The Distribution of Human Papillomavirus Type 30 (HPV30) Lineages Among Women With Normal Cervical Cytology in China

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Research

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Abstract

Background: Human papillomavirus type 30 (HPV30) is involved in cervical diseases. In human immunodeficiency virus (HIV) positive women, the prevalence HPV30 is almost the same as HPV16. However, HPV30 has seldom been investigated. In order to better understand the prevalence and intratype lineage distribution of HPV30 in China, HPV30 infection among women with normal cytology was investigated.

Methods: Prevalence of HPV30 was investigated by the screening of type specific polymerase chain reaction (PCR); intratype lineage distribution was performed by the phylogenetic analysis of the L2 DNA sequences of HPV30 isolates; the diversity of genetic variants of HPV30 isolates was also evaluated.

Results: (1) The infection rate of HPV30 was 0.56% (9/1600). (2) All the nine HPV30 isolates belonged to lineage A, none belonged to lineage B. (3) Compared with the HPV30 prototype reference, 87 variations including 79 substitutions, four insertions and four deletions were observed in this study. (4) Sample 4 contained a C base deletion of the E2 gene resulting in an amino acid sequence shift. (5) Sample had a truncated L2 protein.

Conclusions: The infection rate of HPV30 is 0.56% in this study. All of the HPV30 isolates belongs to lineage A. Natural L2 and E2 defective isolates of HPV30 were found.

Background

Cervical cancer (CC) is one of the most common malignant cancers among women worldwide^{3,4}. If the primary and secondary prevention programs are not carried out in low-income and middle-income countries, over 44 million women will be diagnosed as CC in the next 50 years⁵. The oncogenic human papillomavirus (HPV) types associated with all CCs are phylogenetically clustered in one clade includes of species Alpha-5 (A5), Alpha-6 (A6), Alpha-7 (A7), Alpha-9 (A9) and Alpha-11 (A11) of the Alpha genera⁶. Among the A6 HPVs, relatively high prevalence of Human papillomavirus type 53 (HPV53), Human papillomavirus type 56 (HPV56) and Human papillomavirus type 66 (HPV66) were investigated in the research on worldwide distribution of HPV in 2005 which account for about 6%, 36% and 21% infection rate that of Human papillomavirus type 16 (HPV16), respectively. Human papillomavirus type 30 (HPV30) was originally detected in a tissue specimen from laryngeal carcinomas, and was also detected in genital lesions⁷. In taxonomy, HPV30 is classified into the A6 specie of Alpha genera together with HPV53, HPV56 and HPV66 as well. But HPV30 is always a relatively rarely reported genotype worldwide, with few data available to characterize its prevalence, lineage distribution, and disease association.

In recent years, with the double detection of Human immunodeficiency virus (HIV) and HPV, it has been found that the prevalence of HPV in HIV positive women is higher than in HIV negative women. Especially in HIV positive women, the prevalence of HPV30 is almost the same as HPV16^{1,2}, which suggests the importance of the research of HPV30. This may also provide a good basis for the future HIV vaccine strategy.

There are only 18 representative whole genome sequences of HPV30 in the NCBI database. Variants of HPV30 have been classified as six phylogenic lineages based on the total sixteen whole genome sequences, these are lineage A1-A5 and B^{6,8}. Lack of data on HPV30, on the one hand may be due to the low prevalence of HPV30 in women, on the other hand may be due to the limitations of detection technique. In early studies, HPV30 was mainly detected in the esophageal carcinogenesis and genital tract specimens by Southern Blot. In subsequent studies HPV30 detection was performed mostly by general primer PCR and direct sequencing of the PCR products.

Both the prevalence and lineage distribution of HPV30 are still nebulous. In this study, we screened and confirmed HPV30 infection among healthy women, to investigate the distribution of HPV30. Therefore, the prevalence of HPV30 was investigated by type specific PCR among women with normal cervical cytology; lineage distribution of HPV30 was investigated based on the analysis of L2 DNA sequences; this study also analyzed the diversity of HPV30 genetic variants and the HPV30 proteins. This study may provide basic data for future studies on viral fundamental research and prevention.

