Infectious Agents and Cancer

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Risk of residual/recurrent cervical diseases in HPV-positive women post-conization depends on HPV integration status

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Abstract

Background It is crucial to identify post-operative patients with HPV infection who are at high risk for residual/recurrent disease. This study aimed to evaluate the association between HPV integration and clinical outcomes in HPV-positive women after cervical conization, as well as to identify HPV integration breakpoints.

Methods This retrospective study analyzed data of 791 women who underwent cervical conization for cervical intraepithelial neoplasia grades 2–3 (CIN2-3) between September 2019 and September 2023, sourced from the Fujian and Hubei cervical lesion screening cohorts. Among these, 73 women with HPV infection post-conization underwent HPV integration test within 3 months after a positive HPV test. HPV integration test was performed using the high-throughput viral integration detection (HIVID), a sensitive method for genome-wide survey of HPV integration breakpoints.

Results Among the 73 participants with HPV infection post-conization, 10 cases (13.7%) were positive for HPV integration. The logistic regression analysis showed a higher residual/recurrent lesions risk in patients with HPV integration (OR = 3.917, $p = 0.048$). According to the Kaplan-Meier analysis, age ≥ 45 years ($p = 0.016$) and HPV integration ($p = 0.035$) were associated with a higher risk of residual/recurrent CIN at the 1-year follow-up. HPV 52 accounted for the majority of HPV integration genotype (3/10, 30.0%). Surprisingly, HPV 16 had the highest number of HPV average integration sequencing reads ($n = 129$), followed by HPV 31, 58, 52, 59, 35, and 39. The study also identified 13 HPV breakpoints, including TP63, TLR4, USP10, etc.

Conclusions HPV integration was identified as an independent risk factor for residual/recurrent CIN in HPV-positive women post-conization. Women with positive HPV integration should pay attention to careful post-treatment follow-up.

Keywords HPV integration, Cervical intraepithelial neoplasia, Post-treatment surveillance

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Background

Cervical cancer is a fully preventable disease but is the leading cause of cancer death in 36 low-income and middle-income countries (LMICs) [1, 2]. Alarmingly, more than 600,000 women worldwide were diagnosed with cervical cancer in 2020 [3]. The main cause of cervical cancer and its precursors is persistent infection with human papillomaviruses (HPV) [4]. The lifetime probability of HPV infection in sexually active adults is reported to be approximately 85–90% [5]. In most cases, HPV infection is cleared spontaneously by the human immune system within 1–2 years [5]. Approximately 10% of infected individuals develop persistent infection. HPV persist in squamous cells and replicate throughout the cell cycle through the epithelium [5]. The process from high-risk HPV (HR-HPV) persistent infection to cervical precancerous lesions and then to cervical cancer usually takes 10–20 years. Therefore, appropriate screening and treatment of cervical precancerous lesions play vital roles in preventing and eliminating cervical cancer.

According to the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines and WHO recommendations (screen, triage and treat approach) [6, 7], primary HPV screening programs for cervical cancer have been implemented in China using a cytology triage. The recommended treatment for cervical intraepithelial neoplasia 2 or worse (CIN2+) involves colposcopy coupled with one of two cervical conization procedures: (1) loop electrosurgical excision procedure (LEEP) or (2) cold knife conization (CKC) [6]. Both the 2019 ASCCP guidelines and the Chinese Expert Consensus on Cervical Cancer Screening and Abnormal Management recommend HPV-based testing at 6 months after conization in CIN2-3 patients [6, 8]. However, approximately 10% of patients with negative margins still have a risk of persistent HPV infection after conization [9], and 5–25% of patients suffer from recurrent or persistent high-grade lesions after conization [10, 11]. In particular, those who are positive for HPV 16 are prone to persistent HPV infection after conization [9]. It is acceptable for high-grade squamous intraepithelial lesion (HSIL) patients with HR-HPV infection to undergo reconization. However, only 17.1-36.4% of the secondary surgical pathologic findings were confirmed as residual/recurrent CIN2+ [9, 12]. Accordingly, an HPV test result-driven screening program would inevitably lead to high false-positive rates, which would result in unnecessary colposcopies and cervical biopsies. Therefore, a test that can accurately predict recurrent/residual disease after conization is urgently needed.

HPV DNA integration into the host genome is considered one of the most important risk factors for cervical carcinoma development. Following infection, the virus can remain in its episomal form or be integrated into the host genome. Compared with the half-life of episomal transcripts, the longer half-life of integrated viral transcripts favors their immortalization and transformation into cancer cells [13]. In 2015, whole-genome sequencing and high-throughput viral integration methods identified as many as 3667 HPV integration breakpoints in 26 CINs, 104 cervical carcinomas and 5 cell lines [14]. HPV integration breakpoints occurred in 97.8% of the cervical cancer samples with HPV infection [15]. However, the integration breakpoints of post-operative HPV infection remain unclear.

It remains unclear whether HPV integration increases the risk of residual/recurrent lesions among HPV-positive women post-conization. Therefore, this study aimed to retrospectively identify the HPV integration breakpoints and evaluate the association between HPV integration and clinical outcomes in HPV-positive women post-cervical conization.

