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Lineages of Oncogenic Human Papillomavirus Types Other Than Type 16 and 18 and Risk for Cervical Intraepithelial Neoplasia

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Background Data on clinical outcomes of infection with variants of oncogenic human papillomavirus (HPV) types other than HPV16 and HPV18 are rare. We investigated intratypic variations in non-HPV16/18 oncogenic types and their corresponding relationships with cervical intraepithelial neoplasia grades 2–3 (CIN2/3).

Methods Study subjects were women who were positive for one or more of 11 non-HPV16/18 oncogenic types. Subjects were followed every six months for two years for detection of HPV and cervical lesions. Variant lineages were defined by sequencing the 3′ part of the long control region and the entire E6/E7 region of HPV genome. Lineage-associated risk of CIN2/3 was assessed using logistic regression with generalized estimating equations.

Results A total of 4591 type-specific HPV infections among 2667 women were included in the analysis. The increase in risk of CIN2/3 was statistically significant for women with HPV31 A or B compared with C variants, HPV33 A1 compared with B variants, HPV45 A3 or B2 compared with B1 variants, HPV56 B compared with A2 variants, and HPV58 A1 or A3 compared with C variants. For these five types, the adjusted odds ratio associated with CIN2/3 was 2.0 (95% confidence interval [CI] = 1.5 to 2.6) for infections with single-type high-risk (HR) variants, 1.7 (95% CI = 1.0 to 2.7) for infections with two or more types but only one HR variant, and 5.3 (95% CI = 3.1 to 8.4) for infections with HR variants of two or more types as compared with those with single-type non-HR variants. The likelihood of CIN2/3 was similar for women with HPV16 infection and for those with HPV58 A1 variant infection.

Conclusions These findings suggest that for a given HPV type, intratypic nucleotide changes may alter phenotypic traits that affect the probability of neoplasia.

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Human papillomavirus (HPV) is an etiologic agent for cervical cancer and its precursor, cervical intraepithelial neoplasia grades 2–3 (CIN2/3) (1–4). To date, nearly 170 HPV types have been characterized based on the isolation of complete genomes (5); 12 are classified as oncogenic types (6). For a given type of HPV, continued evolution makes the genome further diversified; viral isolates that differ by less than 2% in the L1 region are termed "variants" (7). Whereas HPV types are known to differ in tissue tropisms, biologic behaviors, and oncogenic potentials, much less is known about whether lineage classification of the variants reflects genetic traits of the virus and clinical outcomes of the infection.

Studies of intratypic variations of the HPV genome have focused mainly on HPV16 and HPV18 (8–20). Data on the clinical relevance of infections with variants of non-HPV16/18 oncogenic types are rare and inconsistent (21–31). It remains largely undetermined whether there are any variants of non-HPV16/18 oncogenic types that possess oncogenicity similar to HPV16, the type that confers the highest risk of cervical cancer.

Infections with multiple types are common, especially among young women and women with cytologic abnormalities (32–34). An increased risk of cervical lesions among women infected with more than one vs only one type of HPV has been observed in some studies (34–39), but not in others (40–43). The reason for this difference in findings is not understood; whether variants of the coexisting HPV types play a role deserves consideration.

To address these questions, we examined risk of CIN2/3 associated with lineage of non-HPV16/18 oncogenic types among women who participated in the Atypical Squamous Cells of Undetermined Significance (ASC-US) and Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS).

Methods

Subjects and Study Design

Study subjects were women enrolled in ALTS who were followed every six months for two years for detection of HPV and cervical lesions. A detailed description of the ALTS design and study population is available elsewhere (44,45). All participants provided written informed consent when they were enrolled in ALTS.

Women were eligible for the present study if they had one or more of 11 non-HPV16/18 oncogenic types (ie, HPV31/33/35/39/45/51/52/56/58/59/68) detected in their cervical swab sample(s) at any time during the trial by polymerase chain reaction (PCR)–based reverse-line blot assay (46). For each type of infection, variant testing was performed on the first positive sample. The reason for testing the first rather than all positive samples is that women virtually always maintain the same variant in the life-span of the infection (26). In total, 5558 type-specific infections, whether initially detected at the same visit or not, were identified among 3002 women. One hundred and eighty-five infections were excluded because of a lack of remaining sample for variant testing. We additionally excluded two infections with a greater than 300 bp deletion in the region analyzed and 780 infections for which we were unable to PCR-generate DNA fragments for sequencing, leaving 4591 from the 2667 women in the analysis.

The primary endpoint used in this study was CIN2/3 histologically confirmed by the panel of expert pathologists. The episode of CIN2/3 was considered to be variant-related only if it was diagnosed at visits where infection with the corresponding type of HPV was concurrently detected. During the trial, outcomes of interest were measured repeatedly. A woman could have CIN2/3 detected at more than one visit. The redetected episode was essentially a result of recurrence (or new occurrence), rather than persistence of a previous lesion, as virtually all CIN2/3 cases were treated by loop electrosurgical excision procedure. One episode of CIN2/3 could be related to one type of HPV and another episode to other types. If two or more types were concurrently detected at the time of diagnosis, the lesion was considered to be related to multiple types. The study protocol was approved by the institutional review board at University of Washington.

