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Ranking the attribution of high-risk genotypes among women with cervical precancers and cancers: a cross-sectional study in Ningbo, China

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Abstract

Background The region-specific importance of carcinogenic HPV genotypes is required for optimizing HPV-based screening and promoting appropriate multivalent HPV prophylactic vaccines. This information is lacking for Ningbo, one of the first cities of China's Healthy City Innovation Pilot Program for Cervical Cancer Elimination. Here, we investigated high-risk HPV (HR-HPV) genotype-specific distribution and attribution to biopsy-confirmed cervical intraepithelial neoplasia grade 2 or worse (CIN2+) before mass vaccination in Ningbo, China.

Methods A total of 1393 eligible CIN2+ archived blocks (including 161 CIN2, 1107 CIN3, and 125 invasive cervical cancers [ICC]) were collected from 2017 to 2020 in Ningbo. HR-HPV DNA was genotyped using the SPF_{10}-DEIA-$LiPA_{25}$ version 1 detection system and the SureX HPV 25X Genotyping Kit. Genotype-specific attribution to CIN2+ was estimated using a fractional contribution approach.

Results Ranking by the attributable proportions, HPV16 remained the most important genotype in both cervical precancers and cancers, accounting for 36.8% of CIN2, 53.2% of CIN3, and 73.3% of ICC cases. Among cervical precancers, HPV52 (17.3% in CIN2, 12.7% in CIN3) and HPV58 (13.9%, 14.9%) ranked second and third, while HPV33 (8.3%, 7.9%) and HPV31 (6.5%, 4.1%) ranked fourth and fifth, respectively. However, among ICCs, HPV18 (5.7%) accounted for the second highest proportion, followed by HPV33 (5.4%), HPV58 (4.0%), and HPV45 (3.2%). HPV18/45 together accounted for 46.8% of adenocarcinomas, which was slightly lower than that of HPV16 (47.7%). The remaining HR-HPV genotypes (HPV35/39/51/56/59/66/68) combined accounted for only 6.7% of CIN2, 2.9% of CIN3, and 4.2% of ICC.

Conclusions With Ningbo's strong medical resources, it will be important to continue HPV16/18 control efforts, and could broaden to HPV31/33/45/52/58 for maximum health benefits. However, different strategies should be proposed for other HR-HPV genotypes based on their lower carcinogenic risks.

Keywords Human papillomavirus, Cervical intraepithelial neoplasia, Cervical cancer, Genotype distribution, Attribution

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Introduction

Persistent infection with high-risk human papillomavirus (HR-HPV) is a prerequisite for the onset and progression of cervical precancers and cancers. Real-world studies have demonstrated the protective effects of prophylactic vaccination against major HR-HPV genotypes to precancerous lesions and cancer [1–3]. Meanwhile, randomized clinical trials have proven the efficacy of HPV-based cervical cancer screening in preventing invasive cervical carcinoma by detecting and treating its precursors, such as cervical intraepithelial neoplasia grade two (CIN2) and grade three (CIN3) [4, 5]. However, urgent issues regarding the clinical use of vaccination and HPV-based screening remains unsolved, including the appropriate choice of multivalent vaccines and optimal management of women with positive HPV results.

In China, five prophylactic HPV vaccines have already been on the market [6], including two bivalent vaccines targeting HPV16/18, one quadrivalent vaccine targeting HPV6/11/16/18, and one nonavalent vaccine targeting HPV6/11/16/18/31/33/45/52/58. For HPV-based testing, currently available commercial assays can typically detect 14 HR-HPV genotypes (HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68), either entirely or partially differentiated [7]. At the practical level, however, the risk of progression from infection to cervical precancer and cancer differs substantially by HR-HPV genotypes [8], and geographic heterogeneity in the importance of these specific carcinogenic genotypes also exists [9–12]. Therefore, to optimize vaccination and HPV-based screening strategies in a specific region, it is crucial to understand the HR-HPV genotype-specific contribution to high-grade cervical lesions and cancers there.

Ningbo is a coastal city located in Zhejiang Province, one of the most developed provinces in China. From 2011 to 2015, the average health resource distribution index of Zhejiang ranked just after that of Beijing, Shanghai, and Tianjin [13]. Within Zhejiang, Ningbo, along with Hangzhou, has the most concentrated health resources [14]. With its strong economy and abundant health resources, Ningbo is well positioned as a pioneering demonstration city for large-scale multivalent HPV vaccination and more precise HPV-based screening and management, ultimately leading to cervical cancer elimination. Since 2017, Ningbo has initiated free HPV-based screening for women aged 35–64 years [15], coinciding with the introduction of HPV vaccines in the same year. [16] However, vaccine coverage is still extremely low, at slightly above 5% among women aged 9 to 45 years in 2020. [17] Furthermore, no studies have reported the effectiveness of HPV-based screening in real-world settings in Ningbo.

Given the current situation, we described and estimated the HR-HPV genotype-specific distribution and attribution to biopsy-confirmed cervical intraepithelial neoplasia grade two or worse (CIN2+) collected from 2017 to 2020 in Ningbo. Our findings might provide valuable insights for policymakers on clinically significant HR-HPV genotypes and help optimize cervical cancer prevention and control interventions tailored to Ningbo and similar populations.

