	5.0 (0.9, 23.6)	2.2 (0.6, 7.5)	4.0 (1.9, 8.5)	2.2 (1.1, 4.5)
≥CIN3	6.1 (5.0, 7.5)	5.4 (4.7, 6.3)	14.2 (10.8, 18.4)	15.1 (12.3, 18.4)	8.0 (4.5, 13.7)	7.9 (5.3, 11.7)	2.7 (0.9, 7.6)	9.0 (5.9, 13.4)	15.0 (5.2, 36.0)	5.4 (2.3, 12.0)	6.7 (3.7, 11.9)	4.4 (2.6, 7.3)
AIS	0.1 (0.0, 0.5)	0.4 (0.2, 0.7)	0.3 (0.1, 1.7)	1.0 (0.4, 2.2)	0.0 (0.0, 2.7)	0.4 (0.1, 2.0)	0.9 (0.2, 4.9)	2.7 (1.2, 5.7)	0.0 (0.0, 16.1)	0.0 (0.0, 4.0)	0.0 (0.0, 2.5)	0.0 (–0.0, 1.2)
SCC/ADC	0.0 (–0.0, 0.3)	0.2 (0.1, 0.4)	0.0 (0.0, 1.2)	0.2 (0.0, 1.1)	0.0 (0.0, 2.7)	0.4 (0.1, 2.0)	0.0 (–0.0, 3.3)	1.3 (0.5, 3.9)	0.0 (0.0, 16.1)	0.0 (0.0, 4.0)	0.0 (0.0, 2.5)	0.0 (–0.0, 1.2)
<b>Normal cytology</b>												
CIN2	2.8 (2.0, 4.0)	1.7 (1.2, 2.2)	4.7 (2.6, 8.5)	1.3 (0.6, 3.1)	3.0 (1.0, 8.5)	6.3 (3.7, 10.4)	2.6 (0.7, 9.0)	1.2 (0.3, 4.2)	0.0 (0.0, 21.5)	1.4 (0.3, 7.7)	1.8 (0.5, 6.2)	2.7 (1.3, 5.5)
≥CIN3	4.7 (3.5, 6.1)	3.0 (2.4, 3.7)	11.4 (7.8, 16.4)	8.2 (5.8, 11.4)	6.1 (2.8, 12.6)	4.8 (2.6, 8.7)	2.6 (0.7, 9.0)	4.7 (2.4, 9.1)	14.3 (4.0, 39.9)	1.4 (0.3, 7.7)	7.1 (3.6, 13.4)	2.7 (1.3, 5.5)
AIS	0.1 (0.0, 0.5)	0.2 (0.1, 0.5)	0.5 (0.1, 2.6)	1.1 (0.4, 2.7)	0.0 (0.0, 3.7)	0.0 (0.0, 1.8)	0.0 (0.0, 4.8)	0.6 (0.1, 3.3)	0.0 (0.0, 21.5)	0.0 (0.0, 5.2)	0.0 (0.0, 3.3)	0.0 (0.0, 1.5)
SCC/ADC	0.0 (0.0, 0.4)	0.0 (0.0, 0.2)	0.0 (0.0, 1.8)	0.0 (0.0, 1.0)	0.0 (0.0, 3.7)	0.0 (0.0, 1.8)	0.0 (0.0, 4.8)	0.6 (0.1, 3.3)	0.0 (0.0, 21.5)	0.0 (0.0, 5.2)	0.0 (0.0, 3.3)	0.0 (0.0, 1.5)
<b>Abnormal cytology</b>												
CIN2	9.3 (6.7, 12.7)	5.5 (4.0, 7.6)	11.5 (6.8, 18.7)	8.3 (4.8, 14.0)	15.4 (7.2, 29.7)	11.4 (5.9, 21.0)	2.9 (0.5, 14.9)	0.0 (0.0, 6.6)	16.7 (3.0, 56.4)	4.3 (0.8, 21.0)	11.1 (4.4, 25.3)	0.0 (–0.0, 6.0)
≥CIN3	10.1 (7.4, 13.6)	14.9 (12.4, 17.8)	19.5 (13.2, 27.7)	33.3 (26.2, 41.4)	12.8 (5.6, 26.7)	17.1 (10.1, 27.6)	2.9 (0.5, 14.9)	22.2 (13.2, 34.9)	16.7 (3.0, 56.4)	17.4 (7.0, 37.1)	5.6 (1.5, 18.1)	11.7 (5.8, 22.2)
AIS	0.3 (0.0, 1.5)	1.1 (0.5, 2.2)	0.0 (0.0, 3.3)	0.7 (0.1, 3.8)	0.0 (0.0, 9.0)	1.4 (0.3, 7.7)	2.9 (0.5, 14.9)	9.3 (4.0, 19.9)	0.0 (0.0, 39.0)	0.0 (0.0, 14.3)	0.0 (0.0, 9.6)	0.0 (–0.0, 6.0)
SCC/ADC	0.0 (–0.0, 1.0)	0.8 (0.3, 1.8)	0.0 (0.0, 3.3)	0.7 (0.1, 3.8)	0.0 (0.0, 9.0)	1.4 (0.3, 7.7)	0.0 (0.0, 10.2)	3.7 (1.0, 12.5)	0.0 (0.0, 39.0)	0.0 (0.0, 14.3)	0.0 (0.0, 9.6)	0.0 (–0.0, 6.0)

