Virology Journal

RESEARCH

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Genetic variations and carcinogenicity analysis of E6/E7 oncogenes in HPV31 and HPV35 in Taizhou, China

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Abstract

Background The purpose of this study was to investigate the genetic variations in the E6 and E7 oncogenes of HPV31 and HPV35, and to explore their potential role in cervical cancer risk among women in Taizhou, China.

Methods Cervical exfoliated cells were collected for HPV genotyping, and only patients with a single infection of either HPV31 or HPV35 were selected for this study. The ABI 3730xl sequencer was utilized to sequence the *E6* and *E7* genes, followed by subsequent sequence alignment, analysis of genetic heterogeneity, and construction of maximum likelihood phylogenetic trees for the sequences of HPV31 and HPV35 using BioEdit and MEGA softwares.

Results From 2013 to 2023, 148 HPV31 *E6/E7* gene sequences and 121 HPV35 *E6/E7* gene sequences were successfully obtained. We identified 16 distinct HPV31 *E6/E7* variants and 5 distinct HPV35 *E6/E7* variants, which have been deposited in GenBank under accession numbers OR540563-OR540578 and OR540579-OR540583, respectively. Phylogenetic analysis revealed that most of the HPV31 variants belonged to sublineage A2 (57.4%), followed by sublineages C2 (26.4%), C3 (14.2%) and B1 (2.0%). The proportion of CIN2 + patients in sublineage A2 was greater than that in other HPV31 sublineages, but the difference was not statistically significant (69.2% vs. 30.8%, $P > 0.05$). The most common variant in A2 was 31CNTZ07, which has a greater risk of CIN2 + than other A2 variants (OR = 3.5, 95% CI = 1.31 to 9.36; $P < 0.05$). In addition, all the HPV35 variants belonged to lineage A, of which 99.2% belonged to sublineage A1. 35CNTZ01 and 35CNTZ03 were the two most common HPV35 variants in our population, but no significant difference in their carcinogenic ability was observed ($P < 0.05$).

Conclusion These data provides a deeper insight into the distribution of geographic/ethnical HPV31 and HPV35 variants, which contribute to the development of multivalent HPV vaccines and diagnostic assays that are suitable for Chinese women.

Keywords Human papillomavirus 31, Human papillomavirus 35, Genetic variability, Phylogenetic analysis, Cervical cancer, E6/E7 oncogenes

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Background

Cervical cancer remains a significant threat to women's health. Approximately 660,000 new cases were diagnosed and 350,000 deaths occurred globally in 2022 [1]. In China, the incidence and mortality rates of cervical cancer are increasing, particularly in rural areas [2]. Persistent infection with high-risk human papillomavirus (HR-HPV) is a principal aetiologic factor for the development of cervical cancer. HPV is a small, double-stranded DNA virus with a genome consisting of approximately 8,000 nucleotides. The early genes *E6* and *E7* encode the primary oncogenic proteins in HPV genotypes and play pivotal roles in tumour initiation and progression [3]. The nomenclature of HPV is established by the International Committee on Taxonomy of Virus (ICTV), but no standards below the species level [4]. A "type" is established when the nucleotide sequence of the *L1* gene differs from that of any other characterized types by > 10%. For example, HPV16 is a type within species *Alphapapillomaviruses* 9 of the genus *Alphapapillomaviruses.*

HPV can be classified into high-risk and low-risk types on the basis of the degree of potential carcinogenicity. Among these, 14 types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) are more likely to cause cervical cancer than other HPV types and are defined as "HR-HPV". Each HPV type can be further classified into variant lineages and sublineages using empirically defined genetic differences of 1.0–10% and 0.5-1.0%, respectively [4, 5]. Notably, our previous studies revealed differences in carcinogenic risk between different genetic variations in HPV genome [6, 7]. Our results showed that the sublineage A4 (Asia) variants of HPV16 carried a significantly increased risk for CIN2 + compared to the A1-3 (European) variants [6]. In particular, the oncogenicity of E6 T178G (D32E), T350G (L90V) and E7 A647G (N29S) variations was associated with the persistent viral infection and cervical cancer risk. Our results showed that the sublineage A3 variants with T20I/G63S substitutions at E7 oncoprotein carried a significantly higher risk for CIN2 + compared to other HPV58 variants [7]. Moreover, our previous epidemiological survey suggested that HPV31 and HPV35 may also cause cervical cancer [8, 9, 10]. However, there are few studies on HPV31-related and HPV35-related genetic variations and their carcinogenicity. Therefore, the purpose of this study was to investigate the genetic variations in the E6/E7 genes of HPV31 and HPV35, conduct an epidemiological analysis of the HPV31 and HPV35 types prevalent in the Taizhou region, and assess their correlation with cervical lesions.