Methods

Study population

All participants were drawn from the Fujian and Hubei cervical lesion screening cohorts, with the study running from September 2019 to September 2023. The participants were eligible when the following inclusion criteria were satisfied: (1) histologically confirmed CIN2-CIN3; (2) underwent cervical conization; (3) with HPV infection after conization; (4) performed HPV integration test within 3 months after the positive HPV test; (5) informed consent. The exclusion criteria are shown below: (1) underwent hysterectomy; (2) history of other malignancies or immune system diseases. Finally, a total of 73 participants with HPV infection after cervical conization underwent a 1-year follow-up (Fig. 1). This study was approved by the Ethics Committees of the Fujian Maternity and Child Health Hospital (No: 2021KLR09009), and all participants provided signed informed consent.

Follow up

Clinical management in our study was based on the 2019 ASCCP guidelines [6]. All women with histologically diagnosed CIN2-CIN3 who underwent conization were tested for HPV test and cervical cytology at 6-month intervals during the initial 2 years following conization. Among them, 73 women with a positive HPV test underwent HPV integration test within 3 months. Then follow-up visits were conducted at 6-month intervals, totaling two rounds of screening. According to the guidelines issued by the Chinese Society of Colposcopy and Cervical Pathology [16], colposcopy and biopsy were performed if any abnormal results were present in post-conization women. No recurrent/residual CIN found after conization was defined as completely negative results of cervical biopsy under colposcopy.

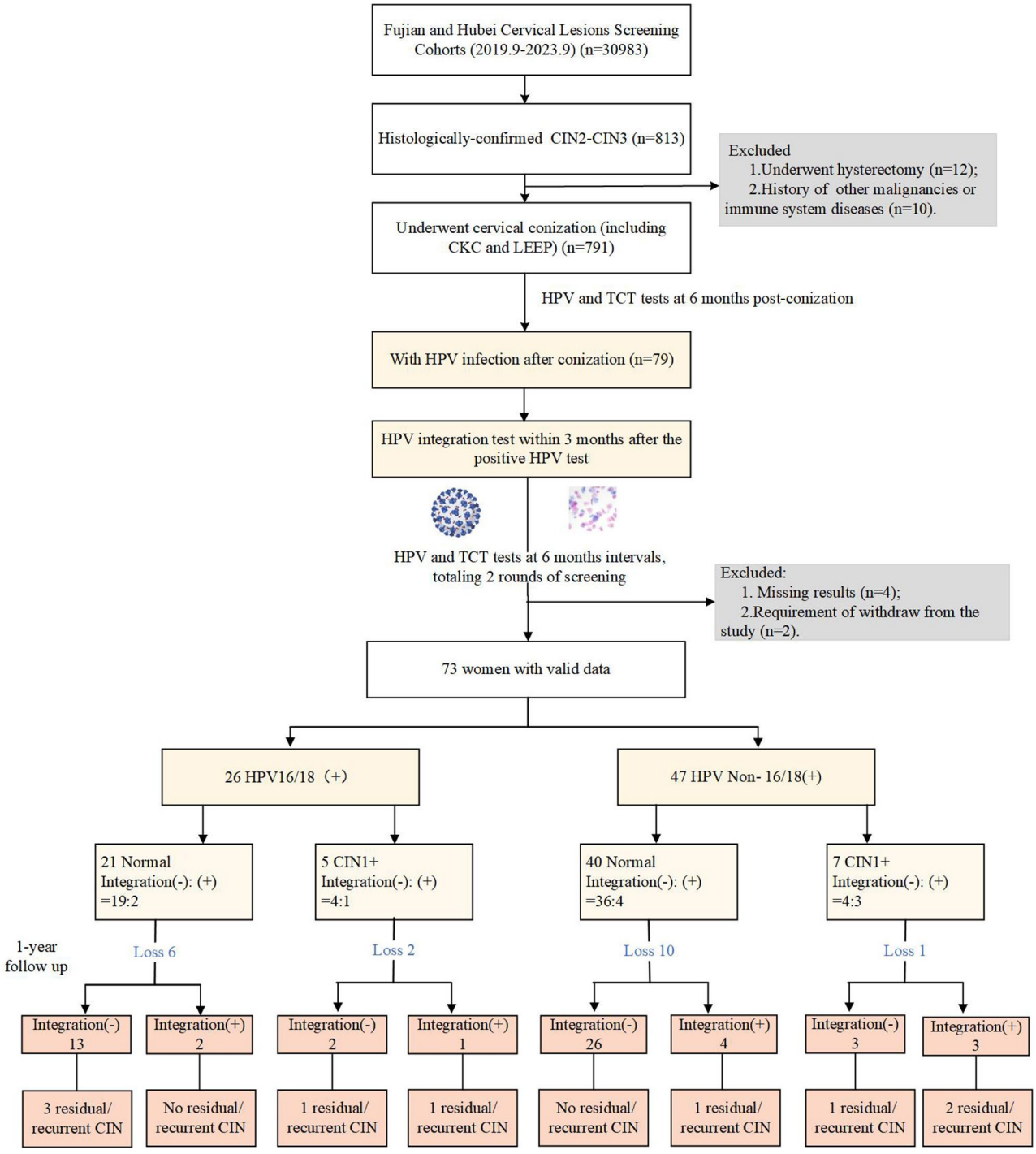

Fig. 1 Flowchart of the Study Protocol. The study included women with histologically confirmed CIN2-CIN3 who underwent cervical conization. Among these, women with HPV infection post-conization were tested for HPV integration within 3 months. Then follow-up visits were conducted at 6-month intervals, totaling two rounds of screening. The flowchart details the distribution of HPV integration results and subsequent outcomes at the 1-year follow-up. No recurrent/residual CIN after conization was defined by completely negative cervical biopsy results under colposcopy. Abbreviations: LEEP, loop electrosurgical excision procedure; CKC, cold knife conization; HPV, human papillomavirus

Specimen collection and management

Cervical samples were collected with a Cervex-Brush by gynecologists. Cervical specimens were placed in a vial containing 20 mL ThinPrep® PreservCyt® medium (Hologic, Waltham, MA, USA) in accordance with standard guidelines for HPV integration test and cytology examination [17]. The samples for HPV testing need to be stored at -20 °C before DNA extraction, and the samples for cytology need to be stored at 4 °C.