<sup>a</sup> HPV52 is considered positive if in the absence of genotypes 33, 35 and 58.

appeared to be a general trend of decreasing risk after 30 years; however, increased risk was observed for ≥CIN3 with HPV18, from 2.6% to 4.7% (2 to 8 women) and HPV45, from 0% to 3.7% (0 to 5 women). The risk for CIN2 compared to ≥CIN3 associated with HPV16 was much lower (4.7% vs 11.4% for women aged 25–29 years and 1.3% vs 8.2% for women aged ≥30 years).

**Abnormal cytology (≥ASC-US).** In women with abnormal cytology, those with HPV16 infection had the highest risk of having ≥CIN3 independent of age (33.3% and 19.5% for women aged ≥30 and 25–29 years, respectively) (Table 4a). In women aged ≥30 years, HPV18 infection was associated with a significant risk of ≥CIN3 (22.2%) and AIS (9.3%) but a very low risk of CIN2. Interestingly, there was no risk of SCC/ADC in women aged <30 years who tested positive for HPV16, HPV18, or any other genotype and no AIS attributable to non-16/18 genotypes. The risk of ≥CIN3 associated with other non-16/18 HPV types was not negligible, particularly in women aged ≥30 years: 17.1% with HPV31 (12 of 70), 17.4% with HPV33 (4 of 23), and 11.7% with HPV52 (7 of 60) (Tables 3 and 4a).

For both age groups and for all populations (independent of cytology status), the absolute risk of ≥CIN2 and ≥CIN3 for HPV 16/18 was significantly greater versus the other high-risk genotypes combined (p = <0.05, Supplemental Fig. 1).

**Discussion**

Data on the age-specific prevalence of HR-HPV genotypes and their associated absolute risk of cervical disease in a large population of women undergoing cervical cancer screening can be applied to assess and improve screening strategies. Over the past decade, there has been debate about which HR-HPV types should be included in genotyping tests in primary screening and which are better suited for pooled panel HR-HPV testing. Because the ATHENA trial is the largest prospective U.S. cervical cancer screening trial with full HR-HPV typing, the data from this trial provide a good opportunity to revisit the genotyping choices made.

In this study, the prevalence of all HR-HPV genotypes was the highest in women aged 25–29 years and decreased with age. These results are similar to those of Wheeler et al.; the highest prevalence of any carcinogenic type in women ≥25 years was found in ages 25–29 years (21.8%) and the lowest prevalence in women >50 years (6.9%) [21]. Further similarity was seen with the data of Hariri et al. [22] in the U.S. and Monsonego et al. [23] in France. The results presented here are also similar to what was reported in a meta-analysis of ≈1 million women worldwide with negative cytology in which the highest prevalence of HPV infection occurred in the youngest women, decreasing progressively with age and then showing a slight increase in women aged ≥55 years [24]. In this current study, we observed the expected decrease in HPV prevalence with age in women who had negative cytology, followed by a plateauing between 50 and 60 years and a decline thereafter.