Materials and methods

Study population and specimen collection

From February 2013 to June 2023, we collected exfoliated cervical cells from women who visited Taizhou Hospital in Zhejiang Province for cervical cancer screening. To ensure the accuracy of the experiment without interference from other HPV types, this study only selected samples infected with HPV31 or HPV35 alone. A total of 150 single HPV31-positive samples and 133 single HPV35-positive samples were selected in this study. All the samples in cell preservation medium were stored at -80 ℃ before DNA extraction.

HPV genotyping

Total DNA was extracted from the stored specimens using a DNA Extraction Kit (#GK0122, GENEray). HPV genotyping was performed using a bead-based multiplex bioGP5+/6 + PCR/MPG assay technology. In short, the biotin-labeled PCR products were captured by HPV type-specific probes attached to color-coded beads; streptavidin-phycoerythrin was used as the reporter that bound to the target; and the HPV genotypes were analyzed using a Luminex200™ analyzer. This assay can simultaneously detects DNA from 27 HPV genotypes (high-risk HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and low-risk HPV6, 11, 26, 40, 42, 43, 44, 53, 55, 61, 81, 82, 83).

Primer design and PCR

Then, using the NCBI primer-BLAST tool, specific primer pairs were designed for the entire E6/E7 regions of HPV31 or HPV35. The specific primer sequences for HPV31 E6/E7 were 31E6E7F 5′-AGGGAGTGACCGAA AGTGGT-3′ and 31E6E7R 5′-ATGTTCCTCCGCTTCC TGTG-3′. The specific primer sequences for HPV35 E6/E7 were 35E6E7F 5′-AGTGACCGAAAACGGTCGTA-3′ and 35E6E7R 5′-GGATCCCCCGTACGTCTACT-3′. The primers used were synthesized by BGI Company (Hangzhou, China).

Sequencing

Based on the reference sequence (GenBank accession number: J04353 for HPV31), the size of the amplified PCR product was 1066 bp, corresponding to nucleotide positions [nt] 30–1095, containing the entire *E6* gene (nt 108–557) and the entire *E7* gene (nt 560–856). Similarly, for HPV35 (GenBank accession number: HQ537708), the size of the amplified PCR product was 944 bp, corresponding to nucleotide sites [nt] 19–962, containing the entire *E6* gene (nt 110–559) and the entire *E7* gene (nt 562–861). The techniques for PCR product purification, visualization, and sequencing were routinely performed. All the data were confirmed using at least two repeated PCR amplifications and sequence analyses.

Phylogenetic analysis

All the obtained nucleotide sequences were aligned using ClustalW multiple alignment (BioEdit Alignment

Editor Software V7.0.9.0), with the prototype reference sequences (J04353 for HPV31 and HQ537708 for HPV35) used as standards for the number of nucleotide locations of HPV31 and HPV35. The phylogenetic tree of HPV31 was generated using 16 complete HPV31 E6/E7 genes obtained in this study and 7 complete HPV31 E6/E7 genes available from NCBI (J04353 (A1), HQ537675 (A2), HQ537676 (B1), HQ537680 (B2), HQ537682 (C1), HQ537684 (C2), and HQ537685 (C3)). The phylogenetic tree of HPV35 was generated using 5 complete HPV35 E6/E7 genes and 3 complete HPV35 E6/E7 genes available from NCBI (HQ537713 (A1), HQ537721 (A2), and MT1217311 (B)). Maximum-Likelihood tree was constructed with the GTR model using MEGA-X software V10.1.7. The data were bootstrap resampled 1000 replicates for tree topology evaluation.

Statistical analysis

All data were analyzed by SPSS 25.0 software (SPSS Inc., Chicago, IL). The relationships between cervical lesion risk and HPV31 or HPV35 variants were analysed using the chi-square test or Fisher precision test. Using CIN2 or worse (CIN2+) as outcome variable and normal histology as control group. Odd ratios (ORs) and relative 95% confidence intervals (95%CI) were calculated. A two-sided *P* value < 0.05 was considered statistically significant.

Results

Characteristics of the study population

The infection rates of the HPV31 and HPV35 types are extremely low among Chinese women. HPV31 and HPV35 infection rates were approximately 7.0% and 5.5%, respectively, either alone or in combination with other types [8, 9, 10, 11]. Between 2013 and 2023, a single HPV31 infection was detected in 150 women (median age 42.0 years; range 20–69 years) and a single HPV35 infection was detected in 133 women (median age 44.0 years; range 21–71 years) were selected in this study. The specific clinical data are presented in Additional Files 1 and 2.