Methods

Study population

The study population consisted of women aged 16–50 years with CIN2+ diagnosed in Ningbo from 2017 to 2020. Clinical information was obtained from Ningbo Women & Children's Hospital. Archived formalin-fixed paraffin-embedded (FFPE) biopsy specimens and hematoxylin and eosin (H&E) stained slides from these women were obtained from the Ningbo Clinical Pathology Diagnosis Center for eligibility assessment. The Ningbo Clinical Pathology Diagnosis Center plays a crucial role in clinical pathology diagnosis for city-level medical institutions in Ningbo. Nearly all pathological specimens collected from clinical practices across Ningbo City were diligently preserved there. To ensure representative and well-preserved specimens, the inclusion criteria for biopsy specimens in this study were as follows: (1) CIN2+ blocks were available as part of routine clinical practice, (2) histological sectioning could be satisfactorily performed, and (3) only one block with the most severe diagnosis could be selected from each woman. This study was approved by the Institutional Review Boards (IRBs)/Ethics Review Committees (ERCs) of the Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS) (Approval No. 20/376-2572). Since only archived specimens and information were used, a waiver for informed consent was approved by the IRBs/ERCs of the CHCAMS for all included patients.

Pathology assessment, tissue sectioning, and diagnosis confirmation

Investigators in Ningbo first retrieved the list of eligible CIN2+ patients from their medical information system and obtained the corresponding archived H&E slides. These slides were then independently reviewed by at least two experienced histopathologists to assess the accuracy of the local diagnoses and preservation of the blocks. Blocks that were diagnosed with non-CIN2+ or had poorly preserved lesions were excluded. Eligible blocks were sectioned using a sandwich technique by local well-trained technicians. Briefly, the first and last paraffin sections were used for histopathological evaluation after H&E staining, while the intermediate paraffin sections were used for HPV DNA testing. Newly stained H&E slides and paraffin sections to be tested were delivered to

the central laboratory at the CHCAMS. The final diagnosis, confirming the presence of CIN2+ lesions, was based on newly stained H&E slides and was made by a senior histopathologist.

HPV DNA testing by SPF_{10}-DEIA-$LiPA_{25}$ version 1 detection system and SureX HPV 25X Genotyping Kit

HPV DNA was detected and genotyped by the SPF_{10}-PCR-DEIA-$LiPA_{25}$ version 1 detection system (DDL Diagnostic Laboratory, Rijswijk, the Netherlands) targeting a 65-bp region of the HPV L1 gene, according to the manufacturer's instructions. This detection system consists of two steps, which respectively depends on two kits. The first step is to detect the presence of HPV DNA using DNA ELISA kit HPV SPF10 version 1 (DEIA kit). DEIA kit can detect the presence of 44 HPV genotypes, including HPV3, 4, 5, 6, 7, 8, 11, 13, 16, 18, 26, 27, 30, 31, 32, 33, 34, 35, 37, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 65, 66, 67, 68, 69, 70, 71, and 74. The second step is to determine the specific genotype for those tested positive with DEIA kit using RHA Kit HPV SPF_{10}-$LiPA_{25}$ version 1 (LiPA kit). LiPA kit can differentiate 25 genotypes, including HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74 [18].

We additionally used the SureX HPV 25X Genotyping Kit (Health Gene Tech, Ningbo, China) on the remaining DNA extracts from 39 samples tested negative with DEIA kit and 21 samples tested positive with DEIA kit but negative with LiPA kit (detection results of these 60 samples under different systems are presented in the supplementary material), due to the inferior sensitivity of the SPF_{10}-PCR-DEIA-$LiPA_{25}$ version 1 detection system for certain HR-HPV genotypes [19, 20]. The SureX HPV 25X Genotyping Kit is a multiplex PCR test that targets the E6/E7 DNA regions of the HPV genome. This test can differentiate HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, 82 and 83.

Statistical analysis

Positive rate and its 95% confidence interval (CI) overall and for specific genotypes (i.e., HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68/73) were described for all CIN2 + cases and stratified by lesion severity. Genotype-specific positivity was defined as the detection of genotype-specific DNA by either SPF_{10}-PCR-DEIA-$LiPA_{25}$ version 1 detection system or SureX HPV 25X Genotyping Kit. Cochran-Armitage test was used to indicate trends in positive rates. Prevalence ratio was calculated for comparisons of positive rates between cervical precancers and cancers. Genotype-specific attribution to a certain lesion grade was calculated by the proportion of single-type infection + (the proportion of multiple-type infections × attribution factor), where attribution factor equals the fraction of single-type infection in that lesion grade caused by the genotype concerned [21]. All the statistical analyses were conducted with R (version 4.3.1; R Core Team, Vienna, Austria), and differences with a p value less than 0.05 were considered to indicate statistical significance.

Results

General characteristics

A total of 1393 women with confirmed CIN2+ blocks were included in the final analysis and underwent genotyping. The median age for all CIN2+ women at the time of diagnosis was 38.0, and the median age at menarche was 14.0. Over 95% were married and premenopausal, and approximately 64% of the women were from outpatient clinics.

We categorized these women into subgroups based on their cervical lesion severity, calendar year of diagnosis, and age at diagnosis. For lesion severity, 161 were confirmed as CIN2, 1107 as CIN3 (including 19 cases of adenocarcinoma in situ), and 125 as invasive cervical cancer (ICC). For age at CIN2 + diagnosis, 32.6% of these women were 16–34 years old, 44.5% were 35–44 years old, and the remaining were 45–50 years old. For calendar year of CIN2 + diagnosis, 532 women were diagnosed in 2017, 293 in 2018, 327 in 2019, and 241 in 2020 (Table 1).