Testing for Variants

An aliquot of 50–100 μL Specimen Transport Medium per sample was used for DNA extraction using QIAamp DNA mini kit (Qiagen, Valencia, CA). HPV DNA fragments were generated by PCR with a set of type-specific external primers, targeting the 3' part of the long control region (LCR) and the entire E6 and E7 region of 11 non-HPV16/18 oncogenic types (see Supplementary Table 1, available online, for primer sequences). PCR products were sequenced with a pair of external primers and a pair of internal primers using BigDye™ Sequencing kit (Applied Biosystems, Foster City, CA). The sequencing reaction was run from both directions. DNA sequences were analyzed using Sequencer™ package (Gene Codes Corp., Ann Arbor, MI). A viral isolate was defined as a distinct variant if, as compared with the prototype and other isolates detected in the study, there were one or more nucleotide alterations in the region analyzed. Lineages of the variants were phylogenetically classified and named according to the alphanumeric nomenclature (47–49). Information on sequence variations of the variants and lineage assignment for each type of HPV is detailed in Supplementary Tables 2–12 and the Supplementary Methods (available online).

Statistical Analyses

The main exposure of interest was lineage of the variants. We assumed that the variant in unexamined type-specific positive samples would be the same as the one detected in the type-specific positive sample tested for each woman. The analysis was performed at the level of individual infections. Thus, women positive for two or more types of interest, whether concurrently at the same visit or not, would be counted multiple times.

Polytomous logistic regression (or unconditional logistic regression when appropriate) (50) with generalized estimating equations (GEE, with the independent working correlation structure) was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of CIN with lineage of the variants. For each type, we arbitrarily chose the low-risk lineage as the reference to compare with others. To ensure that the analyses had reasonable statistical power, lineages detected in 10 or fewer women or those linked to one or less cases were excluded from analyses. Considering limited sample size for some lineages, the 95% CIs were computed using a parametric bootstrap method with 1000 repetitions; the lower and higher bounds were given by the 25th and 975th bootstrap odds ratios, respectively. If one or more parameters could not be estimated in 10% or more bootstrap replicates, the 95% CIs were estimated by jackknife logistic regression clustering on study subject identification (51). The ORs for CIN2/3 were adjusted for self-reported age (18–19, 20–24, 25–29, and ≥30), race (Caucasian vs non-Caucasian), current smoking status (yes vs no), ever receiving treatment for CIN2/3 (yes vs no), and visit number of the first type-specific positive detection (1, 2, 3, 4, and 5), factors that were related to risk of CIN2/3 in this study population (data not shown). The Wald test was used to test a null hypothesis of lack of the association for all lineages.

To examine risk of CIN by single vs multiple types at the level of variants, we included types whose lineages differed statistically significantly in their association with CIN2/3. In the interest of maximizing statistical precision, each type was dichotomized as high-risk (HR) vs non-HR variants. According to the results of individual types, this analysis was restricted to five types, in which HPV31 A/B, HPV33 A1, HPV45 A2/A3/B2, HPV56 A1/B, and HPV58 A1/A3 variants were categorized as HR variants, the rest as non-HR variants. Infection status at individual visits was categorized as positive for: 1) non-HR variants of a single type, 2) non-HR variants of two or more types, 3) HR variants of a single type, 4) one type's HR variants and one or more type's non-HR variants, and 5) HR variants of two or more types. With the same approach, we also examined risk of CIN by variants of single vs multiple types in the context of specific HPV types.

To explore whether any HPV variant approached the carcinogenicity of HPV16, we further compared risk of CIN between women with HPV16 and those with HR variants of non-HPV16/18 oncogenic types. This analysis was restricted to infections initially detected at enrollment, as our previous study of HPV16 variants was conducted only among women with baseline HPV16 infection.

Nonparametric median test was used to examine differences in the median duration of follow-up since the first positive test by lineage of the variants. All statistical analyses were performed using STATA statistical software, version 11 (StataCorp, College Station, TX), and all tests were at the 5% two-sided significance level.

Results

Table 1 shows the distribution of the lineage-specific infections for individual types and the mean and median duration of follow-up since the first positive detection. The median duration of follow-up was longer for women with HPV35 A2, compared with A1, variants (P = .07) and HPV52 A, compared with B, variants (P = .05). The length of follow-up by lineages of other types did not differ statistically significantly.

Variant-related CIN2/3 was histologically confirmed among 548 women at a total of 577 visits, including 521 at a single visit, 25 at two visits, and two at three visits. The increase in the cumulative risk of CIN2/3 was statistically significant for women with HPV31 A or B compared with C variants, HPV33 A1 compared with B

Table 1. Distribution of lineage-specific human papillomavirus infections and duration of follow-up since the first type-specific positive detection