For HPV31, a total of 148 (98.7%) sequences of the entire *E6* and *E7* genes from HPV31 were successfully obtained. Among 148 women, 80 (54.1%) underwent colposcopy biopsy for histological diagnosis, including 46 with normal histology, 8 with CIN1, 14 with CIN2, 12 with CIN3, and no cases of cervical cancer. The characteristics of the study population are shown in Table 1.

For HPV35, a total of 121 (91.0%) sequences of the entire *E6* and *E7* genes from HPV35 were successfully obtained. Among 121 women, 79 (65.3%) underwent colposcopy biopsy for histological diagnosis, including 61 with normal cervices, 6 with CIN1, 8 with CIN2, 4 with CIN3, and no cases of cervical cancer. The characteristics of the study population are shown in Table 2.

Variations in the HPV31 *E6* and *E7* genes

Compared with J04353, all the HPV31 samples presented nucleotide variations. No premature termination codons or frameshift mutations were identified. Figure 1 depicts all the changes in the nucleotide and amino acid (AA) sequences in the entire E6 and E7 regions of the HPV31 sublineages. In total, we obtained 16 different genetic variants of HPV31, denoted 31CNTZ01-31CNTZ16, which have been submitted to the GenBank database under accession numbers OR540563-OR540578. 31CNTZ07 (27.7%, 41/148) was the most common genetic variant, followed by 31CNTZ15 (25%, 37/148) and 31CNTZ04 (19.6%, 29/148). Of which, the AA

Table 1 The frequency of HPV31 sublineages in histopathology grades

HPV31 sublineages	Variants	Normal (*n* = 46)	CIN1 (*n* = 8)	CIN2 (*n* = 14)	CIN3 (*n* = 12)	Cancer (*n* = 0)	Total (*n* = 80)
A2		**24(52.2%)**	**4(50.0%)**	**10(71.4%)**	**8(66.7%)**	**0**	**46(57.5%)**
	31CNTZ01	2(4.3%)	0	0	0	0	2(2.5%)
	31CNTZ02	1(2.2%)	2(25.0%)	0	0	0	3(3.8%)
	31CNTZ04	9(19.6%)	0	2(14.2%)	2(16.7%)	0	13(16.3%)
	31CNTZ05	1(2.2%)	0	0	0	0	1(1.3%)
	31CNTZ06	0	1(12.5%)	0	0	0	1(1.3%)
	31CNTZ07	11(23.9%)	1(12.5%)	7(50%)	6(50.0%)	0	25(31.3%)
	31CNTZ10	0	0	1(7.1%)	0	0	1(1.3%)
B1		**1(2.2%)**	**1(12.5%)**	**0**	**0**	**0**	**2(2.5%)**
	31CNTZ12	1(2.2%)	1(12.5%)	0	0	0	2(2.5%)
C2		**14(30.4%)**	**3(37.5%)**	**4(28.6%)**	**3(25%)**	**0**	**24(30%)**
	31CNTZ15	14(30.4%)	3(37.5%)	4(28.6%)	2(16.7%)	0	23(28.8%)
	31CNTZ16	0	0	0	1(8.3%)	0	1(1.3%)
C3		**7(15.2%)**	**0**	**0**	**1(8.3%)**	**0**	**8(10.0%)**
	31CNTZ13	6(13.0%)	0	0	1(8.3%)	0	7(8.8%)
	31CNTZ14	1(2.2%)	0	0	0	0	1(1.3%)

Table 2 The frequency of HPV35 sublineages in histopathology grades

HPV35 sublineages	Variants	Normal (*n* = 61)	CIN1 (*n* = 6)	CIN2 (*n* = 8)	CIN3 (*n* = 4)	Cancer (*n* = 0)	Total (*n* = 79)
A1		**60(98.4%)**	**6(100.0%)**	**8(100.0%)**	**4(100.0%)**	**0**	**78(98.7%)**
	35CNTZ01	35(57.4%)	3(50.0%)	6(75.0%)	3(75.0%)	0	47(59.5%)
	35CNTZ02	1(1.6%)	0	0	0	0	1(1.3%)
	35CNTZ03	24(39.3%)	1(16.7%)	2(25.0%)	1(25.0%)	0	28(35.4%)
	35CNTZ04	0	2(33.3%)	0	0	0	2(2.5%)
A2		**1(1.6%)**	**0**	**0**	**0**	**0**	**1(1.3%)**
	31CNTZ05	1(1.6%)	0	0	0	0	1(1.3%)