Methods

Cervical specimens

In this study, totally 1600 specimens examined were obtained from outpatient women of the department of obstetric and gynecology at the Chinese PLA General Hospital in Beijing from January 2013 to July 2014. All the 1600 patients aged 15-89 years (median, 41 years) were diagnosed as normal cervical cytology. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

General primers PCR for HPV16 and HPV30 plasmids

HPV30 plasmid was constructed using L1 PCR product (primer 9 and 10) of sample 9 cloned into pMD18-T vector. The HPV16 L1 plasmid was prepared in our laboratory before.

General PCR of PGMY09/11^{9,10}, MY09/11^{11,12}, PI¹³ and FAP59/64¹⁴ were done in a 20 µl reaction mix containing about 50 ng DNA, 4 mmol/L MgCl₂, 100 µmol/L of each dNTP, 0.1 µmol/L of each oligonucleotide primer, and 1 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). The temperature profile used for amplification consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 20 s, annealing at 55°C for 2 min, and extension at 72°C for 1.5 min, which was extended for 5 min in the final cycle.

SKF/R¹⁵ PCR were done in a 20 µl reaction mix containing about 50 ng DNA, 4 mmol/L MgCl₂, 100 µmol/L of each dNTP, 0.1 µmol/L of each oligonucleotide primer, and 1 U ExTaq DNA polymerase (TaKaRa, Dalian). The temperature profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 98°C for 10 s, annealing at 45°C for 30 s, and extension at 72°C for 20 s, which was extended for 5 min in the final cycle.

The PCR products were visualized on 1.5% ethidium bromide-stained agarose gel.

HPV30 detection with polymerase chain reaction (PCR)

HPV30 type specific PCR was performed with primer 1 and 2 (Table 1), with an amplicon size of 240 bp. Briefly, the reaction in a 50 µl reaction mix containing about 50 ng DNA, 2 mmol/L MgCl₂, 100 µmol/L of each dNTP, 0.1 µmol/L of each oligonucleotide primer, and 1 U ExTaq DNA polymerase (TaKaRa, Dalian). The temperature profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 20 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, which was extended for 5 min in the final cycle. The PCR products were visualized on 1.5% ethidium bromide-stained agarose gel.

Table 1
List of primer sequences used for HPV30 PCRs

Primer name	Sequence 5' to 3'	Position of genome	Length (nt)
1	ATCTTTGTGAGGTACAAGAAACATC	151..175	25
2	TTT TAGTTAATGCCACTAGGCTTG	367..390	24
3	GTGTGCCGGGAAGGAGTAAA	1195..1214	20
4	TAGGCCGTGTACCTAACGGA	4494..4513	20
5	CAGACACCACCAGACAGTCC	3486..3505	20
6	CCTCTAAGCTTGTGGCAGCT	6874..6893	20
7	TGGGTTTGGTGCCATGGATT	6260..6279	20
8	GTATTTGCTGTGGTGGCGTG	1399..1418	20
9	ACACAACCTTTAACCTTTTCACCATCGG	5058..5084	27
10	GACACAAACAATCACCGCCGCTTG	7145..7168	24
PGMY11-A	GCACAGGGACATAACAATGG	6597..6616	20
PGMY11-B	GCGCAGGGCCACAATAATGG	6597..6616	20
PGMY11-C	GCACAGGGACATAATAATGG	6597..6616	20
PGMY11-D	GCCCAGGGCCACAACAATGG	6597..6616	20
PGMY11-E	GCTCAGGGTTTAAACAATGG	6597..6616	20
PGMY09-F	CGTCCCAAAGGAAACTGATC	7026..7045	20
PGMY09-G	CGACCTAAAGGAAACTGATC	7026..7045	20
PGMY09-H	CGTCCCAAAGGAAACTGATC	7026..7045	20
PGMY09-I	GCCAAGGGGAAACTGATC	7026..7045	18
PGMY09-J	CGTCCCAAAGGATACTGATC	7026..7045	20
PGMY09-K	CGTCCAAGGGGATACTGATC	7026..7045	20
PGMY09-L	CGACCTAAAGGGAATTGATC	7026..7045	20
PGMY09-M	CGACCTAGTGGAATTGATC	7026..7045	20
PGMY09-N	CGACCAAGGGGATATTGATC	7026..7045	20
PGMY09-P	GCCCAACGGAAACTGATC	7026..7045	18
PGMY09-Q	CGACCCAAGGGAAACTGGTC	7026..7045	20
PGMY09-R	CGTCCTAAAGGAAACTGGTC	7026..7045	20
HMB01	GCGACCCAATGCAAATTGGT	7026..7045	20
MY11	GCMCAGGGWCATAAAYAATGG	6597..6616	20
MY09	CGTCCMARRGGAWACTGATC	7026..7045	20
PI-1	GCICAGGGICATAAIAATGG	6597..6616	20
PI-2	CGTCCIAIIGGAIACCTGATC	7026..7045	20
FAP59	TAACWGTIGGICAYCCWTATT	5779..5799	21
FAP64	CCWATATCWVHCATITCICCATC	6240..6262	23
SKF1	AAATATCCAGATTATCTRAARATG	6339..6362	24
SKF2	AAATATCCTGATTATTTTRGGMATG	6339..6362	24
SKR1	ATACCATAGAYCCACTRGG	6534..6552	19
SKR2	AAACYATAGAGCCACTWGG	6534..6552	19