Type	Lineage or sublineage	Women, No. (%)*	Duration, mo. (95% confidence interval)		P†
			Mean	Median	
HPV31	A	196 (41.7)	17.3 (16.0 to 18.9)	21.9 (19.2 to 23.4)	.22
	B	99 (21.1)	18.6 (16.6 to 20.5)	23.8 (20.4 to 24.1)	
	C	175 (37.2)	17.1 (15.7 to 18.5)	22.0 (17.4 to 23.7)	
HPV33	A1	152 (69.7)	16.9 (15.4 to 18.5)	22.2 (18.2 to 23.3)	.10
	A2	23 (10.6)	21.2 (17.8 to 24.5)	24.2 (21.3 to 25.5)	
	B	43 (19.7)	17.1 (14.3 to 20.0)	23.3 (15.5 to 24.2)	
HPV35	A1	332 (85.8)	16.6 (15.6 to 17.5)	18.8 (17.7 to 22.4)	.07
	A2	55 (14.2)	19.0 (16.6 to 21.4)	23.4 (18.1 to 24.2)	
HPV39	A	476 (99.4)	17.6 (16.8 to 18.4)	19.9 (18.7 to 22.4)	-
	B	3 (0.6)	14.5	13.2	
HPV45	A1	127 (37.2)	16.6 (15.1 to 18.6)	22.4 (17.8 to 23.9)	.70
	A2	49 (14.4)	15.5 (124 to 18.6)	19.4 (8.1 to 23.8)	
	A3	18 (5.3)	14.8 (10.5 to 19.0)	16.9 (8.1 to 23.7)	
	B1	52 (15.3)	17.6 (14.6 to 20.5)	22.2 (17.6 to 24.3)	
	B2	92 (27.0)	17.4 (15.5 to 19.4)	22.1 (18.1 to 23.6)	
	C	3 (0.9)	10.2	6.2	
HPV51	A1	415 (73.3)	18.2 (17.3 to 19.1)	22.9 (20.4 to 23.4)	.24
	A2	114 (20.1)	18.7 (17.1 to 20.4)	23.4 (19.2 to 23.9)	
	A3	28 (5.0)	16.2 (12.6 to 19.8)	17.6 (13.0 to 23.5)	
	B	9 (1.6)	20.1	22.9	
HPV52	A	636 (93.1)	18.1 (17.4 to 18.8)	22.6 (20.6 to 23.1)	.05
	B	32 (4.7)	16.1 (12.6 to 19.6)	17.7 (11.7 to 19.4)	
	C	9 (1.3)	23.8	23.7	
	D	6 (0.9)	19.9	21.4	
HPV56	A1	38 (9.4)	17.9 (14.9 to 20.8)	22.5 (17.7 to 23.9)	.14
	A2	126 (31.3)	19.5 (18.0 to 21.0)	23.6 (22.7 to 24.0)	
	B	239 (59.3)	17.5 (16.3 to 18.7)	21.8 (19.0 to 23.6)	
HPV58	A1	54 (14.3)	19.3 (17.0 to 21.5)	22.4 (18.1 to 23.8)	.22
	A2	228 (60.3)	19.0 (17.8 to 20.1)	23.3 (22.1 to 23.7)	
	A3	17 (4.5)	21.4 (18.1 to 24.8)	23.8 (20.2 to 24.8)	
	B1	3 (0.8)	19.6	24.9	
	B2	18 (4.8)	18.1 (13.8 to 22.5)	22.8 (12.8 to 25.0)	
	C	49 (13.0)	16.4 (13.5 to 19.2)	19.7 (13.3 to 23.6)	
	D1	1 (0.3)	26.5	26.5	
	D2	8 (2.1)	17.2	18.2	
HPV59	A	48 (12.0)	17.6 (14.8 to 20.4)	19.9 (14.9 to 24.2)	.89
	B	353 (88.0)	17.7 (16.7 to 18.6)	22.2 (19.0 to 23.4)	
HPV68	A	10 (3.8)	15.7	16.9	.93
	B	4 (1.5)	8.1	8.9	
	C	60 (22.6)	18.3 (15.8 to 20.7)	20.6 (17.6 to 23.8)	
	D	7 (2.6)	19.3	24.5	
	E	125 (47.2)	16.8 (15.2 to 18.5)	18.8 (17.0 to 23.3)	
	F	48 (18.1)	15.3 (12.3 to 18.2)	18.8 (10.4 to 23.2)	
	G	11 (4.2)	18.1 (10.2 to 26.0)	18.9 (9.0 to 26.1)	

* A woman was counted more than once if she was positive for multiple non-human papillomavirus16/18 oncogenic types. - = not done, because there were not enough valid cases for the median test. HPV = human papillomavirus.

† P values are based on two-sided nonparametric median tests. Lineages detected in 10 women or fewer (without 95% confidence interval provided) were excluded from the testing because they were not included in analyses of lineage-associated risk of cervical lesion.

variants, HPV45 A3 or B2 compared with B1 variants, HPV56 B compared with A2 variants, and HPV58 A1 or A3 compared with C variants (Table 2). HPV45 A2 and HPV56 A1 variants tended to be associated with higher risk also, although a difference was not statistically significant. A globe testing for a null hypothesis of lack of the association for all lineages was statistically significant for HPV31 ($P = .02$) and HPV58 ($P < .001$).

Of 27 women with two or more diagnoses of variant-related CIN2/3, 20 (74.1%) had lesions redetected at nonconsecutive visits; 18 (66.7%) were positive for at least one different non-HPV16/18 oncogenic type between the initial and repeated diagnoses. The risk estimates remained similar when the episodes of CIN2/3 redetected at consecutive visits and/or at visits positive for identical HPV type(s) were excluded from analyses (data not shown). Given that risks of CIN2/3 differed statistically significantly by some lineages of five HPV types, we wanted to know whether coexistent HR variants of these types would be associated with even higher risk of CIN2/3.