Polymerase chain reaction-reverse dot blot (PCR-RDB) HPV genotyping test

The PCR-RDB HPV genotyping kit (YaNeng Biosciences, Shenzhen, China) [18] can detect 23 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, 83, 6, 11, 42, 43, and 81). This method and kit have been approved by the China Food and Drug Administration (Approval number 20,020,515). The procedures were conducted according to the manufacturer's instructions.

Liquid-based cytology

All liquid-based cytology specimens were analyzed using the Bethesda System (TBS) [19], independently by two experienced cytopathologists. The results were classified based on the TBS grading system as cervical atypia squamous cells with undetermined significance (ASC-US), atypical squamous cells-cannot rule out high-grade lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), HSIL, squamous cervical cancer (SCC), atypical glandular cells (AGC) and adenocarcinoma. If the diagnosis was different, the cervical samples were evaluated again, and a consensus diagnosis was obtained.

HPV integration test

HPV integration test was performed using the high-throughput viral integration detection (HIVID), a sensitive method for genome-wide survey of HPV integration breakpoints [14]. According to the manufacturer's instructions, DNA was extracted from cervical exfoliated cells in the 20 mL ThinPrep® PreservCyt® medium (Hologic, Waltham, MA, USA) using the TIANamp Genomic DNA Kit (No: 3304-9). Briefly, the methodology involved designing sequence-capture probes targeting 18 distinct HPV genome sequences (types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82). The HIVID pipeline was used for breakpoint identification. PCR and Sanger sequencing were utilized to verify all potential HPV integration breakpoints. The sequences of the fusion genes were characterized using the NCBI human MEGA-BLAST database alignment tool.

Histology

Women who were HPV-16/18 positive with or without abnormal cytological results (with a grade higher than ASC-US) needed to be referred for colposcopy and punch biopsy. Women with a punch biopsy diagnosis greater than HSIL underwent LEEP and CKC. Specimens were then fixed in 10% formalin, and march paraffin embedding was performed. Subsequently, 4-μm-thick histological sections were cut and stained with hematoxylin and eosin. In accordance with atypical hyperplasia, cells were accounted for in the range of squamous epithelium full-thickness and were histologically examined and classified according to the Lower Anogenital Squamous Terminology (LAST) system and CIN system [20, 21].

Statistical analysis

The measurement data are presented as the mean ± standard deviation in this study. The associations of reproductive history, type of HPV infection, and pathology results with HPV integration status were assessed by the chi-square test or Fisher's exact test. The logistic regression was used to identify independent risk factors associated with recurrence/residual disease. The hazard ratios (HR) were calculated. The forest plots for the regressions above were plotted by GraphPad Prism v9.4.1, along with the Kaplan–Meier curve using the log-rank test. The data analysis was conducted with SPSS 22.0 software (IBM Corporation, Armonk, NY, USA). P values less than 0.05 were considered to indicate statistical significance, and all tests were two-sided.

Results

Clinical features in participants with post-conization HPV infection

Among the 73 participants with post-conization HPV infection, 10 cases (13.7%) were positive for HPV integration. The average age of women with HPV integration was 37.80 ± 9.80 years old, and the average age of women without HPV integration was 40.41 ± 10.51 years old. In addition, 11 cases (15.1%) were postmenopausal women. Among the types of post-operative HPV infection, 26 cases (35.6%) were positive for HPV16/18 and 47 cases (64.4%) were non-16/18HPV infection. The rate of residual/recurrent CIN at 1 year was significantly higher in the HPV integration group (4/10, 40.0%) compared to the HPV non-integration group (5/44, 11.4%), with a p-value of 0.028 (Table 1).

Distribution of HPV integration sequencing reads according to HPV genotypes

HPV 52 occupied the most integration sites among post-treatment women (3/10, 30.0%), followed by HPV 16 (2/10, 20%), HPV 31 (1/10, 10%), HPV 35 (1/10, 10%), HPV 39 (1/10, 10%), HPV 58 (1/10, 10%), and HPV 59 (1/10, 10%). Unexpectedly, HPV 16 had the highest number of HPV average integration sequencing reads