HR-HPV genotype-specific distribution among different grades of cervical lesions

HR-HPV positive rates were high across all lesion severities (P_{trend} = 0.071, Table 2): 95.7% (95% CI 91.2–98.2%) for CIN2, 98.3% (95% CI 97.3–99.0%) for CIN3, and 98.4% (95% CI 94.3–99.8%) for ICC. Among women who tested positive for HR-HPV, proportion of single-type infection was the lowest in CIN2 at 87.0% (95% CI 80.7–91.9%) and the highest in ICC at 93.5% (95% CI 87.6–97.2%). No statistical correlation was found between cervical lesion severity and the proportion of single-type infections (P_{trend} = 0.052, Table 2).

Distribution of HR-HPV genotypes varied by lesion severity and histopathology. For lesion severity, the five most prevalent genotypes, in decreasing order, were HPV16, 52, 58, 31, and 33 for CIN2; HPV16, 58, 52, 33, and 31 for CIN3; and HPV16, 18, 33, 58, and 45 for ICC. For ICC histopathology, 71 were squamous cell carcinoma (SCC), 18 were adenocarcinoma (ADC), 32 were microinvasive carcinoma (MIC), and four could not be definitively classified by H&E slides. For SCC, the most common genotype was HPV16, followed by HPV33, 18, 51, and 58. For women with ADC, positive rates of HPV18 (Prevalence Ratio = 0.1 [0–0.4]) and 45 (0.1 [0–0.8]) were approximately ten times greater than those

Table 1 Demographic characteristics of women with CIN2 + lesions

Characteristics	Total (N = 1393)	CIN2 (N = 161)	CIN3[†] (N = 1107)	ICC (N = 125)	*P*[‡]
Age at CIN2 + diagnosis, years, n(%)					< 0.001
Median (P25, P75)	38.0 (33.0, 44.0)	38.0 (33.0, 43.0)	38.0 (32.5, 44.0)	43.0 (38.0, 47.0)	
16–34 yrs	454 (32.6)	61 (37.9)	378 (34.1)	15 (12.0)	
35–44 yrs	620 (44.5)	67 (41.6)	493 (44.5)	60 (48.0)	
45–50 yrs	319 (22.9)	33 (20.5)	236 (21.3)	50 (40.0)	
Age at menarche, years, n(%)					< 0.001
Median (P25, P75)	14.0 (13.0, 14.0)	14.0 (13.0, 14.0)	14.0 (13.0, 14.0)	14.0 (13.0, 14.0)	
12–13 yrs	457 (33.7)	70 (45.2)	348 (32.2)	39 (32.0)	
14–15 yrs	862 (63.5)	85 (54.8)	704 (65.2)	73 (59.8)	
> 15 yrs	38 (2.8)	0 (0.0)	28 (2.6)	10 (8.2)	
Missing	36 (2.6)	6 (3.7)	27 (2.4)	3 (2.4)	
Menopausal status, n(%)					0.897
Premenopause	1312 (96.7)	149 (97.4)	1047 (96.6)	116 (96.7)	
Perimenopause	29 (2.1)	2 (1.3)	25 (2.3)	2 (1.7)	
Postmenopause	16 (1.2)	2 (1.3)	12 (1.1)	2 (1.7)	
Missing	36 (2.6)	8 (5.0)	23 (2.1)	5 (4.0)	
Marital status, n(%)					0.238
Unmarried	38 (2.8)	2 (1.3)	33 (3.1)	3 (2.5)	
Married	1291 (95.8)	147 (97.4)	1031 (95.8)	113 (94.2)	
Separated/Divorced/Widowed	18 (1.3)	2 (1.3)	12 (1.1)	4 (3.3)	
Missing	46 (3.3)	10 (6.2)	31 (2.8)	5 (4.0)	
Patient source, n(%)					< 0.001
Outpatient	899 (64.5)	125 (77.6)	718 (64.9)	56 (44.8)	
Inpatient	499 (35.5)	36 (22.4)	389 (35.1)	69 (55.2)	
Calendar year at CIN2+ diagnosis, n(%)					< 0.001
2017	532 (38.2)	69 (42.9)	432 (39.0)	31 (24.8)	
2018	293 (21.0)	51 (31.7)	189 (17.1)	53 (42.4)	
2019	327 (23.5)	29 (18.0)	277 (25.0)	21 (16.8)	
2020	241 (17.3)	12 (7.5)	209 (18.9)	20 (16.0)	

CIN2, cervical intraepithelial neoplasia grade two; CIN3, cervical intraepithelial neoplasia grade three; ICC, invasive cervical cancer; P25, 25th percentile; P75, 75th percentile

[†] 19 cases of adenocarcinoma in situ were included

[‡] Fisher's exact test was used for age at menarche and menopausal status and Chi-square test was used for other variables

among women with SCC (Table 3). For women with MIC, HPV16 remained the most common genotype, followed by HPV33/52/58 and HPV31/56.

HR-HPV genotype-specific attributions to cervical precancers and cancers

Genotype-specific attributable proportions to cervical precancers and cancers are depicted in Fig. 1. HPV16 accounted for the highest proportion across all lesion severities, with 36.8% in CIN2, 53.2% in CIN3, and 73.3% in ICC. This was followed by HPV52 (17.3%), 58 (13.9%), 33 (8.3%), 31 (6.5%), and 18 (5.5%) in CIN2. The other genotypes were each responsible for less than 2% of CIN2 cases. The ranking was similar for CIN3, HPV16 were followed by HPV58 (14.9%), HPV52 (12.7%), HPV33 (7.9%), HPV31 (4.1%), and HPV18 (2.2%). The other genotypes were each responsible for less than 1% of CIN3 cases. However, the ranking differed for ICC. HPV18 and HPV33 had the second (5.7%) and third (5.4%) highest proportions of ICC, respectively, followed by HPV58 and HPV45. These four genotypes were each responsible for 3–6% of the ICCs, while the remaining genotypes were each responsible for less than 2% of the ICCs. In terms of ICC histopathology, HPV18 and 45 accounted for nearly half of the ADC cases, and the remaining cases were attributed to HPV16.