Infection with HPV31, HPV33, HPV45, HPV56, and/or HPV58 was detected among 1689 women at a total of 2921 visits (2551 positive for a single type and 370 positive for two or more types). Relative to infections with non-HR variants of a single type, the adjusted OR associated with CIN2/3 was 2.0 (95% CI = 1.5 to 2.6) for infections with HR variants of a single type, 1.7 (95% CI = 1.0 to 2.7) for infections with two or more types but only one being classified as a HR variant, and 5.3 (95% CI = 3.1 to 8.4) for infections with HR variants of two or more types (Table 3). Relative to infections with HR variants of a single type, risks of CIN2/3 did not change appreciably for those with coexistent non-HR variants of other types ($OR_{adjusted} = 0.9$, 95% CI = 0.5 to 1.3) but were statistically significantly higher for those with coexistent HR variants of other types ($OR_{adjusted} = 2.6$, 95% CI = 1.6 to 4.2). With CIN3 as the endpoint, the increase in risk associated with HR variants of multiple types was even more substantial, almost three-fold higher as compared with risk associated with HR variants of a single type.

To address whether the above observation was by chance because of differential distributions of type-specific infections across exposure categories, we further compared risks by variants of single vs multiple types in the context of type-specific infection. As shown in Table 4, risks of CIN2/3 were comparable between infections with HR variants of the index type with and without coexistent non-HR variants of other types; the highest risk was consistently associated with HR variants of both index and coexisting type(s).

Prevalent infection with HR variants of HPV31, HPV33, HPV45, HPV56, and HPV58 at enrollment was detected in 193, 89, 96, 174, and 48 women, respectively. As reported previously (13), 822 ALTS participants were positive for HPV16 at enrollment. Overall, women with HPV16 were more likely to have a diagnosis of CIN2/3 or CIN3 than were those with HR variants of non-HPV16/18 oncogenic types, except for those with HPV31 lineage B or HPV58 lineage A1 and A3 (Table 5). The likelihood of having a diagnosis of CIN2/3 or CIN3 was similar between women with any variants of HPV16 and those with HPV58 A1 variants. As compared with women with HPV16, those with HPV31 B variants had a comparable risk of CIN2/3 but not CIN3 alone, and those with HPV58 A3 variants had higher risk of CIN2, but no CIN3 detected. Results remained similar when women (or visits) with coexisting types compared were excluded from analyses (data not shown).

Discussion

In this study, we examined the clinical outcomes of infection with variants of 11 non-HPV16/18 oncogenic types. The main findings were: 1) the association between risk of CIN2/3 and lineages of five non-HPV16/18 oncogenic types, 2) the extra risk of CIN2/3 associated with coinfection with HR variants of multiple types relative to individual types, and 3) the comparable frequencies of having a diagnosis of CIN2/3 or CIN3 alone between women with HPV16 and those with HPV58 lineage A1.

Published reports regarding clinical relevance of infection with lineages of non-HPV16/18 oncogenic types are rare. Our results for lineages of HPV31, HPV33, and HPV58 basically agree with the trends previously suggested by others (27–30). Additionally, we observed that risks of CIN2/3 differed statistically significantly by lineages of HPV45 and HPV56. We did not see appreciable differences in risk of CIN2/3 by lineages of the other six types. One interpretation for the lack of association could be small sample size for particular lineages. For example, the study in Asia by Chang et al. showed that the lineages B and C accounted for 99% of 280 HPV52 infections and the increased risk of ≥CIN3 was more associated with the C compared with B variants (27). However, in our study population, the lineages B and C were detected only in 4.7% and 1.3% of 685 HPV52 infections, respectively. It is also possible that sequence variations in the region analyzed were not sensitive enough for distinction of the heterogeneity of the variants, as some sublineages identified by whole genome analyses could not be distinguished by analyses of our target region. Thus, a possibility that certain unrecognized sublineages might possess oncogenic potentials either stronger or weaker than what was observed for the lineage overall cannot be ruled out. A third and more plausible interpretation is that genetic changes accumulated so far in the process of lineage fixation after the emergence of these HPV types might not be sufficient to lead to alteration of oncogenicity. We hypothesize that over time, as the quantity of the lineage-defining variations increases, some of them might be eventually shaped to make certain lineages more aggressive than others.

As an important contribution to understanding the natural history of HPV infection, our study demonstrated a variant-dependent role of infection with multiple non-HPV16/18 oncogenic types in the development of cervical lesions. The increase in risk of CIN2/3 associated with HR compared with non-HR variants was anticipated as dichotomization of these variants, based on the known results of individual types. However, the variant-dependent role of multiple HPV types cannot be simply explained by the grouping because the clustering of lineages (and types) was unrestrained. The results remained similar when analyses were performed in the context of specific HPV types. Although underlying mechanisms for the variant-dependent role of multiple HPV types are currently unknown, the findings may in part explain why the association of cervical lesions with multiple types was seen in some studies but not in others.

Another interesting observation is the HPV16-like behavior of HPV58 A1 variants. This may in part explain why risks of cervical

Table 2. Risk of cervical intraepithelial neoplasia associated with lineage-specific human papillomavirus infection