	1 3 4	1 7 6	2 6 1	2 8 5	2 9 7	2 9 9	3 2 0	3 2 6	4 0 4	4 0 7	4 2 8	4 4 6	4 4 5	5 2 0	5 3 7	5 8 0	6 2 6	6 7 0	6 9 5	7 4 3	7 8 7	7 8 8	8 2 6	Total (n=148)	Sub-lineages	GenBank accession numbers
Ref J04353	T	C	A	C	A	A	A	A	G	A	A	A	A	C	C	G	C	C	G	A	T	C	C			
31CNTZ01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	T	.	2	A2	OR540563
31CNTZ02	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	4		OR540564
31CNTZ03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	C	.	.	1		OR540565
31CNTZ04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	29		OR540566
31CNTZ05	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	2		OR540567
31CNTZ06	.	T	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	1		OR540568
31CNTZ07	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	41		OR540569
31CNTZ08	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	.	1		OR540570
31CNTZ09	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	T	2		OR540571
31CNTZ10	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	G	.	.	.	1		OR540572
31CNTZ11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	G	.	.	.	1		OR540573
31CNTZ12	.	.	C	.	G	.	T	.	.	.	.	.	G	T	.	.	.	T	A	G	.	.	.	3	B1	OR540574
31CNTZ13	.	.	.	T	.	.	T	.	A	.	G	.	.	T	.	A	.	T	A	G	.	.	.	20	C3	OR540575
31CNTZ14	.	.	.	T	.	.	T	.	A	G	G	.	.	T	.	A	.	T	A	G	.	.	.	1		OR540576
31CNTZ15	.	.	.	T	.	.	T	G	A	.	G	.	.	T	.	A	.	T	A	G	.	.	.	37	C2	OR540577
31CNTZ16	.	.	.	T	.	.	T	G	A	.	G	.	.	T	G	A	.	T	A	G	.	.	.	2		OR540578
Ref aa	P	Y	I	H	T	T	S	V	L	L	Q	E	K	A	R	T	H	V	E	K	I	R	I			
aa position	9	2 3	5 2	6 0	6 4	6 4	7 1	7 3	9 9	1 0 0	1 0 7	1 3 3	1 2 3	1 3 8	1 4 4	7	2 3	3 7	4 6	6 2	7 6	7 7	8 9			
aa mutation	.	.	L	Y	A	A	.	.	.	.	.	D	R	V	G		Y		K	E		C				
	E6															E7										

Note: novel HPV31 variants highlighted in bold, novel nucleotide substitutions are highlighted in gray.

Fig. 1 Genetic variability of HPV31 *E6* and *E7* nucleotide sequences in Taizhou area, Southeast China. Numbering refers to the first nucleotide of the HPV31 prototype reference sequence (GenBank: J04353). Each row indicates the variant identification and the nucleotide sequence alignment compared to the reference

variant sequences of 31CNTZ04 were consistent with that of 31CNTZ07. Seven (43.8%, 7/16) variants were novel HPV31 E6/E7 variants, which are highlighted in bold in Fig. 1.

Altogether, 24 single nucleotide substitutions were identified in the HPV31 E6/E7 region, with 12 (50.0%, 12/24) nonsynonymous substitutions and 7 (29.2%, 7/24) novel discovered substitutions. "Nonsynonymous substitution" refers to the substitution of encoded amino acids caused by single nucleotide substitution in DNA sequences. The nonsynonymous substitutions included A261C (I52L), C285T (H60Y), A297G (T64A), A299G (T64A), A446C (E113D), A475G (K123R), C520T (A138V), and C537G (R144G) in the E6 sequence and C626T (H23Y), G695A (E46K), A743G (K62E), and C788T (R77C) in the E7 sequence. To the best of our knowledge, the nucleotide substitutions A299G (T64A), A320G, A446C (E113D), C537G (R144G), C670T, C788T (R77C) and C826T have not been previously reported.

Variations in the HPV35 *E6* and *E7* genes

Compared with HQ537708, all the HPV35 samples presented nucleotide variations. No premature termination codons or frameshift mutations were identified. Figure 2

	1	1	2	3	3	6	7	Total (n=121)	Sub-lineages	GenBank accession numbers
	2	3	7	4	7	7	4			
	7	6	4	1	0	5	8			
Ref HQ537708	T	T	A	T	G	T	G			
35CNTZ01	C	C	.	C	.	.	.	75	A1	OR540579
35CNTZ02	C	C	G	C	.	.	.	3		OR540580
35CNTZ03	C	C	.	C	C	.	.	40		OR540581
35CNTZ04	C	C	.	C	.	.	A	2		OR540582
35CNTZ05	.	.	.	.	.	C	.	1	A2	OR540583
Ref aa	A	P	R	W	T	T	E			
aa position			5	7	8	3	6			
	6	9	5	8	7	8	3			
aa mutation	.	.	.	R	.	.	K			
	E6					E7				

Note: novel HPV35 variants highlighted in bold, novel nucleotide substitutions are highlighted in gray.