Table 1 Characteristics of patients according to the HPV integration status

Characteristics		HPV integration (*n* = 10)	HPV non-integration (*n* = 63)	*p*
Age		37.80 ± 9.80	40.41 ± 10.51	0.846
HPV vaccination, n [%]	Yes	0 (0.0)	12(19.0)	/
	No	10(100.0)	51(81.0)	
Reproductive history, n [%]	Yes	5(50.0)	48(76.2)	0.085
	No	5(50.0)	15(23.8)	
Reproductive needs, n [%]	Yes	6(60.0)	24(38.1)	0.191
	No	4(40.0)	39(61.9)	
Endocervical gland invasion, n [%]	No invasion	6(60.0)	47(74.6)	0.278
	Invasion	4(40.0)	16(25.4)	
Margin status, n [%]	Negative	8(80.0)	58(92.1)	0.229
	Positive	2(20.0)	5(7.9)	
Post-operative type of HPV infection, n [%]	Single infection	6(60.0)	49(77.8)	0.226
	Multiple infections	4(40.0)	14(22.2)	
Post-operative HPV genotype, n [%]	HPV-16/18(+)	3(30.0)	23(36.5)	0.493§
	Non-16/18 HPV(+)	7(70.0)	40(63.5)	
Residual/recurrent diseases at 1 year, n [%]	No Residual/ recurrent CIN	6(60.0)	39(88.6)	0.028
	Residual/recurrent CIN	4(40.0)	5(11.4)	

Footnotes: No recurrent/residual CIN found after conization was defined as completely negative results of cervical biopsy under colposcopy. § is Fisher's exact probability method, and the rest are all performed via the chi-square test

(*n* = 129), followed by HPV 31, 58, 52, 59, 35, and 39 (Fig. 2a).

HPV integration was an independent risk factor for residual/recurrent lesions

The median follow-up time from conization to the HPV integration test was 6.48 months, to the first round of screening after the integration test was 12.27 months, and to the second round of screening after the integration test was 19.09 months. 40% of women with post-operative HPV integration experienced residual/recurrent lesions, whereas only 11.4% of women without HPV integration had residual/recurrent lesions at 1-year endpoint (Fig. 2b-c).

Further logistic regression analyses showed a higher risk of residual/recurrent lesions in patients with HPV integration (OR = 3.917, $p = 0.048$) (Fig. 3a). In addition, we found that the recurrent/residual rate of CIN in patients ≥ 45 years old was significantly higher than that in patients <45 years old ($p = 0.033$) (Table 2). The rate of recurrent/residual CIN had no significant correlation with HPV genotypes. At the end of 12 months, 88.6% (39/44) women without HPV integration experienced no recurrent/residual CIN, compared to only 60.0% (6/10) of women with HPV integration. HPV integration ($p = 0.028$) was a risk factor for residual lesions in HPV-positive women after cervical conization. The Kaplan–Meier analysis showed age ≥ 45 years ($p = 0.016$), HPV integration ($p = 0.035$) had a higher recurrent/residual rate of CIN (Fig. 3b-f).

Sensitivity and specificity of residual/recurrent diseases

We calculated sensitivity and specificity of residual/recurrent diseases for HPV integration test alone and HPV&TCT co-testing (Table 3). The HPV&TCT co-testing after cervical conization had a higher sensitivity (91.67%). In contrast, HPV integration test had a higher specificity (90.2%). HPV integration test increased the specificity for residual/recurrent diseases.

Distribution of the HPV integration sites in the host chromosome and genes

We identified 13 integration sites among 10 women with HPV integration after cervical surgery (Fig. 4). Chromosomes 3 (*n* = 2), Chromosomes 16 (*n* = 2) and chromosomes 9 (*n* = 2) were identified as the most common HPV integration sites in our study. There were 13 types of HPV breakpoints, including the *ZNF670-ZNF695* gene, *LINC02237*, *TP63*, *GABARAP*, *CCK*, *LYZL4*, *PTPRD* and *USP10* in women with abnormal pathology results, *TRIML1*, *LINC01060*, *TLR4*, *LINC02578* and *GABARAPL2* in normal pathology group.

Discussion

Although HPV infection after CIN2 + treatment is an independent risk factor for the recurrence and progression of lesions, not all women with HPV infection will be confirmed as residual/recurrent CIN2+. Hence, evaluating the association between HPV integration and residual/recurrent CIN in HPV-positive women after cervical conization is of great significance. Age ≥ 45 years, HPV integration were risk factors for residual/recurrent