Type	Lineage or sublineage	No. of women*	No. of positive visits	CIN2		CIN3		CIN2/3		
				No. (per 100 visits) (%)	OR (95% CI)	No. (per 100 visits) (%)	OR (95% CI)	OR (95% CI)	$OR_{adjusted}$ (95% CI)†	*P*‡
HPV31	C	175	347	19 (5.5)	1.0 (referent)	14 (4.0)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.02
	A	196	349	30 (8.6)	1.7 (1.0 to 3.4)	32 (9.2)	2.5 (1.4 to 5.3)	2.1 (1.4 to 3.3)	1.7 (1.0 to 2.8)	
	B	99	175	17 (9.7)	2.1 (1.0 to 4.4)	21 (12.0)	3.4 (1.7 to 7.9)	2.6 (1.5 to 4.5)	2.2 (1.3 to 4.1)	
HPV33	B	43	97	4 (4.1)	1.0 (referent)	4 (4.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.21
	A1	152	240	16 (6.7)	1.8 (0.6 to 8.2)	29 (12.1)	3.3 (1.5 to 13.2)	2.6 (1.2 to 7.8)	2.5 (1.1 to 9.0)	
	A2	23	46	3 (6.5)	1.7 (0.4 to 10.4)	4 (8.7)	2.3 (0.5 to 10.1)	2.0 (0.6 to 7.5)	1.8 (0.5 to 7.3)	
HPV35	A2	55	90	7 (7.8)	1.0 (referent)	5 (5.6)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.98
	A1	332	585	38 (6.5)	0.8 (0.4 to 2.4)	41 (7.0)	1.3 (0.6 to 5.2)	1.0 (0.6 to 2.2)	1.0 (0.5 to 2.3)	
HPV39	A	476	810	36 (4.4)	-	38 (4.7)	-	-	-	
	B	3	8	1 (12.5)	-	0	-	-	-	
HPV45	B1	52	98	2 (2.0)	1.0 (referent)	3 (3.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.13
	A1	127	228	11 (4.8)	2.5 (0.3 to 18.5)	11 (4.8)	1.7 (0.5 to 6.5)	2.0 (0.8 to 7.8)	2.0 (0.6 to 6.3)	
	A2	49	86	3 (3.5)	1.9 (0.2 to 18.9)	8 (9.3)	3.3 (0.8 to 13.7)	2.7 (0.8 to 12.1)	3.1 (0.9 to 11.3)	
	A3	18	41	7 (17.1)	9.6 (1.3 to 70.5)	0	-	3.8 (1.2 to 19.3)	4.3 (1.1 to 17.1)	
	B2	92	160	11 (6.9)	3.7 (0.5 to 27.7)	11 (6.9)	2.5 (0.7 to 9.8)	3.0 (1.1 to 13.2)	3.3 (1.1 to 10.6)	
	C	3	4	1 (25.0)	-	0	-	-	-	
HPV51	A3	28	49	2 (4.1)	1.0 (referent)	2 (4.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.64
	A1	415	644	33 (5.1)	1.3 (0.2 to 9.4)	35 (5.4)	1.4 (0.2 to 9.9)	1.3 (0.5 to 6.7)	1.1 (0.3 to 3.9)	
	A2	114	173	13 (7.5)	2.0 (0.3 to 15.3)	11 (6.4)	1.7 (0.2 to 12.9)	1.8 (0.7 to 10.3)	1.4 (0.4 to 5.3)	
	B	9	17	0	-	1 (5.9)	-	-	-	
HPV52	A	636	1260	55 (4.4)	1.0 (referent)	62 (4.9)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.15
	B	32	62	4 (6.5)	1.6 (0.3 to 3.6)	5 (8.1)	1.7 (0.4 to 3.7)	1.7 (0.7 to 3.1)	1.9 (0.7 to 4.1)	
	C	9	17	2 (11.8)	-	0	-	-	-	
	D	6	15	1 (6.7)	-	1 (6.7)	-	-	-	
HPV56	A2	126	195	4 (2.1)	1.0 (referent)	4 (2.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.12
	A1	38	60	4 (6.7)	3.5 (0.8 to 17.6)	2 (3.3)	1.7 (0.2 to 14.7)	2.6 (0.6 to 8.4)	3.0 (0.9 to 9.7)	
	B	239	370	15 (3.3)	2.1 (0.7 to 8.9)	16 (4.3)	2.2 (0.6 to 7.7)	2.1 (1.0 to 6.2)	2.3 (1.0 to 5.5)	
HPV58	C	49	108	2 (1.9)	1.0 (referent)	2 (1.9)	1.0 (referent)	1.0 (referent)	1.0 (referent)	<.001
	A1	54	97	11 (11.3)	7.8 (1.1 to 57.7)	13 (13.4)	9.3 (1.2 to 70.8)	8.5 (3.4 to 34.9)	10.6 (2.7 to 42.1)	
	A2	228	457	12 (2.6)	1.5 (0.2 to 10.9)	20 (4.4)	2.4 (0.3 to 17.7)	2.0 (0.8 to 8.1)	1.9 (0.5 to 6.9)	
	A3	17	29	6 (20.7)	13.6 (1.7 to 110.1)	0	-	6.8 (1.9 to 32.5)	8.7 (1.7 to 45.0)	
	B2	18	49	4 (8.2)	4.7 (0.5 to 44.3)	1 (2.0)	-	3.0 (0.8 to 13.6)	3.3 (0.7 to 15.9)	
	B1	3	4	0	-	0	-	-	-	
	D1	1	1	0	-	0	-	-	-	
	D2	8	19	3 (15.8)	-	0	-	-	-	
HPV59	A	48	68	2 (2.9)	1.0 (referent)	3 (4.4)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.75
	B	353	606	18 (3.0)	1.0 (0.1 to 7.4)	30 (5.0)	1.1 (0.3 to 4.8)	1.1 (0.5 to 4.7)	1.2 (0.4 to 3.3)	

(*Table continues*)

Table 2 (Continued).