Our finding that HPV16 was the most prevalent single HPV genotype in the general population and was 2–3 times more prevalent than HPV18 is consistent with that of Wheeler et al. [21] HPV16 was also found to be the most prevalent among those genotypes classified as oncogenic by Hariri et al. [22] and Monsonego et al. [23] in women aged ≥25 years.

In addition to evaluating prevalence, it is valuable to know the risk for high-grade disease conferred by specific genotypes when assessing screening strategies. This is particularly important in women with negative cytology results because HPV genotyping can be useful in triaging to colposcopy. In women aged ≥30 with negative cytology, we observed that HPV16 and HPV18 conferred the highest risk for ≥CIN3, followed by HPV31. In women 25–29 years, only HPV33 among all genotypes demonstrated a higher risk (14.3%) than that of HPV16 for ≥CIN3, but this estimate was based on only 2 of 14 cases. The risk for HPV16 was considerably higher than the risk associated with HPV18;

**Table 4b**  
Absolute risk by HPV genotype (hierarchical ranking) and age group.

	HPV35, % (95% CI)		HPV39, % (95% CI)		HPV45, % (95% CI)		HPV58, % (95% CI)		Other GTs, % (95% CI) <sup>a</sup>	
	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year	25–29 years	≥30 year
<b>Overall population</b>										
CIN2	7.2 (3.1, 15.9)	3.5 (1.6, 7.4)	1.1 (0.2, 6.0)	1.3 (0.4, 3.8)	1.6 (0.3, 8.7)	0.6 (0.1, 3.4)	9.4 (4.4, 19.0)	2.8 (1.2, 6.3)	2.2 (1.1, 4.2)	1.4 (0.8, 2.3)
≥CIN3	4.3 (1.5, 12.0)	3.5 (1.6, 7.4)	3.3 (1.1, 9.2)	3.5 (1.8, 6.7)	0.0 (0.0, 5.9)	4.3 (2.1, 8.7)	1.6 (0.3, 8.3)	1.7 (0.6, 4.8)	1.4 (0.6, 3.1)	0.8 (0.4, 1.6)
AIS	0.0 (0.0, 5.3)	0.0 (0.0, 2.2)	0.0 (0.0, 4.1)	0.0 (0.0, 1.6)	0.0 (0.0, 5.9)	0.0 (0.0, 2.3)	0.0 (0.0, 5.7)	0.0 (0.0, 2.1)	0.0 (–0.0, 1.0)	0.0 (–0.0, 0.4)
SCC/ADC	0.0 (0.0, 5.3)	0.0 (0.0, 2.2)	0.0 (0.0, 4.1)	0.4 (0.1, 2.4)	0.0 (0.0, 5.9)	0.0 (0.0, 2.3)	0.0 (0.0, 5.7)	0.0 (0.0, 2.1)	0.0 (–0.0, 1.0)	0.0 (–0.0, 0.4)
<b>Normal cytology</b>										
CIN2	5.8 (2.0, 15.6)	0.8 (0.1, 4.4)	1.3 (0.2, 7.0)	0.5 (0.1, 2.9)	1.9 (0.3, 9.9)	0.7 (0.1, 4.1)	1.9 (0.7, 5.6)	1.4 (0.6, 3.6)	1.4 (0.6, 3.6)	1.0 (0.5, 1.9)
≥CIN3	3.8 (1.1, 13.0)	0.8 (0.1, 4.4)	2.6 (0.7, 9.0)	3.1 (1.4, 6.6)	0.0 (–0.0, 6.8)	3.7 (1.6, 8.4)	0.6 (0.1, 3.6)	0.4 (0.1, 2.0)	0.4 (0.1, 2.0)	0.6 (0.3, 1.4)
AIS	0.0 (0.0, 6.9)	0.0 (–0.0, 3.0)	0.0 (0.0, 4.8)	0.0 (0.0, 1.9)	0.0 (–0.0, 6.8)	0.0 (0.0, 2.8)	0.0 (0.0, 2.4)	0.0 (0.0, 1.3)	0.0 (0.0, 1.3)	0.0 (0.0, 0.5)
SCC/ADC	0.0 (0.0, 6.9)	0.0 (–0.0, 3.0)	0.0 (0.0, 4.8)	0.0 (0.0, 1.9)	0.0 (–0.0, 6.8)	0.0 (0.0, 2.8)	0.0 (0.0, 2.4)	0.0 (0.0, 1.3)	0.0 (0.0, 1.3)	0.0 (0.0, 0.5)
<b>Abnormal cytology</b>										
CIN2	11.8 (3.3, 34.3)	10.4 (4.5, 22.2)	0.0 (0.0, 21.5)	5.7 (1.6, 18.6)	0.0 (0.0, 32.4)	0.0 (0.0, 12.9)	25.0 (8.9, 53.2)	7.7 (2.1, 24.1)	4.6 (1.8, 11.2)	3.6 (1.7, 7.7)
≥CIN3	5.9 (1.0, 27.0)	10.4 (4.5, 22.2)	7.1 (1.3, 31.5)	5.7 (1.6, 18.6)	0.0 (0.0, 32.4)	7.7 (2.1, 24.1)	0.0 (0.0, 24.3)	7.7 (2.1, 24.1)	4.6 (1.8, 11.2)	1.8 (0.6, 5.2)
AIS	0.0 (0.0, 18.4)	0.0 (0.0, 7.4)	0.0 (0.0, 21.5)	0.0 (0.0, 9.9)	0.0 (0.0, 32.4)	0.0 (0.0, 12.9)	0.0 (0.0, 24.3)	0.0 (0.0, 12.9)	0.0 (0.0, 4.2)	0.0 (0.0, 2.3)
SCC/ADC	0.0 (0.0, 18.4)	0.0 (0.0, 7.4)	0.0 (0.0, 21.5)	0.0 (0.0, 14.5)	0.0 (0.0, 32.4)	0.0 (0.0, 12.9)	0.0 (0.0, 24.3)	0.0 (0.0, 12.9)	0.0 (0.0, 4.2)	0.0 (0.0, 2.3)