HR-HPV genotype-specific positive rates and attributable proportion to CIN2+ over calendar year

HR-HPV positive rate was relatively low in 2017 (96.2%) and then increased slightly afterwards (99.3% in 2018,

Table 2 HR-HPV genotype-specific positive rate[†] among women with CIN2+ lesions stratified by lesion severity (n [%, 95%CI])

HPV genotype	CIN2 (n = 161)	CIN3[‡] (n = 1107)	ICC (n = 125)	Total (n = 1393)	P_{trend}[§]	Prevalence ratio[¶] (ICC:HG)
HR-HPV[*]	154 (95.7 [91.2–98.2])	1088 (98.3 [97.3–99.0])	123 (98.4 [94.3–99.8])	1365 (98.0 [97.1–98.7])	0.071	–
Single-type infection[**]	134 (87.0 [80.7–91.9])	994 (91.4 [89.5–93.0])	115 (93.5 [87.6–97.2])	1243 (91.1 [89.4–92.5])	0.052	–
HPV16	61 (37.9 [30.4–45.9])	595 (53.7 [50.8–56.7])	92 (73.6 [65.0–81.1])	748 (53.7 [51.0–56.3])	**< 0.001**	**2.9 (1.9–4.5)**
HPV18	10 (6.2 [3.0–11.1])	31 (2.8 [1.9–4.0])	9 (7.2 [3.3–13.2])	50 (3.6 [2.7–4.7])	0.926	**2.1 (1.0–4.6)**
HPV31	15 (9.3 [5.3–14.9])	65 (5.9 [4.6–7.4])	3 (2.4 [0.5–6.9])	83 (6.0 [4.8–7.3])	**0.014**	0.4 (0.1–1.2)
HPV33	15 (9.3 [5.3–14.9])	101 (9.1 [7.5–11.0])	9 (7.2 [3.3–13.2])	125 (9.0 [7.5–10.6])	0.566	0.8 (0.4–1.6)
HPV35	4 (2.5 [0.7–6.2])	13 (1.2 [0.6–2.0])	0 (0 [0–2.9])	17 (1.2 [0.7–2.0])	0.055	–
HPV39	1 (0.6 [0.0–3.4])	4 (0.4 [0.1–0.9])	0 (0 [0–2.9])	5 (0.4 [0.1–0.8])	0.389	–
HPV45	1 (0.6 [0.0–3.4])	6 (0.5 [0.2–1.2])	4 (3.2 [0.9–8.0])	11 (0.8 [0.4–1.4])	**0.028**	**4.5 (1.3–15.8)**
HPV51	5 (3.1 [1.0–7.1])	16 (1.4 [0.8–2.3])	3 (2.4 [0.5–6.9])	24 (1.7 [1.1–2.6])	0.530	1.2 (0.4–4.3)
HPV52	32 (19.9 [14.0–26.9])	164 (14.8 [12.8–17.0])	3 (2.4 [0.5–6.9])	199 (14.3 [12.5–16.2])	**< 0.001**	**0.1 (0–0.4))**
HPV56	3 (1.9 [0.4–5.3])	6 (0.5 [0.2–1.2])	1 (0.8 [0–4.4])	10 (0.7 [0.3–1.3])	0.222	1.3 (0.2–10.9)
HPV58	24 (14.9 [9.8–21.4])	175 (15.8 [13.7–18.1])	6 (4.8 [1.8–10.2])	205 (14.7 [12.9–16.7])	**0.034**	**0.2 (0.1–0.5)**
HPV59	2 (1.2 [0.2–4.4])	3 (0.3 [0.1–0.8])	2 (1.6 [0.2–5.7])	7 (0.5 [0.2–1.0])	0.880	3.3 (0.6–17.5)
HPV66	2 (1.2 [0.2–4.4])	8 (0.7 [0.3–1.4])	0 (0 [0–2.9])	10 (0.7 [0.3–1.3])	0.222	–
HPV68/73	1 (0.6 [0.0–3.4])	6 (0.5 [0.2–1.2])	0 (0 [0–2.9])	7 (0.5 [0.2–1.0])	0.493	–

CIN2+, cervical intraepithelial neoplasia grade two or worse; CI, confidence interval; CIN2, cervical intraepithelial neoplasia grade two; CIN3, cervical intraepithelial neoplasia grade three; ICC, invasive cervical cancer; HG, high-grade cervical precancers (CIN2 and CIN3); HR-HPV, high-risk human papillomavirus

[†] Denominator was respectively all study cases

[‡] 19 cases of adenocarcinoma in situ were included

[§] Cochran Armitage Test. P_{trend} values are in bold for those less than 0.05

[¶] Logistic regression adjusting age. Prevalence ratio with a 95% CI that does not cross 1 are in bold

[*] DNA positive for at least one genotype of 14 high-risk HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68/73)

[**] Denominator was HR-HPV-positive cases

98.8% in 2019, 99.2% in 2020; $P_{trend} = 0.003$). HPV16, 52, 58, and 33 remained the most common genotypes regardless of calendar year. Specifically, HPV16 accounted for over 50% of CIN2 + cases each year (Table 4), followed by HPV52 and HPV58, which together accounted for nearly 30% of CIN2+. HPV33 alone accounted for 7% ~ 8% of CIN2+ annually.