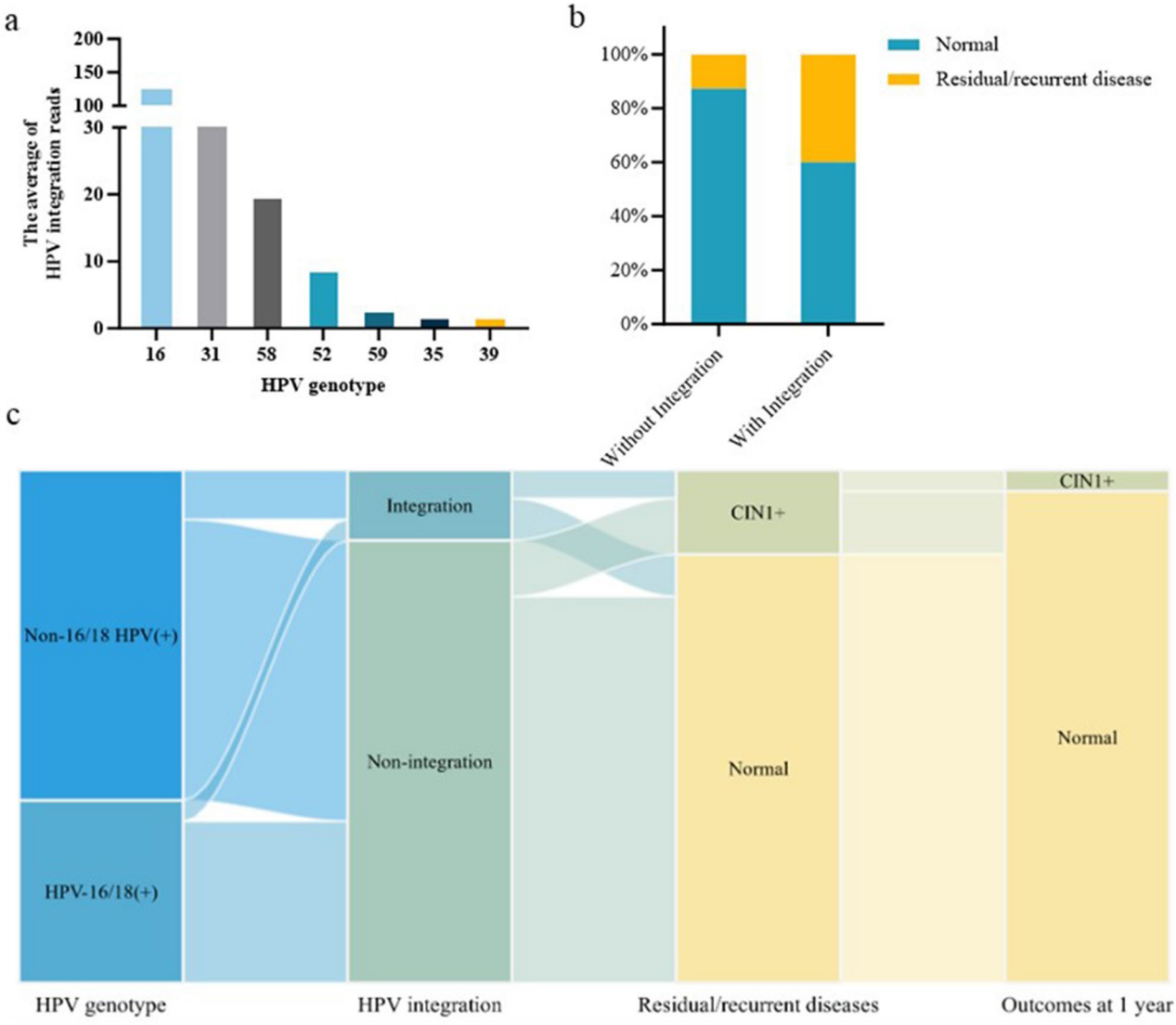

Fig. 2 Distribution of HPV genotypes, HPV integration status, and residual/recurrent lesions. (**a**) The average number of HPV integration reads detected in women with post- conization HPV infection. HPV16 had the highest number of reads across 10 samples followed by HPV (31, 58, 52, 59, 35, and 39) in decreasing order of their read counts. (**b**) Incidence rate of residual/recurrent lesions in women with HPV integration. (**c**) Sankey diagram illustrating the transitions between HPV genotypes, HPV integration status, and residual/recurrent lesions in post-conization women. The width of the flow lines represents the proportion of women transitioning from one category to another

lesions in HPV-positive women after cervical conization. The study also identified 13 types of integration genes in women with HPV infection after cervical conization.

Age is a critical factor influencing the clearance of HPV and the recurrence of lesions. Previous studies have shown that patients younger than 25 years old have a higher likelihood of cervical lesion regression [22], whereas the persistence of HPV infection after treatment is more common in senior women, with age being a significant risk factor for persistent infection [23]. Our study demonstrated that at the 12-month follow-up, the recurrent/residual rate of CIN in patients ≥ 45 years old was significantly higher than that in patients <45 years old. Another study identified that being aged 45 or older (OR = 3.5, 95% CI = 1.3–9.9) is an independent predictor of recurrent CIN within 6 years after treatment [24]. Therefore, our findings support the recommendation [25, 26] that post-menopausal women with high-grade CIN should undergo more extensive and thorough excisions and be closely monitored during follow-up. This could be attributed to the lower immune function in senior women compared to younger women [27], which partly explains the lower regression rate of pre-cancerous lesions with increasing age.

In recent years, the literature has demonstrated that HPV vaccination could have a significant protective effect in women surgically treated for HPV disease and could also impact disease recurrence. The SPERANZA project,