Fig. 2 Genetic variability of HPV35 *E6* and *E7* nucleotide sequences in Taizhou area, Southeast China. Numbering refers to the first nucleotide of the HPV35 prototype reference sequence (GenBank: HQ537708). Each row indicates the variant identification and the nucleotide sequence alignment compared to the reference

depicts all the changes in the nucleotide and amino acid sequences in the entire E6 and E7 regions of the HPV35 sublineages. In total, we obtained 5 different genetic variants of HPV35, denoted 35CNTZ01-35CNTZ05, which have been submitted to the GenBank database under accession numbers OR540579-OR540583. Notably, 35CNTZ01 (62.0%, 75/121) and 35CNTZ03 (33.1%, 40/121) were the two most common genetic variants, but the AA variant sequences were consistent with each other. One (20.0%, 1/5) belonged to the novel HPV35 E6/E7 variants, which are highlighted in bold in Fig. 2.

Overall, 7 single nucleotide substitutions were identified in the HPV35 E6/E7 region, with 2 (28.6%, 2/7) nonsynonymous substitutions and 1 (14.3%, 1/7) novel discovered substitution. The nonsynonymous substitutions included T341C (W78R) in the E6 sequence and G748A (E63K) in the E7 sequence. A743G was observed in the E7 sequence of all the HPV31 variants obtained in this study. To the best of our knowledge, the nucleotide substitutions of A274G have not been previously reported.

Phylogenetic construction

For HPV31, the phylogenetic tree was constructed from 16 HPV31 E6/E7 sequences obtained from this study, along with 7 reference sequences from GenBank (Fig. 3). According to the phylogenetic tree, we observed that HPV31 variants belonged to sublineage A2, B1, C2, and C3, but no sublineages A1, B2, or C1 in Taizhou. Sublineage A2 (57.4%, 85/148) was the most commonly detected, followed by C2 (26.4%, 39/148), C3 (14.2%, 21/148), and B1 (2.0%, 3/148), respectively. For HPV35, the phylogenetic tree was constructed from 5 HPV35 E6/E7 sequences obtained from this study, along with 3 reference sequences from GenBank (Fig. 4). All the HPV35 E6/E7 variants belonged to lineage A, of which 99.2% belonged to sublineage A1 (120/121).

Risk association with cervical lesions

Our data suggested that the proportion of CIN2 + patients in sublineage A2 was greater than that in other HPV31 sublineages, but this difference was not statistically significant (69.2% vs. 30.8%, $P > 0.05$). The most common variant in A2 was 31CNTZ07 (48.2%, 41/85), which was associated with a greater risk of CIN2 + than other A2 variants (OR = 3.5, 95% CI = 1.31 to 9.36; $P < 0.05$) (Table 1). Moreover, 35CNTZ01 (62.0%, 75/121) and 35CNTZ03 (33.1%, 40/121) were the two most common HPV35 variants in our population. However, no significant difference in their carcinogenic ability was noted ($P < 0.05$) (Table 2). In addition, no significant trends were observed between the nucleotide substitutions of the HPV31 or HPV35 E6/E7 variants and the risk of cervical lesions.

Discussion

Cervical cancer is the only type of tumour with a well-defined aetiology that can be prevented through HPV screening and vaccination. However, owing to public issues such as the lack of national immunization