Notably, attributable proportions of HPV18 and HPV31 changed over the calendar year. HPV18 accounted for 1.7% of CIN2+ in 2017 but increased to about 3–4% in 2018–2020. Conversely, HPV31 accounted for over 4% of CIN2+ from 2017 to 2019 but decreased to 1.8% in 2020. Additionally, Cochran-Armitage trend test revealed a statistically significant decrease in the positive rate of HPV31 over the four years ($P_{trend} = 0.018$), whereas no significant trend was observed for that of HPV18.

Discussion

The importance of HPV genotypes varies substantially depending on their carcinogenicity and geographic distribution. To advance cervical cancer prevention and control strategies in Ningbo, we determined the predominant HR-HPV genotypes among local women who were diagnosed with high-grade cervical precancers and cancers. The findings from our study can be summarized as follows: (1) HPV16 was the most prevalent genotype with the highest attribution in both cervical precancers and cancers; (2) HPV18 and 45 were similarly important in adenocarcinoma (ADC), together accounting for 46.8% of ADC; (3) HPV31/33/52/58 each ranked second to fourth in terms of attributable proportions in cervical precancers, collectively accounting for approximately 40% of precancers and over 10% of invasive cervical cancer (ICC); and (4) HPV35/39/51/56/59/66/68 individually accounted for less than 2% of precancers or cancers and collectively accounted for less than 5% of ICC. These findings could inform the prioritization of specific genotype to be targeted in Ningbo's future genotype-based interventions.

A meta-analysis summarizing the HPV genotype distribution among 8786 Chinese CIN women [22] showed that in CIN2/3, the overall HPV positive rate was 87%, and the top five HPV genotypes in descending order were HPV16 (45.7%), 58 (15.5%), 52 (11.7%), 33 (9.4%), and 31 (4.3%). Compared to this, our study had a higher HPV positive rate (98%) among CIN2/3 population, but the genotype distribution was completely consistent: HPV16 (51.7%), 58 (15.7%), 52 (15.4%), 33 (9.1%), and 31 (6.3%).

Table 3 HR-HPV genotype-specific positive rate[†] among women with ICC[‡] (n [%, 95%CI])

HPV genotype	SCC (n = 71)	ADC (n = 18)	MIC (n = 32)	Prevalence ratio§ (SCC:ADC)
HR-HPV[¶]	71 (100.0 [94.9–100.0])	17 (94.4 [72.7–99.9])	32 (100.0 [89.1–100.0])	–
Single-type infection*	68 (95.8 [88.1–99.1])	15 (88.2 [63.6–98.5])	29 (90.6 [75.0–98.0])	–
HPV16	55 (77.5 [66.0–86.5])	9 (50.0 [26.0–74.0])	27 (84.4 [67.2–94.7])	**3.3 (1.1–9.8)**
HPV18	3 (4.2 [0.9–11.9])	6 (33.3 [13.3–59.0])	0 (0 [0–10.9])	**0.1 (0–0.4)**
HPV31	2 (2.8 [0.3–9.8])	0 (0 [0–18.5])	1 (3.1 [0–16.2])	–
HPV33	7 (9.9 [4.1–19.3])	0 (0 [0–18.5])	2 (6.3 [0.8–20.8])	–
HPV35	0 (0 [0–5.1])	0 (0 [0–18.5])	0 (0 [0–10.9])	–
HPV39	0 (0 [0–5.1])	0 (0 [0–18.5])	0 (0 [0–10.9])	–
HPV45	1 (1.4 [0–7.6])	3 (16.7 [3.6–41.4])	0 (0 [0–10.9])	**0.1 (0–0.8)**
HPV51	3 (4.2 [0.9–11.9])	0 (0 [0–18.5])	0 (0 [0–10.9])	–
HPV52	1 (1.4 [0–7.6])	0 (0 [0–18.5])	2 (6.3 [0.8–20.8])	–
HPV56	0 (0 [0–5.1])	0 (0 [0–18.5])	1 (3.1 [0–16.2])	–
HPV58	3 (4.2 [0.9–11.9])	3 (16.7 [3.6–41.4])	2 (6.3 [0.8–20.8])	0.7 (0.1–7.4)
HPV59	0 (0 [0–5.1])	0 (0 [0–18.5])	0 (0 [0–10.9])	–
HPV66	0 (0 [0–5.1])	0 (0 [0–18.5])	0 (0 [0–10.9])	–
HPV68/73	0 (0 [0–5.1])	0 (0 [0–18.5])	0 (0 [0–10.9])	–

ICC, invasive cervical cancer; SCC, squamous cell carcinoma; ADC, adenocarcinoma; MIC, microinvasive carcinoma; CI, confidence interval; HR-HPV, high-risk human papillomavirus

[†] Denominator was respectively all study cases

[‡] The HPV infection status of the four other pathological types of ICC cases is not presented in the table. These four cases include: one case of poorly differentiated adenosquamous carcinoma infected with HPV59, and three case of carcinoma with uncertain pathology classification (one case infected with HPV16, one case infected with HPV59, and one case was HPV negative)

[§] Logistic regression adjusting age. Prevalence ratio with a 95% CI that does not cross 1 are in bold