Type	Lineage or sublineage	No. of women*	No. of positive visits	CIN2		CIN3		CIN2/3		
				No. (per 100 visits) (%)	OR (95% CI)	No. (per 100 visits) (%)	OR (95% CI)	OR (95% CI)	$OR_{adjusted}$ (95% CI)†	*P*‡
HPV68	E	125	191	5 (2.6)	1.0 (referent)	7 (3.7)	1.0 (referent)	1.0 (referent)	1.0 (referent)	.81
	C	60	109	6 (5.5)	2.2 (0.6 to 7.9)	4 (3.7)	1.0 (0.2 to 4.7)	1.5 (0.6 to 4.0)	1.1 (0.4 to 3.1)	
	F	48	92	1 (1.1)	-	7 (7.6)	2.1 (0.6 to 7.3)	1.4 (0.5 to 3.8)	1.1 (0.4 to 3.3)	
	G	11	16	1 (6.3)	-	2 (12.5)	3.9 (0.4 to 40.0)	3.4 (0.8 to 16.3)	2.6 (0.3 to 20.0)	
	A	10	14	1 (7.1)	-	1 (7.1)	-	-	-	
	B	4	5	0	-	0	-	-	-	
	D	7	10	0	-	2 (20.0)	-	-	-	

* A woman was counted more than once if she was positive for multiple non-human papillomavirus16/18 oncogenic types. - = not done, because lineages were detected only in 10 or fewer women or linked to ≤1 case. CI = confidence interval; CIN = cervical intraepithelial neoplasia; HPV = human papillomavirus; OR = odds ratio.

† Adjusted for age, race, current smoking status at enrollment, having a previous CIN2/3 treatment and visit number of the first type-specific positive detection.

‡ Two-sided Wald test for a null hypothesis of lack of the association for all lineages.

Table 3. Risk of cervical intraepithelial neoplasia associated with variants of single vs multiple human papillomavirus types

No. of types (variants)*	No. of positive visits	CIN2		CIN3		CIN2/3	
		No. (per 100 visits) (%)	OR (95% CI)	No. (per 100 visits) (%)	OR (95% CI)	OR (95% CI)	$OR_{adjusted}$ (95% CI)†
1 (non-HR)	1303	47 (3.6)	1.0 (referent)	50 (3.8)	1.0 (referent)	1.0 (referent)	1.0 (referent)
≥2 (non-HR)	98	2 (2.0)	0.6 (0.1 to 3.9)	4 (4.1)	1.0 (0.2 to 2.4)	0.8 (0.2 to 1.6)	0.8 (0.2 to 1.6)
1 (HR) and ≥1 (non-HR)	188	17 (9.0)	2.7 (1.5 to 4.7)	7 (3.7)	1.0 (0.3 to 2.0)	1.8 (1.1 to 2.8)	1.7 (1.0 to 2.7)
1 (HR)	1248	82 (6.6)	2.0 (1.3 to 2.9)	93 (7.5)	2.1 (1.5 to 3.1)	2.0 (1.6 to 2.7)	2.0 (1.5 to 2.6)
≥2 (HR)†	84	10 (11.9)	4.4 (2.1 to 9.3)	16 (19.1)	6.7 (3.4 to 11.7)	5.6 (3.4 to 8.8)	5.3 (3.1 to 8.4)

* Women could move from one exposure category to other(s) depending on infection status at follow-up visits. CI = confidence interval; CIN = cervical intraepithelial neoplasia; HR = high-risk variants, including human papillomavirus (HPV) 31 A/B, HPV33 A1, HPV45 A2/A3/B2, HPV56 A1/B and HPV58 A1/A3 variants; non-HR = non–high-risk variants, the rest variants of these five type; OR = odds ratio.

† Adjusted for age, race, current smoking status at enrollment, having a previous CIN2/3 treatment, and visit number of the first positive detection for any of these five types.

Table 4. Risk of cervical intraepithelial neoplasia associated with variants of the index human papillomavirus type with or without coexistence of variants of other types