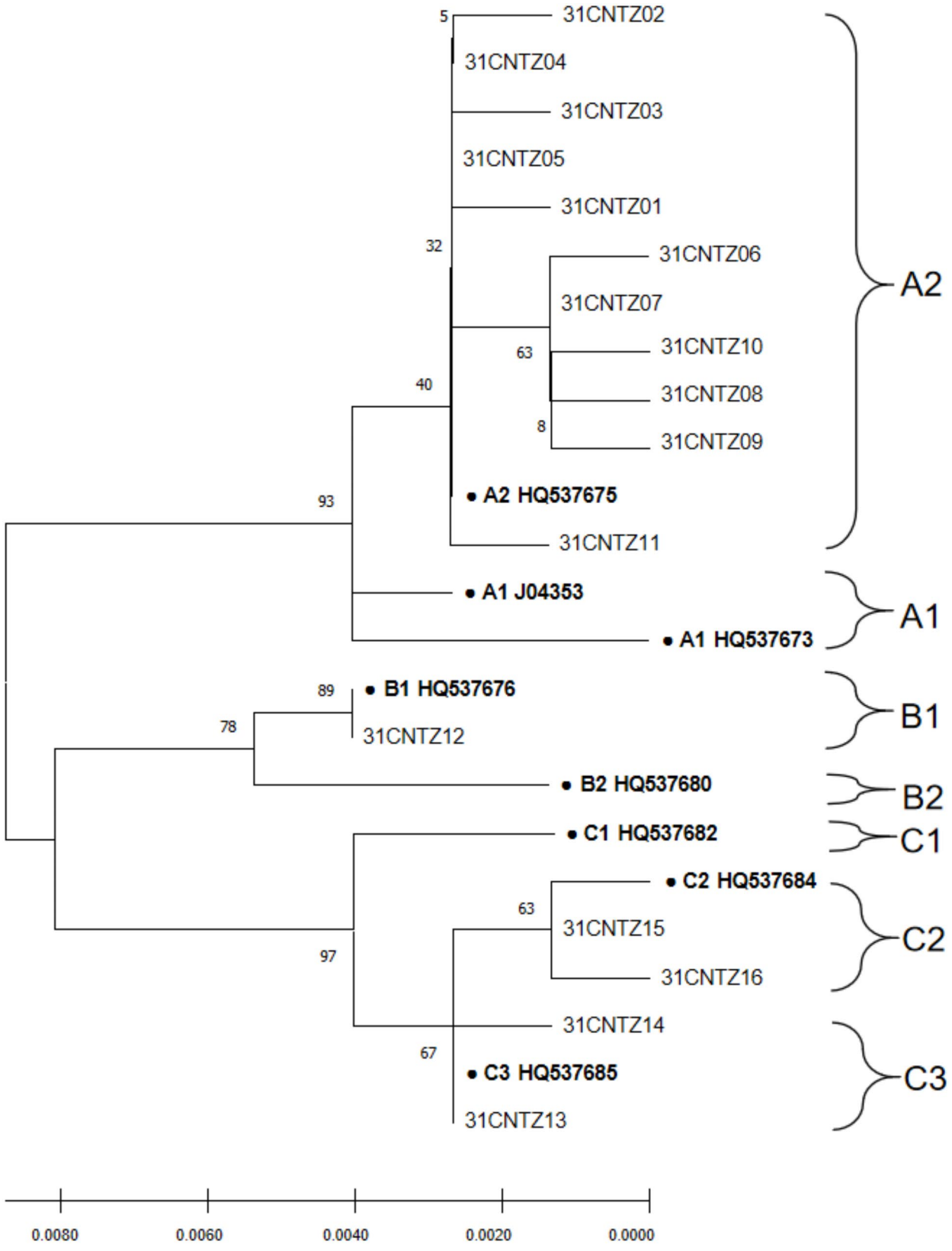

Fig. 3 Phylogenetic tree of the HPV31 E6/E7 variants. Maximum-likelihood analysis (with MEGA-X) of *E6* and *E7* nucleotide sequences was inferred from 16 obtained HPV31 variants and 7 reference sequences. Numbers below branches indicate bootstrap values