[¶] DNA positive for at least one genotype of 14 high-risk HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68/73)

[*] Denominator was HR-HPV-positive cases

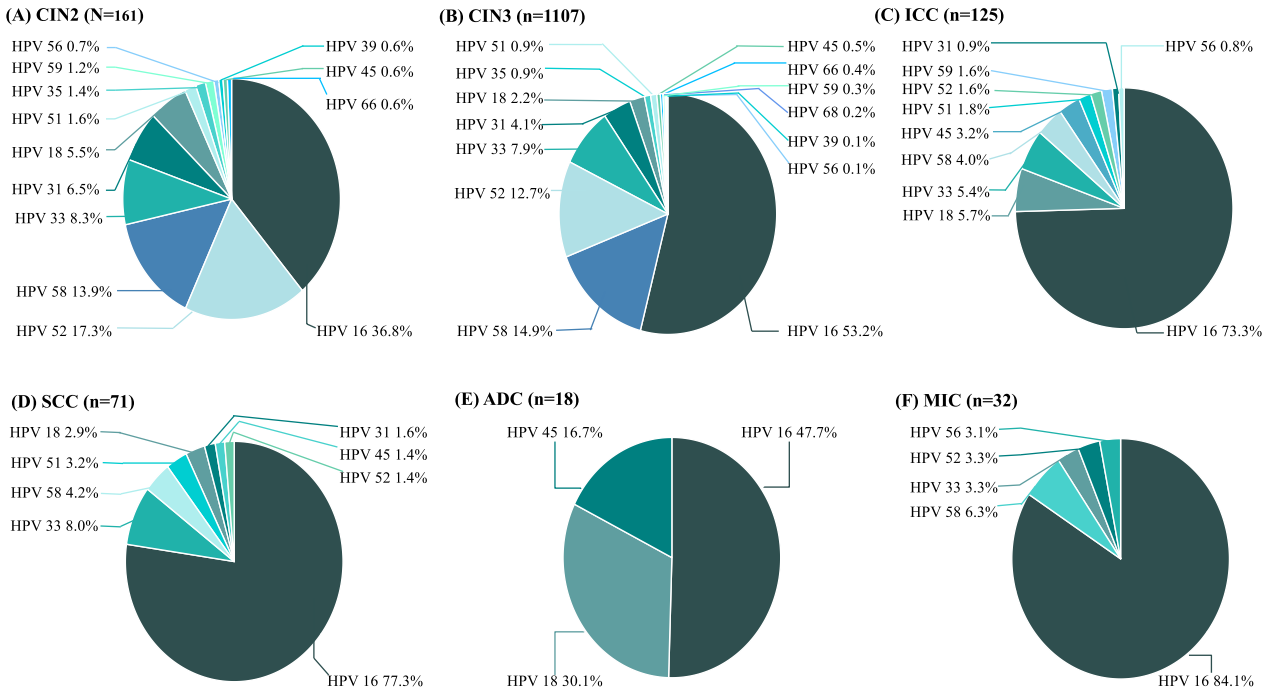

Fig. 1 Genotype-specific attributable proportions to cervical precancer and cancer. CIN2, cervical intraepithelial neoplasia grade two; CIN3, cervical intraepithelial neoplasia grade three; ICC, invasive cervical cancer; SCC, squamous cervical cancer; ADC, adenocarcinoma; MIC, microinvasive carcinoma; HPV, human papillomavirus. The attributable proportion for genotypes that does not present in the figure is zero

Table 4 HR-HPV genotype-specific positive rate and attribution among women with CIN2 + lesions stratified by calendar year

HPV genotype	2017 (n = 532)		2018 (n = 293)		2019 (n = 327)		2020 (n = 241)	
	Positive rate n (%)[†]	Attributable proportion	Positive rate n (%)[†]	Attributable proportion	Positive rate n (%)[†]	Attributable proportion	Positive rate n (%)[†]	Attributable proportion
HPV16	281 (52.8)	52.2	151 (51.5)	50.7	174 (53.2)	52.9	142 (58.9)	58.5
HPV18	11 (2.1)	1.7	19 (6.5)	4.7	11 (3.4)	3.2	9 (3.7)	3.1
HPV31	37 (7.0)	4.8	23 (7.9)	4.4	16 (4.9)	4.3	7 (2.9)	1.8
HPV33	46 (8.7)	7.1	32 (10.9)	9.6	27 (8.3)	7.9	20 (8.3)	6.8
HPV35	4 (0.8)	0.4	2 (0.7)	0.7	9 (2.8)	1.8	2 (0.8)	0.8
HPV39	2 (0.4)	0.2	0	0	2 (0.6)	0	1 (0.4)	0.4
HPV45	6 (1.1)	0.9	4 (1.4)	1.4	1 (0.3)	0.31	0	0
HPV51	8 (1.5)	0.8	5 (1.7)	0.8	8 (2.5)	1.7	3 (1.2)	1.2
HPV52	79 (14.9)	12.3	35 (12.0)	9.7	47 (14.4)	13.0	38 (15.8)	14.1
HPV56	2 (0.4)	0	4 (1.4)	0.4	3 (0.9)	0.6	1 (0.4)	0
HPV58	86 (16.2)	15.4	49 (16.7)	15.2	40 (12.2)	11.6	30 (12.5)	11.1
HPV59	2 (0.4)	0.4	3 (1.0)	1.0	2 (0.6)	0.6	0	0
HPV66	4 (0.8)	0	0	0	4 (1.2)	0.9	2 (0.8)	0.8
HPV68/73	1 (0.2)	0	3 (1.0)	0.7	1 (0.3)	0	2 (0.8)	0.4
Overall	–	96.2	–	99.2	–	98.7	–	99.0