Other HPV30 PCRs to obtain the full length of HPV30 genome(primer 3 and 4, primer 5 and 6, primer 7 and 8) were also done in a 50 µl reaction mix containing about 50 ng DNA, 4 mmol/L MgCl₂, 100 µmol/L of each dNTP, 0.1 µmol/L of each oligonucleotide primer, and 1 U ExTaq DNA polymerase (TaKaRa, Dalian). The temperature profile consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles with denaturation at 95°C for 20 s,

annealing at 55°C for 30 s, and extension at 72°C for 3 min, which was extended for 5 min in the final cycle. The PCR products were visualized on 1.0% ethidium bromide-stained agarose gel and purified by using Qiagen gel extraction kit (Qiagen, Hilden, Germany). Directly sequencing of the PCR products was performed on conventional Sanger sequencing (ABI 3730, TSINGKE Biologically Technolog).

Data analysis

HPV30 variants were investigated by the comparisons to the reference sequence of prototype sequence (HPV30 X74474), which belongs to the European lineage.

All the whole genome of HPV30 were downloaded from NCBI database¹⁶.

DNAStar software ((Version 7.1.0) was used to edit and combine sequence.

The Molecular Evolution Genetic Analysis (MEGA) Software program (Version 6.0) was used for the phylogenetic tree analysis. The neighbor-joining tree was built by using Maximum Composite Likelihood model. Bootstrap test (1,000 bootstrap replicates) was used to evaluate the phylogenetic groups.

Ethical approval and consent to participants

Each sample donor was informed of the study aims to obtain the informed consent. This study was approved by the Ethical Review Committee of National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (ID No: IVDC2014-008). Women in this study signed a consent form before their participation.

Results

Amplification efficiency of HPV30 comparison with general primer sets

To assess the capacity of HPV30 detection, HPV16 was used as a control. Five sets of general primers targeting L1 gene including PGMY09/11, MY09/11, PI, FAP59/64 and SKF/R were used to detect HPV16 and HPV30 plasmids at the same time.

PGMY09/11 and SKF/R PCR were able to detect 1000 copies of HPV16 plasmid respectively; MY09/11, PI and FAP59/64 PCR were able to detect 100 copies of HPV16 plasmid respectively. PGMY09/11, MY09/11 and PI PCR were unable to detect HPV30 respectively, while FAP59/64 PCR was able to detect 100 copies of HPV30 plasmid, SKF/R PCR was able to detect 1000 copies of HPV30 plasmid. In summary, HPV16 could be detected by all the five sets of primers, while HPV30 could be detected by the FAP59/64 and SKF/R (Fig. 1).

Detection of HPV30 among women with normal cervical cytology

HPV30 was screened in the 1600 cervical specimens from women with normal cytology by HPV30 type specific PCR (Fig. 2A). The positive control was a mixture of the 1600 samples. The identification was confirmed by the results of a single amplicon band of 240 bp, after screening, nine samples (numbers 1-9) showed the expected bands (Fig. 2B). Therefore, the infection rate of HPV30 was 0.56% (9/1600) in China.