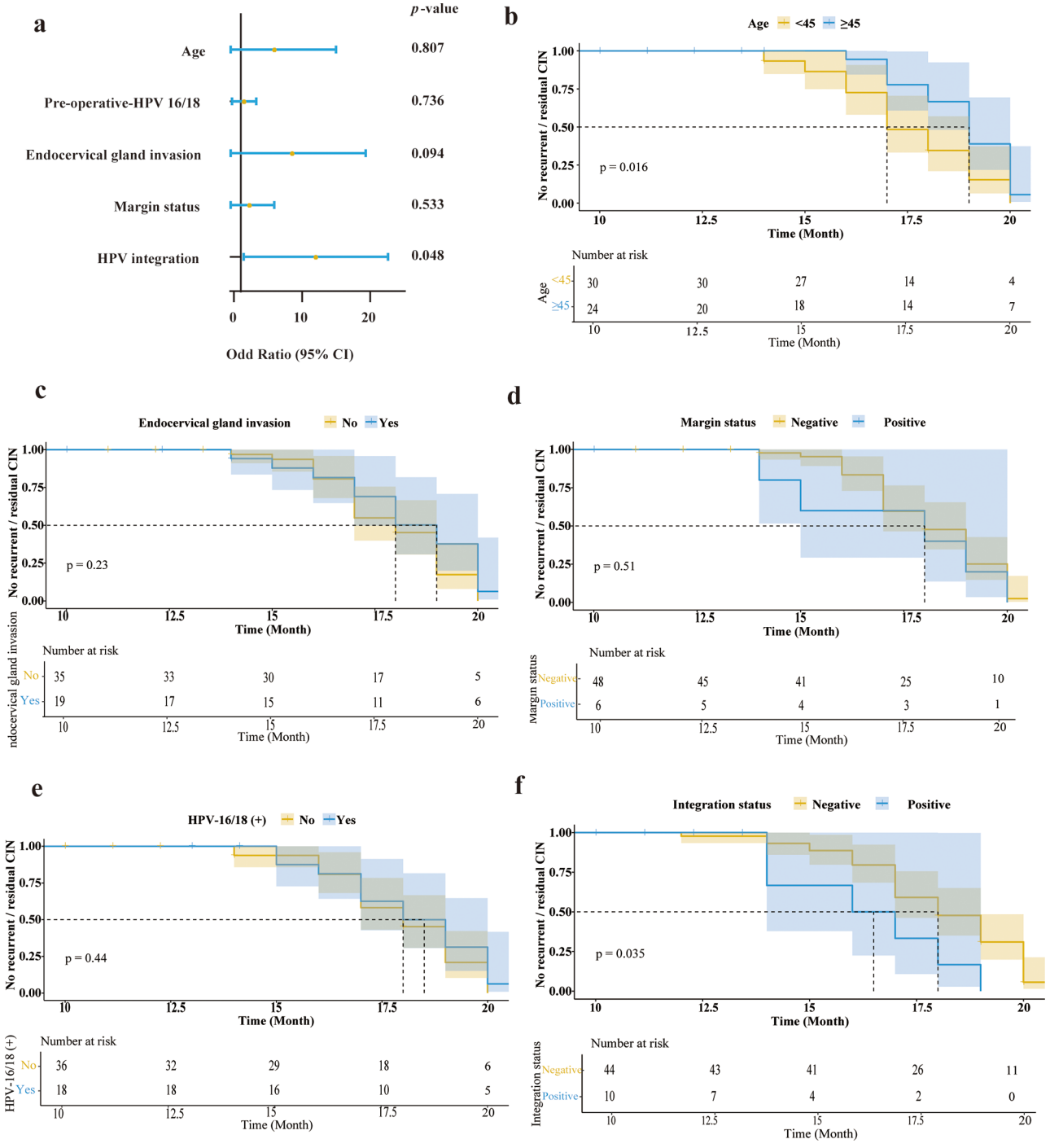

Fig. 3 HPV integration was related to residual/recurrent CIN. (**a**) Forest plot of the OR for residual/recurrent CIN based on logistic regression analysis. (**b-f**) Kaplan–Meier curves showing differences in residual/recurrent CIN rates between different groups

a prospective case-control study, reported a reduction in disease recurrences (CIN2+) in the vaccinated group following surgical treatment [28]. However, due to the low coverage rate of the HPV vaccine in China, the proportion of vaccinated women in the population enrolled in this study was relatively small. Further analysis of the effect of HPV vaccination on HPV integration status is warranted in future research.

HPV integration into the host genome is a critical etiological event in cervical carcinogenesis and progression [29]. Analysis of data from The Cancer Genome Atlas showed that HPV integration occurs in > 80% of HPV-positive cervical cancers [30]. In HPV 16 positive

Table 2 The outcomes in patients with HPV infection post-treatment according to the characteristics ($N=54$)

Characteristics		6 months follow-up			12 months follow-up		
		No recurrent /residual CIN	Recurrent /residual CIN	*p*	No recurrent /residual CIN	Recurrent /residual CIN	*p*
Age	<45	29(61.7%)	1(14.3%)	0.036	28(62.2%)	2(22.2%)	0.033
	≥45	18(38.3%)	6(85.7%)		17(37.8%)	7(77.8%)	
HPV genotypes	HPV-16/18(+)	16(34.0%)	2(28.6%)	0.775	15(33.3%)	3(33.3%)	1.000
	Non-16/18 HPV(+)	31(66.0%)	5(71.4%)		30(66.7%)	6(66.7%)	
Integration status	Without integration	41(87.2%)	3(42.9%)	0.005	39 (86.7%)	5(55.6%)	0.028
	With integration	6(12.8%)	4(57.1%)		6(13.3%)	4(44.4%)	

Notes: 54 cases from the population had a complete one-year follow-up. No recurrent/residual CIN found after conization was defined as completely negative results of cervical biopsy under colposcopy

Table 3 The performance of HPV&TCT co-testing and HPV integration test for identifying residual/recurrent CIN

	HPV & TCT co-testing % (95% CI)	HPV integration test % (95% CI)
Sensitivity	91.67% (60.0-99.5%)	33.3% (11.2-64.5%)
Specificity	14.8% (7.3-29.5%)	90.2% (79.1-96.0%)
PPV	17.5% (9.4-30.9%)	40.0% (13.7-72.6%)
NPV	90.0% (54.11-99.48%)	87.3% (76.0-94.0%)

Footnotes: Thresholds: cytology ≥ LSIL, or cytological ASC-US with any HR-HPV (+), or cytologically normal (NILM) but HPV-16/-18 (+); HPV integration (+)

Abbreviations: 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive values

precancerous cervical lesions, HPV DNA integration rate was 7.4% [31]. In HPV-positive oropharyngeal squamous cell carcinomas, the incidence of viral integration is lower, and the rate of HPV integration in other anogenital cancers is not as well documented [32]. We found that the HPV integration rate was 13.7% in women with HPV infection after cervical surgery. A study reported a recurrence rate of 10.7% (6 out of 56) among HPV integration-positive patients [33], which is similar to our data. In our study, HPV integration was detected after conization treatment, which may explain the lower integration rate compared to previous studies conducted on preoperative cervical cancer patients.