CIN2+, cervical intraepithelial neoplasia grade two or worse; HR-HPV, high-risk human papillomavirus

[†] Denominator was respectively all study cases. The sum of the percentages of each HPV genotype is not necessarily equal to 100% because a result may be counted more than once in cases where the sampled lesion contained multiple HPV types

Numerical difference in positive rates should mainly be attributed to different HPV detection methods and sample types. Among the nine studies included in the meta-analysis, eight used exfoliated cell samples instead of the FFPE used in our study, and the HPV detection methods were primarily PCR-based with longer primers, such as MY09/11 and GP5/6.

We found four studies reported the HPV genotype distribution among women with CIN and cervical cancer in Zhejiang province, where Ningbo is located, during the periods of 2004–2006 [23], 2007–2008 [24], 2008–2013 [25], and 2012–2014 [26]. The samples tested in these studies were all exfoliated cells, and three of them used the HPV GenoArray Diagnostic Kit (HybriMax, Chaozhou Hybribio Limited Corp., Chaozhou, China), while the other study used an MY09/11 primers-based PCR method. Similarly, due to the differences in sample types and the use of more sensitive SPF_{10}-DEIA-$LiPA_{25}$ detection system [27], our study had the highest overall HPV positive rates both in ICC and in CIN2/3 compared to these studies. Our study describes the genotype distribution among women with CIN2+ diagnosed in 2017–2020. Although our study and the above four studies covered different time periods, HPV16, 18, 31, 33, 52, and 58 remained the common five genotypes found in women with ICC and CIN2/3. Among ICC, positive rate of these five genotypes across different studies were: 56–73.6% for HPV16, 7.2–12.2% for HPV18, 2.4–4.4% for HPV31, 1.7–7.2% for HPV33, 2.2–8.6% for HPV52, and 4.8–11.2% for HPV58; Among CIN2/3, the positive rates were respectively: 42.3–51.7% for HPV16, 3.2–5.2% for HPV18, 4.6–7.0% for HPV31, 8.4–12.8% for HPV33, 2.3–15.4% for HPV52, and 15.7–20.7% for HPV58. Except for HPV16, which was the most dominant genotype in ICC and CIN2/3 across all studies, the distribution of other genotypes varied slightly depending on the study. The positive rates of these genotypes generally did not fluctuate much over the years. The possible reasons for the numerical change, like the positive rate of HPV52 among CIN2/3, could be related to the sample size and the study population besides the detection methods and sample types.

Due to the shared mode of sexual transmission of all alpha HPV genotypes, the concurrent presence of multiple genotypes is common, especially among women younger than 25 years old and older than 65 years old. [28] It is challenging to determine which genotype is truly carcinogenic and warrants greater concern based solely on its positive rate in the population. From a virologic perspective, to be confirmed as a definite human carcinogen, one HPV genotype must be transcriptionally active in a tumor [29]. Previous studies have suggested that only a single genotype is transcriptionally active and pathogenic in lesions that tested positive

for multiple genotypes [21, 30]. Therefore, we adopted the fractional allocation method reported by Insinga et al., which assigns weights to the contribution of each genotype in muti-type infected lesions based on their presence as single-type infections. We then ranked the importance of individual HR-HPV genotypes by their estimated attributable proportions to cervical precancers and cancers to indicate which genotype should be of concern to health policymakers.

In Ningbo, HPV16 (alpha 9 species) could attribute to 77.3% of SCC during the period from 2017 to 2020, which is consistent with a nationwide study in 2009 reporting that HPV16 accounted for 76.7% of SCC [11]. The International Agency for Research on Cancer (IARC) also lists HPV16 as the foremost carcinogen among all carcinogenic HPV genotypes and reports that 60% of SCC could be singularly attributed to this genotype worldwide [31]. The above evidence highlights the importance of HPV16 and suggests that controlling HPV16 infection should remain a top priority in future large-scale vaccination and HPV-based cervical screening in Ningbo.

HPV18 and 45 were rare in women with cervical precancers but accounted for nearly 50% of ADC in our study. A retrospective cross-sectional worldwide study has suggested that ICC cases with HPV18 or HPV45 infection tended to present at an earlier age, indicating a shorter time for progression to invasive cancer, sometimes even without transformation through the preinvasive stages [32]. These two genotypes are also categorized with the second highest attributable risk of cancer by IARC [31]. Screening has been less effective in preventing adenocarcinomas than in preventing squamous cancers [33]. Given the sizable contribution of HPV18 and HPV45 to ADC, these two genotypes should not be overlooked in future genotype-based interventions.

Five non-HPV16 alpha 9 HR-HPV (HPV31/33/35/52/58) genotypes are categorized as the third highest attributable risk group by the IARC [31] and are at medium risk of developing cervical cancer [34]. A study analyzing HPV detection data from one million cervical samples in Belgium from 2006 to 2014 indicated that testing for HPV16/18 combined with HPV31, 33, 45, and 52 at certain viral load thresholds could predict 86.5% of cervical cancers occurring within a year after testing [35]. This study found comparable effectiveness by testing for all 14 HR-HPV genotypes, which predicted 89.4% of cervical cancers [35]. Additionally, the specificity also increased considerably in the former testing algorithm. However, the Belgium study did not find HPV58 to be predictive of any cervical cancer. This discrepancy could be attributed to regional differences in the distribution of HPV58, which has a much greater prevalence in high-grade cervical lesions in East Asia than in Europe [36].