<sup>a</sup> Other GTs includes genotypes 51, 56, 59, 66, 68, 73, and 82.

however, the risk for HPV16 and most other genotypes decreased with age while the risk for HPV18 increased substantially in women ≥ 30 years. These data, considered with the significant contribution of HPV16 and HPV18 to invasive cancer, support genotyping specifically for HPV16 and 18 and pooled testing of the other non-16/18 HPV types in cotesting, as is supported by the current guidelines [6]. Previous analyses of the ATHENA data indicated that the absolute risk of ≥CIN3 at baseline in women ≥30 years with negative cytology was found to be approximately 5 times higher in women with positive results for HPV16 and 2 times higher with HPV18 positive results when compared to women testing positive for the non-16/18 genotypes [12]. In addition, Schiffman et al. [19] reported the 3-year cumulative risk for ≥CIN3 in 18,000 women ≥30 years with negative cytology and positive HR-HPV results. They observed the highest risk for HPV16 (10.6%), followed by HPV33 (5.9%) and HPV18 (5.9%), HPV31 (4.5%), HPV52 (3.8%), and HPV45 (1.7%). With the exception of lower baseline risks for HPV33 and HPV52 and a higher risk for HPV45, our risk determination in women ≥30 years with negative cytology shows trends similar to these longitudinal observations.

In women with abnormal cytology, HPV16 was also the driver of the majority of ≥CIN3, the risk being very high both in women aged ≥30 years and in women aged 25–29 years. Interestingly, HPV18 also drove the risk of ≥CIN3 (including AIS) in women with abnormal cytology aged ≥30 years.