Six (sample 1, 3 and 6-9) of the nine HPV30 isolates successfully retrieved HPV30 full-length sequences, while the other three (sample 2, 4 and 5) retrieved the full-length E2-L2 sequences with partial E1 and L1 sequences.

Lineage distribution of HPV30 isolates

According to the previous HPV30 lineages studies, the whole genome sequences of HPV30 submitted to GenBank were separated into six distinct lineages A1, A2, A3, A4, A5 and B⁶.

The phylogenetic analysis was firstly conducted according to multiple nucleotide sequence alignments of whole genomes, including the six HPV30 genomes (sample 1, 3 and 6-9) identified in this study and other 18 known representative HPV30 variant lineages (for NCBI accession numbers see Fig. 3A).

Then the phylogenetic trees were constructed using the partial genome sequences of L2 DNA of the six HPV30 isolates identified in this study and the 18 NCBI downloaded HPV30 sequences. The classification result of L2 DNA was consistent with the result according to the full-length six isolates of HPV30

Finally, according to the phylogenetic tree of L2 DNA, among the nine HPV30 isolates (1-9) obtained in this study, six (sample 1, 4-6, and 8-9) belonged to lineage A1, two (sample 2 and 4) belonged to lineage A4, and the last one (sample 3) belonged to lineage A5, none belonged to lineage A2, A3 or lineage B (Fig. 3C).

Summary of the mutations of HPV30 isolates

Compared with the HPV30 prototype reference (GenBank: X74474), 87 variations in the nine HPV30 genomes identified in this study were observed (Table 3), including 79 substitutions, four insertions and four deletions. Among the six (sample 1, 3 and 6-9) which successfully retrieved HPV30 full-length sequences, the number of substitutions range from 12 to 39 (Table 2). Insertions and deletions were observed in E2, L2 and LCR, but not in other ORFs (E6, E7, E1, E5 and L1). The specific mutation sites of each isolate were also shown.

Table 2 Calculation of HPV30 DNA variations by genome region and open reading frames (ORFs)

Discussion

Co-infection both with HIV and HPV as well is an important issue. Compared to HIV-negative women, HIV-infected women have a higher prevalence of HPV^[14, 21–23]. Immune-suppression caused by HIV increases the persistence of HPV, and the risk for lesions and cancer in the cervix and other sites. As pregnancy is a unique immunological state, pregnancy is also associated with increased HPV prevalence, and with more rapid progression to intraepithelial lesion. HPV infections were 2.3-times more frequent in pregnant women than in non-pregnant women. Among the HPVs in the HIV positive women, the infection rate of HPV30 has increased^{1,2}. The prevalence of HPV30 even increased up to about 14.6% in the HIV positive women of South Africa and Kenya.² Two case of HPV30 positive sample was detected in 92 pregnant women¹⁷. These may suggest HPV30 could be an important HPV type in immunosuppressive women. Further researches are needed to analysis the role of HPV30 infection in the disease development of immunosuppressed people. Nowadays, none of the successfully marketed HPV vaccines protects against infection and disease related to HPV30. As the infection rate of HPV30 has increased in the HIV positive women, the vaccine needed to be redeveloped and HPV30 needed to be added in HIV positive women, for prolonging the rapid and slow progress of HIV infection in HIV and HPV30 co-infection women.

HPV30 is a relatively rarely reported genotype worldwide, with few data available to characterize its prevalence and lineage distribution, even in women with normal cervical cytology. This study speculates that the reason why HPV30 is less studied is due to the problem of HPV detecting. HPV typing is mostly based on the L1 sequences. The detection methods have mainly relied on PCR amplification by general primers which target the conserved regions of L1 gene of the virus genome, followed by HPV type identification of direct sequencing, or type-specific oligonucleotide probes hybridization. However, these consensus primers have limitations, particularly in the variability in detection sensitivity among different HPV types. Taken an example, MY09/11 PCR was able to detect 10 copies of HPV 31, but the detection limit was 10^2 for HPV16, and 10^4 for HPV52¹¹. As shown in Fig. 1, MY09/11 primer sets PCR lacked specificity for HPV30. We supposed that some HPV types like HPV30 could be missed in previous studies of which HPVs were detected by general primer sets. Next-generation sequencing (NGS) bypasses or reduces many problems due to PCR-based HPV detection approaches. By the use of NGS, the existence of HPV30 infection was also confirmed in lower genital tract of women^{2,18,19}. The technical limitations may partially explain the reason why some HPV types like HPV30 are not detected in clinical specimen.