Above non-HPV16 alpha 9 genotypes, excluding HPV35, are already covered by nonavalent vaccines. These genotypes (HPV31/33/52/58) combined accounted for a significant proportion of cervical precancers and ICCs in our study. However, HPV35 had a minimal contribution (1.4% in CIN2, 0.9% in CIN3, and zero in ICC) to cervical lesions. A population-based study in Ningbo similarly reported that only 0.5% of women who attended clinics from 2019 to 2021 were infected with HPV35 [37]. These findings suggest that controlling HPV31/33/52/58 infections might provide considerable additional protection for women in Ningbo, while HPV35 appears to be less important in both the general population and women with cervical lesions.

In 2022, the IARC reported that for women with cervical cancer, HPV39/51/56/59/66/68 have negligible attributable risk, each of which is responsible for less than 1% of cancers [31]. Notably, the estimated attributable risk for HPV66 was zero. These findings are consistent with our study of women with cervical cancer. However, the population-based study in Ningbo [37] revealed that the prevalence of these genotypes among all HPV-positive individuals reached as high as 25.9%. Current HPV testing assays mainly distinguish HPV16/18 separately but detect the universal gene region of the remaining 12 HR-HPV genotypes. Thus, in the general population, using HPV-based testing assays covering HPV39/51/56/59/66/68 will yield a high proportion of positive test results with a low probability of progression to cancer. We recommend that future HPV-based testing assays distinguish HPV16/18/31/33/45/52/58 and combine or exclude HPV39/51/56/59/66/68 for general population screening.

To the best of our knowledge, this study is the first to rank the importance of HR-HPV genotypes using biopsy-confirmed CIN2+ blocks and attributable proportion estimation in Ningbo. The data from our study could provide direct insights for upgrading current prevention and control measures. Second, HPV genotyping based on tissue specimens can more accurately indicate the pathogenic risk associated with HR-HPV genotypes. This is because exfoliated cell samples, which are commonly used for genotyping, represent comprehensive infections in the vagina-cervix areas, but most of these infections do not persist or progress to disease. However, the fixation process and long-term preservation of formalin-fixed paraffin-embedded blocks can cause nucleic acid degradation and fragmentation. This can potentially fail the detection of HPV DNA using PCR assays, resulting in reduced sensitivity of current PCR-based HPV genotyping assays

in tissues. To minimize the impact of DNA degradation and fragmentation on the genotyping results, we applied the SPF_{10}-DEIA-$LiPA_{25}$ detection system. This system targets the smallest amplicon compared to any available HPV DNA genotyping system and is particularly suitable for detection in tissues [27]. Meanwhile, we employed a sandwich sectioning technique and strict pathology confirmation process to ensure the sample's qualification for testing and reduce the possibility of sample loss.

One limitation of our study is that we provided HR-HPV genotyping data for only one city. China is a vast country with disparities in economic development and the allocation of medical resources among cities and regions. Due to regional variations in HR-HPV genotype-specific distribution, it is necessary to analyze region-specific HPV infection patterns to tailor cervical cancer prevention and control policies accordingly. Another limitation is that we reported cross-sectional information for HPV genotyping. When future large-scale vaccine programs are implemented, ongoing assessments will be needed to monitor any changes in HR-HPV genotype distribution and attribution over time to ensure efficient resource utilization.

In conclusion, it is important to prioritize HPV16/18 control efforts, and the focus could be broadened to HPV31/33/45/52/58 in resource-rich settings, such as Ningbo, for maximum health benefits. However, HPV39/51/56/59/66/68 should be considered with caution for HPV-based testing assays and vaccines due to their lower carcinogenic risks.

Abbreviations

ADC	Adenocarcinoma
CI	Confidence interval
CIN2	Cervical intraepithelial neoplasia grade two
CIN3	Cervical intraepithelial neoplasia grade three
CIN2+	Cervical intraepithelial neoplasia grade two or worse
FFPE	Formalin-fixed paraffin-embedded
H&E	Hematoxylin and eosin
HR-HPV	High-risk human papillomavirus
ICC	Invasive cervical cancer
MIC	Microinvasive carcinoma
SCC	Squamous cell carcinoma

Supplementary Information

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Additional file1

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Author contributions

F.Z. and S.H. conceived and designed the study. S.C. and S.H. accessed and verified all reported data. S.C., J.Y., and S.H. contributed to the analysis of this study. S.C. drafted the manuscript. All authors have critically revised the manuscript for intellectual content and approved the final version of the manuscript. All authors had final responsibility for the decision to submit for publication.

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Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Boards (IRBs)/Ethics Review Committees (ERCs) of the Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS) (Approval No. 20/376-2572). Since only archived specimens and information were used, a waiver for informed consent was approved by the IRBs/ERCs of the CHCAMS for all included patients.

Competing interests

Fanghui Zhao received grants from GlaxoSmithKline Biologicals, Merck & Co., and Xiamen Innovax Biotech to the Cancer Hospital/Chinese Academy of Medical Sciences to undertake clinical trials on the human papillomavirus (HPV) vaccine. Feng Guo and Susanne Hartwig are employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA. Susanne Hartwig owns stock in Merck & Co., Inc., Rahway, NJ, USA. The other coauthors declare no competing interests.

Availability of data and materials

No datasets were generated or analysed during the current study.

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