Cervical cancer with HPV integration is associated with increased levels of E6/E7 proteins, enhanced tumor aggressiveness, and immune evasion [34]. The integrated

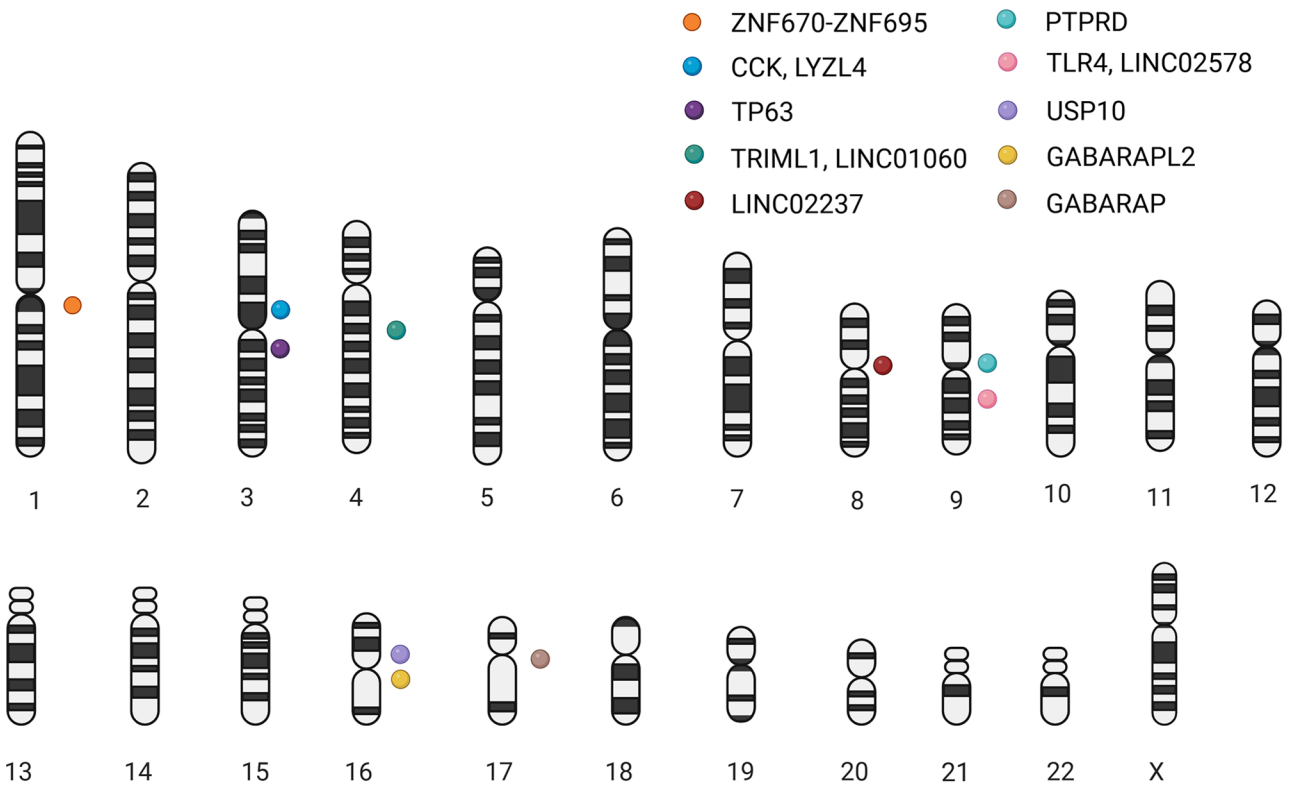

Fig. 4 Schematic representation of all HPV integration sites in the human genome detected across exfoliated cervical cell samples. Each dot represents an HPV integration site

form of the virus is a severely unfavorable predictive factor, and the survival rate of such patients is significantly lower than that of patients with the episomal form of the virus [35]. HPV integration is also associated with distant metastasis in cervical cancer [36]. In a study of head and neck squamous cell carcinoma (HNSCC), integration-negative tumors presented significantly improved immune cellular characteristics, including CD4+, CD3+, CD8 + T cells, NK cells, and B cells, compared to integration-positive tumors [37]. This may explain the better survival rate for HPV integration-negative patients. Currently, most studies focus on HPV integration detection in cancer tissues, with few studies on the triage of HPV-positive women. In 2023, an observational cohort study evaluated the performance of an HPV integration test for the triage of HPV-positive women. The results showed that at 1-year follow-up, the progression rate in HPV integration-positive women was higher than in HPV integration-negative women (12.0% versus 2.1%, odds ratio 5.6, 95% CI, 2.6–11.9) [38]. These findings suggest that HPV viral integration status is an important and potentially useful clinical biomarker, which will need confirmation in larger, prospective validation studies.