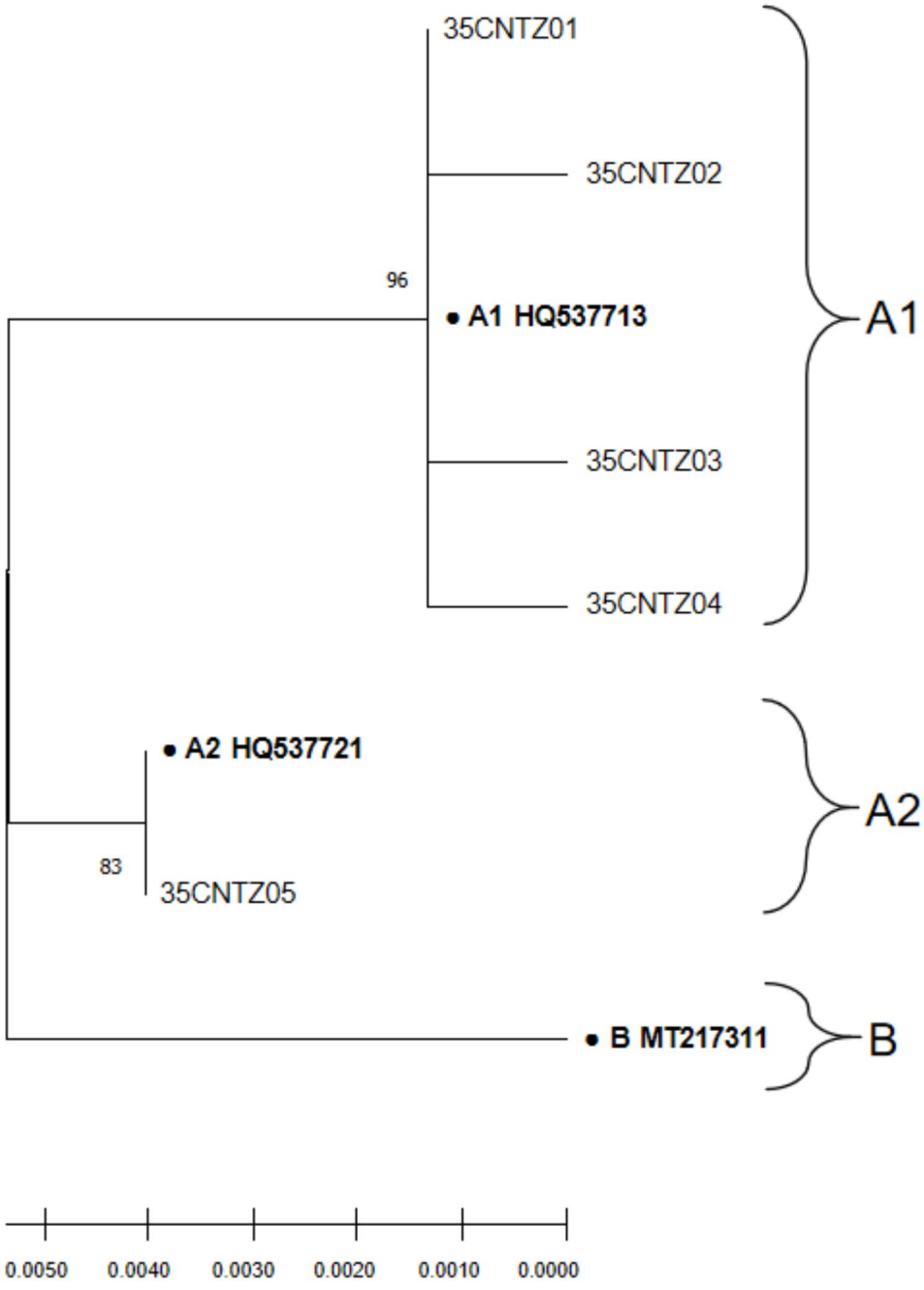

Fig. 4 Phylogenetic tree of the HPV35 E6/E7 variants. Maximum-likelihood analysis (with MEGA-X) of *E6* and *E7* nucleotide sequences was inferred from 5 obtained HPV35 variants and 3 reference sequences. Numbers below branches indicate bootstrap values

programs and insufficient supply of HPV vaccines in China, HPV screening remains the main measure for cancer prevention. Moreover, the 9-valent HPV vaccine (9vHPV) might not provide protection against all HPV-related infections beyond the nine types covered by the vaccine [12]. The current 9vHPV (Gardasil 9) contains HPV31 type but not cover HPV35 type. In the postvaccination era, we need to focus on cervical lesions and cervical cancer caused by nonvaccine HPV types. Notably, owing to the extremely low infection rates of HPV31 (0.7%) and HPV35 (0.5%) in the general population [9], little is known about the genetic variation of these two high-risk HPV types in China. In this study, we investigated the genetic variations in the *E6/E7* oncogenes of HPV31 and HPV35, constructed phylogenetic trees of HPV31 and HPV35 variants in the Taizhou region, and explored their relationship between genetic variants and

the risk for cervical cancer among the Taizhou-based population.

As is well known, the distribution of HPV variants depends on geographical origin, ethnicity, and vaccination coverage [3, 4, 5, 8, 12]. In this study, we observed that 57.4% of the HPV31 *E6/E7* variants belonged to sublineage A2, followed by C2 (26.4%), C3 (14.2%) and B1 (2.0%), but no sublineages A1, B2, or C1 in Taizhou region, Southeast China. In addition, Yu et al. [13] reported that HPV31 lineage A (49.0%) was the most commonly detected, followed by lineages C (47.0%) and B (4.0%) in Northeast China. In Southwest China, Zhang et al. [14] reported that the proportions of HPV31 lineages A, B and C were 54.0%, 8.2%, and 37.8%, respectively. The above data suggested that the distribution trend of HPV31 variants was similar in various regions of China, with sublineage A2 accounting for the majority [13, 14]. With respect to the distribution of sublineages HPV31 in other regions of East Asia, sublineage B2 (34.6%) was the most commonly detected, followed by sublineages A2 (26.9%), B1 (19.2%), A1 (9.6%). C1 (3.8%), C2 (3.8%), and C3 (1.9%) in Japan [15]. In West Asia, sublineage A1 (57.1%) was the most commonly detected among Iranian women, followed by sublineages C3 (14.3%), C1 (9.5%), B2 (9.5%), A2 (4.8%), and B1 (4.8%) [16]. In northeastern Brazil, the sublineage distribution of HPV31 (57.2% in lineage A, 5.7% in lineage B and 37.1% in lineage C) was similar to that in China [17]. In Italy, lineage C (65.8%) was detected most frequently, followed by lineage B (29.3%) and lineage A (4.9%) [18]. As mentioned above, the distribution of HPV31 variants varies due to geographic variation and ethnic differences; sublineage A2 is more common in China.