Variants* of			CIN2		CIN3		CIN2/3	
Index type	Coexisting type(s)	No. of positive visits	No. (per 100 visits) (%)	OR (95% CI)	No. (per 100 visits) (%)	OR (95% CI)	OR (95% CI)	$OR_{adjusted}$ (95% CI)†
HPV31								
non-HR	none	282	15 (5.3)	1.0 (referent)	13 (4.6)	1.0 (referent)	1.0 (referent)	1.0 (referent)
non-HR	HR or non-HR	65	4 (6.2)	1.1 (0.3 to 3.1)	1 (1.5)	-	0.8 (0.2 to 1.7)	0.8 (0.2 to 1.8)
HR	non-HR	40	7 (17.5)	3.6 (1.1 to 9.9)	0	-	1.9 (0.6 to 4.3)	1.5 (0.5 to 3.8)
HR	none	439	35 (8.0)	1.6 (1.0 to 3.4)	43 (9.8)	2.3 (1.3 to 4.9)	2.0 (1.3 to 3.2)	1.7 (1.0 to 2.7)
HR	HR	45	5 (11.1)	2.8 (0.7 to 7.7)	10 (22.2)	6.5 (2.5 to 16.0)	4.5 (2.1 to 10.1)	3.8 (1.6 to 8.9)
HPV33								
non-HR	none	115	4 (3.5)	1.0 (referent)	7 (6.1)	1.0 (referent)	1.0 (referent)	1.0 (referent)
non-HR	HR or non-HR	28	3 (10.7)	3.3 (0.7 to 15.0)	1 (3.6)	-	1.6 (0.4 to 4.6)	1.8 (0.5 to 6.4)
HR	non-HR	33	2 (6.1)	1.8 (0.2 to 18.3)	2 (6.1)	1.0 (0.1 to 8.5)	1.3 (0.3 to 4.4)	1.3 (0.3 to 5.9)
HR	none	175	12 (6.9)	2.1 (0.6 to 8.1)	17 (9.7)	1.7 (0.7 to 4.4)	1.9 (1.0 to 4.1)	2.0 (0.8 to 4.7)
HR	HR	32	2 (6.3)	2.6 (0.3 to 25.9)	10 (31.3)	7.4 (2.5 to 21.8)	5.7 (2.3 to 15.0)	7.0 (2.2 to 23.0)
HPV45								
non-HR	none	251	9 (3.6)	1.0 (referent)	13 (5.2)	1.0 (referent)	1.0 (referent)	1.0 (referent)
non-HR	HR or non-HR	79	5 (6.3)	1.7 (0.5 to 5.7)	1 (1.3)	-	0.9 (0.2 to 2.0)	0.8 (0.3 to 2.3)
HR	non-HR	35	3 (8.6)	2.5 (0.7 to 8.7)	2 (5.7)	1.2 (0.2 to 8.6)	1.7 (0.5 to 3.9)	2.3 (0.8 to 6.5)
HR	none	212	14 (6.6)	1.9 (0.8 to 5.5)	11 (5.2)	1.0 (0.4 to 2.5)	1.4 (0.8 to 2.5)	1.6 (0.8 to 3.0)
HR	HR	40	4 (10.0)	3.4 (0.9 to 12.1)	6 (15.0)	3.5 (1.3 to 9.8)	3.5 (1.5 to 7.4)	3.5 (1.5 to 8.4)
HPV56								
non-HR	none	155	3 (1.9)	1.0 (referent)	1 (0.7)	-	1.0 (referent)	1.0 (referent)
non-HR	HR or non-HR	40	1 (2.5)	-	3 (7.5)	-	4.2 (0.8 to 22.7)	4.9 (1.0 to 24.6)
HR	non-HR	64	3 (4.7)	2.6 (0.5 to 11.5)	2 (3.1)	-	3.2 (0.8 to 15.3)	3.4 (0.9 to 13.1)
HR	none	326	9 (2.8)	1.5 (0.4 to 5.8)	12 (3.7)	-	2.6 (1.0 to 12.0)	2.6 (0.8 to 8.3)
HR	HR	40	7 (17.5)	12.1 (2.9 to 52.2)	4 (10.0)	-	14.3 (4.8 to 65.6)	13.6 (3.7 to 50.0)
HPV58								
non-HR	none	500	16 (3.2)	1.0 (referent)	16 (3.2)	1.0 (referent)	1.0 (referent)	1.0 (referent)
non-HR	HR or non-HR	138	5 (3.6)	1.2 (0.4 to 3.4)	7 (5.1)	1.6 (0.6 to 4.3)	1.4 (0.7 to 2.6)	1.4 (0.7 to 3.1)
HR	non-HR	16	2 (12.5)	4.5 (0.7 to 27.9)	1 (6.3)	-	3.4 (1.0 to 11.3)	5.4 (1.2 to 25.1)
HR	none	96	12 (12.5)	4.7 (2.1 to 10.5)	10 (10.4)	4.0 (1.6 to 9.7)	4.3 (2.3 to 8.0)	5.2 (2.5 to 10.9)
HR	HR	14	3 (21.4)	9.8 (2.3 to 42.0)	2 (14.3)	6.5 (0.7 to 57.1)	8.1 (2.3 to 30.7)	12.3 (3.0 to 49.9)

* Women could move from one exposure category to other(s) depending on infection status at follow-up visits. - = not done, because lineages (including the reference group of HPV56 for CIN3) were linked to ≤1 case; CI = confidence interval; CIN = cervical intraepithelial neoplasia; HR = high-risk variants, including HPV31 A/B, HPV33 A1, HPV45 A2/A3/B2, HPV56 A1/B and HPV58 A1/A3 variants; HPV = human papillomavirus; none = no coinfection; non-HR = non–high-risk variants, the rest variants of these five types; OR = odds ratio.

† Adjusted for age, race, current smoking status at enrollment, having a previous CIN2/3 treatment and visit number of first positive detection of the index type.

lesions attributable to HPV58 infection vary geographically. As shown by a meta-analysis (52), HPV58 was ranked as the third most common type in cases of cervical cancer and the second most common type in cases of high-grade cervical lesions in Asia, as compared with a worldwide rank of seventh and sixth, respectively. Among women with normal cytology, however, HPV58 was ranked as the fourth most common type, as compared with a worldwide rank of fifth (53). It is possible that differences in ranks in Asia as compared with those worldwide somewhat reflect the geographic disparity in HPV58-associated risk of cervical lesions, perhaps because of differences in distributions of HR variants. Supporting this notion is evidence that the A1 and A3 variants accounted for 16% and 35% of HPV58 infections in Asia, as compared with an average of 10% and 22%, respectively, worldwide (54).