In this study, by using the HPV30 type specific PCR targeting E6-E7 region (primer 1 and 2), the infection rate of HPV30 of 0.56% (9/1600) in China was determined. Whole genome and partial HPV30 genome of these nine isolates were obtained, which verifies the reliability of the existence of HPV30 infection in these specimens. This infection rate is basically consistent with the 0.5% infection rate of Thailand²⁰. Because of the samples collection, we did not get the samples with abnormal cervical cytology. But as the reference studies^{20,21}, the abnormal group could get a higher infection rate of HPV30 than the normal group.

HPV30 lineages classification was based on the complete genome analysis. The HPV phylogenetic tree in some previous researches were also based on L2 DNA sequences. Based on the analysis of L2 DNA, all HPV30 isolates in China suggested to belong into lineage A (A1, A4 and A5). The HPV30 isolates of Costa Rica distributed to all the A and B lineage groups. The diversities of lineages in different regions suggested the regional characteristic of HPV distribution. Refer to the carcinogenicity diversities of variants HPV16²², and other types⁸, although the carcinogenicity diversities of HPV30 intratype lineage has not been investigated because of the limitation of sample collection. The different risks of cancer progression of HPV30 lineages could be investigated in further studied.

In this study, the fragment deletion of HPV genome sequence in E2 or L2 genes was found in two different samples (E2 in sample 4, L2 in sample 5), which had not been reported in previous studies. The capsid protein of L2 is the minor capsid protein. L2 can be incorporated when co-expressed with L1²⁴. L2 contains a major cross-neutralization epitope in the N-terminus and represents the target of neutralizing antibodies²³. The sample 5 had an N-terminal truncated L2 (196 amino acids) compared with L2 of other isolates of HPV30 (463 amino acids). As the HPV L2 protein contains overlapping binding sites for neutralizing, cross-neutralizing and non-neutralizing antibodies among the N-terminal region²⁵. The L2 of sample 5 could be a naturally defective L2 lack of the of N-terminal function. The sample 4 had a C-base deletion mutation occurred at position 767 of E2 DNA, resulting in the amino acid sequence shift after the position 256. And this frame shift could be the loss of E2 protein function of C-terminal DNA binding.

Conclusion

Our study showed that the infection rate of HPV30 is 0.56% in China; all of the HPV30 isolates belongs to lineage A. Natural L2 and E2 defectives isolate of HPV30 were found. As HPV30 could be an important HPV type in immunosuppressive women, sequence diversity and phylogenies of HPV30 provide basis for future researches on discrete viral, epidemiology, evolution, pathogenicity, and even the vaccines strategy.

Abbreviations

CC: cervical cancer; HIV: Human immunodeficiency virus; HPV: Human papillomavirus; HPV16: Human papillomavirus type 16; HPV30: Human papillomavirus type 30; HPV53: Human papillomavirus type 53; HPV56: Human papillomavirus type 56; HPV66: Human papillomavirus type 66; HR-HPV: high risk HPV; LHR-HPV: low risk HPV; NGS: next generation sequencing; PCR: polymerase chain reaction; PHR-HPV: possible high-risk; TM: transmembrane.

Declarations

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Authors' contributions

Ying Li performed the experiments, analyzed the data and wrote the paper; Yifan Guo collected the samples and performed manuscript drafting; Zhan Wang, Hui Wang, Jiao Wang and Li Zhang performed data curation and manuscript drafting; Jiaosheng Li helped collected the samples and performed manuscript drafting; Hongtu Liu conceived and designed the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Each sample donor was informed of the study aims to obtain the informed consent. This study was approved by the Ethical Review Committee of National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention (ID No: IVDC2014-008). Women in this study signed a consent form before their participation.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