The prevalence of the various genotypes found in disease is also instructive in assessing strategies. Regardless of age group, the prevalence of HPV16 is low in <CIN2 and high in ≥CIN2, highlighting the role of HPV16 in CIN lesions of increasing severity. In fact, HPV16 was responsible for 54.1% and 41.8% of ≥CIN3 cases in women aged 25–29 and ≥30 years, respectively. By comparison, HPV18 was responsible for a lower percentage of ≥CIN3 cases, but a sharp increase was noted after 30 years. Moreover, 50% of the AIS and 50% of the cancers in this study were positive for HPV18 and detection of HPV18 in cervical cancers is second only to HPV16 in large population studies [1]. Additionally, HPV16 and HPV18 cause approximately 83% of cervical adenocarcinomas with a precursor of AIS, which is known to be more frequently missed by cytology screening than is CIN3. This is likely due to limitations in sampling lesions that are often located in the endocervical canal or because the lesions are focal and small in size [25,26]. Moreover, cytologic and colposcopic features of AIS are difficult to differentiate from normal columnar epithelium [27]. In the large cotesting study from Kaiser Northern California, 34% of the ≥CIN3, 44% of the AIS, 29% of the total cervical cancers and 63% of the adenocarcinomas were detected in follow up to cytology-negative/HR-HPV positive cotest results [28]. The fact that 44% of the AIS and 63% of the adenocarcinomas were not detected by cytology but were detected by HPV testing is in contrast to the normal ratio of 20% glandular to 80% squamous cervical cancers [29]. These data argue strongly for the inclusion of HPV testing in primary screening.

In the current study, the contribution of HPV18 to CIN3, AIS, and cervical cancer is disproportionately greater in women aged ≥30 years when compared with all other HR-HPV types; this supports the hypothesis that HPV18-associated lesions are found at a later stage because they are either missed by cytology and/or colposcopy or take longer to develop [8]. These observations reinforce that testing specifically for HPV18 is warranted, as is supported by the current U.S. guidelines [6]. Moreover, testing for HPV18 in women 25–29 years may alert clinicians to be watchful for future development of ≥CIN3 in those young women who test positive.

Although prevalence of non-16/18 genotypes was much lower in women with ≥CIN2 than those with <CIN2, nearly half of ≥CIN2 involved other HR-HPV types, especially in women aged ≥30 years. The risk of ≥CIN3 associated with other genotypes was substantial, particularly for HPV31 and HPV52 in women aged ≥30 years with abnormal cytology. Although ATHENA was not designed to evaluate vaccine efficacy, these data infer that the inclusion of the additional genotypes in the

nonavalent vaccine should further decrease the incidence of high-grade cervical disease. From the diagnostic perspective, the lower overall contribution to cancer of the non-HPV16/18 types again supports their being analyzed as pooled HR-HPV types rather than individually for triage to colposcopy.

The main limitation of this study is that the number of  $\geq$ CIN3 cases for non-HPV16/18 types is relatively low for individual genotypes; therefore, data regarding the risk of  $\geq$ CIN3 associated with these genotypes should be interpreted with caution. Moreover, the mean age of the population was  $\approx$ 40 years and most women had an interval of screening  $<$ 5 years, suggesting that this was a low-risk population. Another important limitation is that this was a cross-sectional analysis without long-term follow-up. However, because all women with either abnormal cytology or positive HPV results at baseline were referred to colposcopy, the majority of disease was detected at baseline. In effect, the baseline disease detection in ATHENA was comparable to what was detected longitudinally in other studies in which colposcopy was not performed until abnormal cytology occurred or HR-HPV was persistently positive.

This study provides the opportunity to determine the usefulness of HPV genotyping by analyzing the prevalence of genotypes and the risks of CIN associated with them in a large population of women undergoing screening in the U.S. Our results confirm the utility of determining the presence of HPV16 and HPV18 in women aged  $\geq$ 30 years as an adjunctive test, particularly in women with negative cytology. The data suggest further that identifying their associated risks may be useful in HPV primary screening starting at age 25 years. For the other HPV genotypes, the risk is much lower in all population categories and does not justify genotyping for these *individual* HR-HPV types in primary screening. However, genotyping for *pooled* HR-HPV (non-HPV16/18) types is indicated, in view of the fact that approximately 30% of invasive cancers are due to these other genotypes.

#### Conflict of interest statement

Dr. Monsonego has served as an advisor for Merck, Sanofi Pasteur MSD, Gen-Probe and Roche Diagnostics. Dr. Cox has served as an advisor and speaker for Roche and Hologic/Gen-Probe, as well as an advisor for Merck, Trovogene, and Zilico. Dr. Sandri has served as an advisor for Roche and Abbott. Dr. Franco has served as an advisor for Roche, Merck, and Becton & Dickinson. Dr. Huh has served as an advisor for Merck. Dr. Behrens and Dr. Yap are employed by Roche.

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