To the best of our knowledge, the present report is one of the first, if not the first study, to comprehensively examine clinical outcomes of infection with variants of 11 non-HPV16/18 oncogenic types in a large-scale longitudinal setting. Unlike the majority of previous studies, which defined variants on the basis of single or a few polymorphisms of the HPV genome (21–24), viral isolates detected in this study were phylogenetically classified as lineages and/or sublineages. This provides a basis to view the variant as a distinct phylogenetic entity and better understand the oncogenic properties of this family of viruses. The findings of intratypic genomic diversity of non-HPV16/18 oncogenic types and lineage-associated risk of CIN2/3, particularly the added risk conferred by infection with HR variants of multiple types and the HPV16-like behavior of HPV58 lineage A1, could be important considerations in the development of prophylactic and therapeutic HPV vaccines.

Several limitations of the study should be addressed. In ALTS, HPV typing was performed only on cervical swab samples. Thus, the variant-related lesion was defined based on HPV status detected in a swab rather than biopsy sample. It is possible that some types might exist in swab samples but not in tissue samples (55), thereby resulting in overestimation of the variant-related lesion. However, no evidence suggests that this would have occurred differentially by lineage. Second, variant lineages were determined on the basis of the partial rather than the whole genome. Although this does not

Table 5. Risk of cervical intraepithelial neoplasia associated with high-risk variants of non–human papillomavirus16/18 oncogenic types as compared to HPV16 infection at enrollment

Type	Lineage or sublineage	No. of women*	No. of positive visits	CIN2		CIN3		CIN2/3	
				No. (per 100 visits) (%)	OR (95% CI)	No. (per 100 visits) (%)	OR (95% CI)	OR (95% CI)	$OR_{adjusted}$ (95% CI)†
HPV16	Any	822	1577	108 (6.9)	1.0 (referent)	295 (18.7)	1.0 (referent)	1.0 (referent)	1.0 (referent)
HPV31	A	124	230	21 (9.1)	1.2 (0.7 to 1.9)	22 (9.6)	0.5 (0.3 to 0.7)	0.7 (0.5 to 0.9)	0.6 (0.4 to 0.9)
	B	69	131	14 (10.7)	1.6 (0.8 to 2.7)	19 (14.5)	0.8 (0.4 to 1.2)	1.0 (0.7 to 1.4)	1.0 (0.6 to 1.6)
HPV33	A1	89	146	11 (7.5)	1.0 (0.4 to 1.9)	20 (13.7)	0.7 (0.4 to 1.2)	0.8 (0.5 to 1.2)	0.7 (0.5 to 1.2)
HPV45	A2	29	61	3 (4.9)	0.6 (0.2 to 1.5)	8 (13.1)	0.6 (0.2 to 1.3)	0.6 (0.3 to 1.2)	0.6 (0.3 to 1.1)
	A3	8	19	3 (15.8)	-	0	-	-	-
	B2	59	103	11 (10.7)	1.4 (0.7 to 2.6)	9 (8.7)	0.4 (0.2 to 0.7)	0.7 (0.4 to 1.1)	0.7 (0.4 to 1.1)
HPV56	A1	28	47	4 (8.5)	1.0 (0.3 to 2.4)	1 (2.1)	-	0.3 (0.1 to 0.7)	0.4 (0.1 to 0.8)
	B	146	231	15 (6.5)	0.8 (0.4 to 1.3)	11 (4.8)	0.2 (0.1 to 0.4)	0.4 (0.2 to 0.5)	0.4 (0.2 to 0.6)
HPV58	A1	35	61	9 (14.8)	2.4 (1.0 to 4.7)	11 (18.0)	1.0 (0.5 to 2.2)	1.4 (0.8 to 2.6)	1.5 (0.8 to 2.7)
	A3	13	25	6 (24.0)	3.4 (1.3 to 8.6)	0	-	0.9 (0.3 to 2.2)	1.3 (0.4 to 3.2)

* A woman was counted more than once if she was positive for multiple types compared. - = not done, because lineages were detected only in 10 or fewer women or linked to ≤1 case; CI = confidence interval; CIN = cervical intraepithelial neoplasia; HPV = human papillomavirus; OR = odds ratio.

† Adjusted for age, race, current smoking status at enrollment, and having a previous CIN2/3 treatment.

appear to cause misclassification of the lineages, as phylogenetic trees reconstructed using sequences of the target region displayed a topology similar to that based on whole genome sequences, some sublineages cannot be distinguished. It remains undetermined whether the variability in other regions linking to the unrecognized sublineages plays a role in defining consequences of the infection. Third, despite the fact that this study included the largest number of infections to date, the sample size for some lineages was too small to provide a reasonably precise estimate of risk. Fourth, because our findings are from a US-based study of consecutively enrolled women with a mildly abnormal Pap smear (18 years of age or older), the frequency of CIN2/3 among these women was higher than would be observed in other screening populations and the distribution of variants was likely different from that observed in other regions of the world. However, these differences do not affect the validity of our relative comparisons of lineage-associated risk of CIN2/3. Replication studies ideally would examine the full range of HPV infections, whether cytologically abnormal or normal. Lastly, while risk estimates for some variants were statistically significantly elevated relative to other variants of the same type, absolute numbers of CIN2/3 cases associated with these variants were not sufficiently large to warrant consideration of changing the format of clinically available HPV tests that detect HPV16 and HPV18 and the 11 high-risk HPV types evaluated in this study.

In summary, our data indicate that among women with non-HPV16/18 oncogenic types, risk of CIN2/3 differed by some but not all lineages, suggesting that for a given type of HPV, continued accumulation of nucleotide changes may alter genetic traits of the virus, leading to lineage-related variations in clinical outcomes. Analysis of infections at the level of lineages furthers our understanding of why the natural history of HPV infections is so variable.

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