References

1. Rahman M, Sasagawa T, Yamada R, Kingoro A, Ichimura H, S. M. High prevalence of intermediate-risk human papillomavirus infection in uterine cervixes of Kenyan women infected with human immunodeficiency virus. *J Med Virol* 2011; **83**(11): 1988-96.
2. Meiring TL, Salimo AT, Coetzee B, et al. Next-generation sequencing of cervical DNA detects human papillomavirus types not detected by commercial kits. *Virology Journal* 2012; **9**(1): 164.
3. Bray F, Ferlay J, Laversanne M, et al. Cancer Incidence in Five Continents: Inclusion criteria, highlights from Volume X and the global status of cancer registration. *International Journal of Cancer* 2015; **137**(9): 2060-71.
4. Pilleron S, Cabaasag CJ, Ferlay J, et al. Cervical cancer burden in Latin America and the Caribbean: Where are we? *International Journal of Cancer* 2020.
5. Simms KT, Steinberg J, Caruana M, et al. Impact of scaled up human papillomavirus vaccination and cervical screening and the potential for global elimination of cervical cancer in 181 countries, 2020-99: a modelling study. *Lancet Oncol* 2019; **20**(3): 1-14.
6. Chen Z, Schiffman M, Herrero R, et al. Classification and evolution of human papillomavirus genome variants: Alpha-5 (HPV26, 51, 69, 82), Alpha-6 (HPV30, 53, 56, 66), Alpha-11 (HPV34, 73), Alpha-13 (HPV54) and Alpha-3 (HPV61). *Virology* 2018; **03**(516): 86-101.
7. Kahn T, Schwarz E, H. zH. Molecular cloning and characterization of the DNA of a new human papillomavirus (HPV 30) from a laryngeal carcinoma. *Int J Cancer* 1986; **37**(1): 61-5.
8. Burk RD, Harari A, Chen Z. Human papillomavirus genome variants. *Virology* 2013; **445**(1-2): 232-43.
9. Gravitt PE, Peyton CL, Alessi TQ, et al. Improved amplification of genital human papillomaviruses. *J Clin Microbiol* 2000; **38**(1): 357-61.
10. Coutlée F, Gravitt P, Kornegay J, et al. Use of PGM1 primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *J Clin Microbiol* 2002; **40**(3): 902-7.
11. Depuydt CE, Boulet GAV, Horvath CAJ, Benoy IH, Vereecken AJ, Bogers JJ. Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. *Journal of Cellular and Molecular Medicine* 2007; **11**(4): 881-91.
12. Resnick RM, Cornelissen MT, Wright DK, et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. *J Natl Cancer Inst* 1990; **82**(18): 1477-84.
13. Husnjak, K, Grce, M, Magdi, L, Paveli K. Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. *Journal of Virological Methods* 2000; **88**(2): 125-34.
14. Ola Forslund, Annika Antonsson, Peter Nordin, Stenquist B, Hansson BGr. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. *Journal of General Virology* 1999; **80**(9): 2437-43.
15. Sasagawa T, Mitsuishi T. Novel polymerase chain reaction method for detecting cutaneous human papillomavirus DNA. *Journal of Medical Virology* 2012; **84**(1): 138-44.
16. Van Doorslaer K, Li Z, Xirasagar S, et al. The Papillomavirus Episteme: a major update to the papillomavirus sequence database. *Nucleic Acids Research* 2017; **45**(D1): D499-D506.
17. Schneider A, Hotz M, Gissmann L. Increased prevalence of human papillomaviruses in the lower genital tract of pregnant women. *Int J Cancer* 1987; **40**(2): 198-201.