The first study to identify the exact HPV integration breakpoints in the human genome was carried out in 1987, when a single integrated copy of the virus was detected between KLF5 and KLF12 in SiHa cells [39]. Since then, many reports have described breakage sites in the human genome caused by HPV. Moreover, Li et al. found that the breakpoints are significantly enriched in the INTRON and PROMOTER regions [40]. Therefore, it might suggest that HPV integration could be directly related to the disruption and alteration of gene function. Rusan et al. described three main pathways of HPV integration into the host genome that can lead to carcinogenesis: loss of function of tumor suppressor genes, increase in oncogene expression, and inter- or intrachromosomal rearrangements [13].

In our study, we found that the integration of the viral genome was almost non-selective large segments were analyzed, finding breakpoints throughout the human genome. Several studies aiming to discover viral integration sites in the genome of host cells have demonstrated frequent integrations in the MYC, TMEM49, and FANCC genes [41], as well as in POU5F1B, FHIT, KLF12, KLF5, HMGA2, LRP1B, LEPREL1, DLG2, and SEMA3D [42]. Consistent with our findings, HPV integration in TP63 genes was recently reported in HPV-positive vulvar cancer patients [43]. USP10, a cytoplasmic ubiquitin-specific protease, deubiquitinates p53, reversing Mdm2-induced p53 nuclear export and degradation [44]. Deletions in PTPRD1 were found to be associated with poor efficacy of EGFR and MEK inhibitors in HNSCC cell lines [45]. Zinc finger proteins (ZNFs) may interact with DNA sequences, RNAs, proteins, and post-translational modifications [46, 47]. ZNF540 expression is highly correlated with HPV infection, rendering ZNF540 a potential biomarker for HNSCC prognosis and treatment [48]. Nevertheless, we also identified viral integration sites not previously reported, such as LYZL4, TRIML1, LINC01060, LINC02578, and GABARAPL2. LINC01060 is an EMT-related long non-coding RNA (lncRNA) that is up-regulated in osteosarcoma; higher LINC01060 expression is linked to a worse prognosis in osteosarcoma patients [49]. Although these genes are involved in other areas, their meaningful impact on cervical cancer is not yet clear.

The results of our HPV integration breakpoints supported previous conclusion that HPV is randomly integrated into the host genome at the beginning. However, the recurrent loci of hot genes in every individual provided a growth advantage for carcinogenesis. Common fragile sites (CFS) are widely located among human chromosomes, which weakens the human genome and enables the integration of carcinogenic viruses. Integration of the virus creates a chromosomal instability via chromosomal translocation, leading to oncogenesis in hosts [50].

Our results indicate that HPV integration-positive is an efficient predictor for the residual/recurrent CIN. To the best of our knowledge, there have been few studies evaluating the association between HPV integration and residual/recurrent lesions after cervical conization [33]. Several study limitations should be noted. First, the follow-up period of this study was short, the risk of recurrent CIN may be underestimated. Secondly, the small sample size limited our further analysis of the performance of HPV integration for the triage of HPV-positive women. Further prospective studies with larger sample sizes in a broader context are needed.

In conclusion, our results point out that HPV integration was a risk factor for residual/recurrent lesions in HPV-positive women after cervical conization. The application of integration hotspots may provide evidence of intensive follow-up and may reduce the incidence of delayed treatment and inadequate treatment for post-treatment patients with HPV infection in clinical practice. More studies are expected to confirm the data of this new method and to evaluate the cost-effectiveness of HPV integration tests in the follow-up of patients after conization.

Abbreviations

ASC-US	Atypical squamous cells of undetermined significance
CIN	Cervical intraepithelial neoplasia
CKC	Cold knife conization
HIVID	High-throughput viral integration detection
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
LEEP	Loop electrosurgical excision procedure cone biopsy

LAST	Lower Anogenital Squamous Terminology
LSIL	Low-grade squamous intraepithelial lesion
NILM	Negative for intraepithelial lesion or malignancy
PCR-RDB HPV test	PCR-reverse dot blot human papillomavirus genotyping test

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13027-025-00637-3 .

Supplementary Material 1

Acknowledgements
The authors express their profound gratitude to Kelvin Stefan Osafo for additions to this work.

Author contributions
Wenyu Lin: Methodology, Formal analysis, Writing– original draft, Writing– review & editing, Visualization. Yuxuan Huang: Methodology, Investigation, Formal analysis, Writing– original draft. Yan Zhang and Lixiang Huang: Investigation, Writing– review & editing. Hongning Cai: Data curation, Investigation; Methodology. Guanxiang Huang: Software, Visualization. Ye Li: Software, Visualization. Qiaoyu Zhang: Methodology. Huifeng Xue: supervision; Binhua Dong: conceptualization, writing– review & editing. Pengming Sun: Conceptualization, Resources, Writing– review & editing, Project administration, Funding acquisition. All authors reviewed the manuscript.

Funding
The study was supported by grants from the National Natural Science Foundation of China (grant no. 82271658), the National Key R&D Program of China (grant no. 2021YFC2701205), Fujian Health Young and Middle-aged Scientific Research Major Project (grant no. 2021ZQNZD011), Fujian Maternity and Child Health Hospital Technology Innovation Project (grant no. YCXM-20-04 and YCXM-20-15), the startup fund for scientific research, Fujian Medical University (grant no. 2023QH2047).

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Ethical approval for the study was obtained from the Ethics Review Committee of Fujian Maternity and Child Health Hospital (No:2021KLR09009), China. The procedures followed were in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association. All women provided informed consent for participation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 11 November 2024 / Accepted: 17 January 2025
Published online: 28 January 2025

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