To further analyze the association between oncogenic risk and HPV31 lineages, our data suggested that the proportion of CIN2 + in A2 was greater than that in other HPV31 sublineages, and 31CNTZ07 was associated with a greater risk of CIN2 + than other A2 variants (OR = 3.5). Previous studies reported that HPV31 sublineages A1 (OR = 1.71), A2 (OR = 2.48) and B2 (OR = 1.89) were more strongly associated with cervical cancer than sublineage C3 [19, 20]. Therefore, these findings helps to explain in part why some HPV31 infections regress spontaneously and others lead to disease progression.

HPV35 is the most prevalent high-risk HPV type in Africa [21], but it is not common in Asia, Europe and North America [9, 22]. Previous study have shown that HIV-positive women were more susceptible to HPV35 infection compared to HIV-negative women (OR = 5.75, 95% CI 2.49–13.252.97, $P < 0.001$) [23]. In this study, we observed that all HPV35 E6/E7 variants belonged to lineage A, of which 99.2% belonged to sublineage A1. Several previous studies have also shown that the A1 sublineage was a common variant in their countries, such as in Mexico, America, Norway, Belgium, Zimbabwe, and Chad [24, 25, 26, 27, 28]. Pinheiro et al. [29] identified a new B lineage among Asian women, which is the predominant prevalent variant in India, accounting for 62.5%. Although the A2 sublineage is not the predominant prevalent variant worldwide, it is significantly associated with CIN3 + among African-American women [29]. African women infected with HPV35 present an up to 10-fold increase in the prevalence of CIN3 [30]. There was no significant correlation between HPV35 variants and cervical lesion in Taizhou. Of note, HPV35 is not covered by the current 9vHPV vaccine. However, the inclusion of HPV35 in HPV vaccines would be very useful in Africa.

To the best of our knowledge, this study was the first comprehensive investigation examining the genetic variation, phylogenetic relationships, and carcinogenic potential of HPV31 and HPV35 in Taizhou, China. Several limitations of the study should be addressed. First, the present study was limited by the number of single HPV31 or HPV35 positive samples, due to the extremely low natural incidence of HPV31 and HPV35 in Taizhou region. However, the number of HPV31 or HPV35 positive samples was by far the largest studied in Southeast China. Second, HPV variants were defined based on partial sequences (E6 and E7 oncogenes) rather than whole genome. However, this does not appear to cause misclassification of the variant lineages. Third, we did not have longitudinal data to evaluate the relationship between genetic variations and persistence throughout infection, but we plan to assess this in our future studies.

Conclusions

In summary, the present study provides a useful data about the geographic/ethnical distribution, genetic variations, phylogenetic relationships, and their carcinogenic potential of in HPV31 and HPV35, which helpful to the development of multivalent HPV vaccines and diagnostic assays suitable for Chinese women.

Abbreviations

bp	Base pair
CIN	Cervical intraepithelial neoplasia
DNA	Deoxyribonucleic acid
HPV	Human papillomavirus
nt	Nucleotide sites
PCR	Polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02650-9.

Supplementary Material 1: Clinical data for HPV31 study in Taizhou area, China

Supplementary Material 2: Clinical data for HPV35 study in Taizhou area, China

Acknowledgements
We appreciate all the patients for their contribution to this study.

Author contributions
H.H.X. and J.H.Y. designed the experiments, performed analysis. H.B.Y. and Z.Y.Y. drafted the manuscript and PCR amplifcation. Y.Q. and J.G. carried out the sample collection and performed HPV genotyping. All authors have read and approved the final manuscript.

Funding
This work was supported by grants from National Natural Science Foundation of China (No.81901625), by Science and Technology Bureau of Taizhou (No.23ywa01), and by Shaoxing University (No.Y20230324). None of the funders had any influence on the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Medical Ethics Review Board of Taizhou Hospital of Zhejiang Province (approval #K20220226), and was carried out in line with the Helsinki Declaration. All participants provided written informed consent for study participation before specimen collection, and the patients' privacy is strictly protected.

Consent for publication
Written informed consent was obtained from all patients for the publication of their medical data.

Competing interests
The authors declare no competing interests.

Received: 13 December 2024 / Accepted: 4 February 2025
Published online: 13 February 2025

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