18. Siqueira JD, Alves BM, Prellwitz IM, et al. Identification of novel human papillomavirus lineages and sublineages in HIV/HPV-coinfected pregnant women by next-generation sequencing. *Virology* 2016; **6**(493): 202-8.
19. Molet L, Girlich D, Bonnin RA, et al. Identification by high-throughput sequencing of HPV variants and quasispecies that are untypeable by linear reverse blotting assay in cervical specimens. *Papillomavirus Res* 2019; **12**(8): 1-6.
20. Lurchachaiwong W, Junyangdikul P, Payungporn S, et al. Human papillomavirus genotypes among infected Thai women with different cytological findings by analysis of E1 genes. *New Microbiol* 2011; **34**(2): 147-56.
21. Ngabo F, Franceschi S, Baussano I, et al. Human papillomavirus infection in Rwanda at the moment of implementation of a national HPV vaccination programme. *BMC Infect Dis* 2016; **24**(16): 1-10.
22. Clifford GM, Tenet V, Georges D, et al. Human papillomavirus 16 sub-lineage dispersal and cervical cancer risk worldwide: Whole viral genome sequences from 7116 HPV16-positive women. *Papillomavirus Research* 2019; **7**: 67-74.
23. Wang JW, Roden RBS. L2, the minor capsid protein of papillomavirus. *Virology* 2013; **445**(1-2): 175-86.
24. Finnen ReL, Erickson KD, Chen XS, Garcea RL. Interactions between Papillomavirus L1 and L2 Capsid Proteins. *Journal of Virology* 2003; **77**(8): 4818-26.
25. Roden RBS, Yutzy WH, Fallon R, Inglis S, Lowy DR, Schiller JT. Minor Capsid Protein of Human Genital Papillomaviruses Contains Subdominant, Cross-Neutralizing Epitopes. *Virology* 2000; **270**(2): 254-7.

Figures

Figure 1

Amplification efficiency of HPV30 comparison with general primer sets. **A** PGMY09/11 PCR; **B** MY09/11 PCR; **C** PI PCR; **D** FAP59/64 PCR; **E** SKF/R PCR.

HPV16 was used as a control. Five sets of general primers targeting L1 gene including PGMY09/11, MY09/11, PI, FAP59/64 and SKF/R were used to detect HPV16 and HPV30 plasmids at the same time. (N) negative control; (L) 100 copies; (M) 1000 copies; (H) 10,000 copies; the numbers of 200, 300, 400 and 500 as well shown on the left of gel image stood for the 200 bp, 300 bp, 400 bp and 500 bp bands of DL500 DNA Marker; Arrow shown on the right of gel image stood for the expected amplicon of 450 bp, 480 bp and 214 bp respectively.

Figure 2

HPV30 was screened among 1600 samples as described in methods. **A** We performed the validation on type specific PCR primers targeting the HPV30 E6-E7 region. The experiment was repeated three times. The simulated sample consisted of 10 ng 293T DNA and plasmid containing HPV30 E6-E7 region. (N) negative control; (1) 100,000 copies; (2) 10,000 copies; (3) 1000 copies; (4) 100 copies; (5) 10 copies. **B** HPV30 was screened among the 1600 women. (N)

negative control; (P) positive control; (1-9) sample numbers 1-9. The numbers of 200 and 300 as well shown on the left of gel image stood for the 200 bp and 300 bp bands of DL500 DNA Marker; Arrow shown on the right of gel image stood for the expected amplicon of 240 bp.

Figure 3

Phylogenetic analysis of the L2 DNA of the HPV30 isolates. Phylogenetic analysis based on the Neighbor-Joining method of the relationships. Phylogenetic analyses were conducted in MEGA 6.0. **A** Sub-lineage classification was based on full genomes from a reported research. **B** The regions of L2 DNA sequence of the sixteen HPV30 isolates available at GenBank and six sequences (1, 3, 6-9) obtained in this study were analyzed. **C** The regions of L2 DNA sequence of the sixteen HPV30 isolates available at GenBank and nine sequences (1-9) obtained in this study were analyzed.

Figure 4

The mutation in the E2 ORF of sample 4. Original sequencing peak maps across the position 767 of the E2 gene of the nine HPV30 isolates were shown. The C base at position 767 of the E2 gene of sample 4 has a deletion mutation compared with the original HPV30 reference (X74474).

Figure 5

The mutation in the L2 ORF of sample 5. **A** The stop codon mutation in the L2 ORF of sample 5. Original sequencing peak map across the region containing C25T mutation of the nine HPV30 isolates was shown. The C to T substitution (C25T) was detected within the L2 gene of sample 5. Correspondingly, this substitution led to the formation of the stop codon (TGA) in the position of the ninth amino acid. **B** Original sequencing peak map across the region containing C insertion at the position of 493-497 mutation of the nine HPV30 isolates. The C insertion at the position of 493-497 (a five bp of CCCCC) of sample 5 compared with the original HPV30 reference (X74474).