View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Kanazawa University Repository for Academic Resources

E6 and E7 variants of human papillomavirus-16 and -52 in Japan, the Philippines, and Vietnam

著者	Ishizaki Azumi, Matsushita Kaori, Hoang Huyen Thi Thanh, Agdamag Dorothy M., Nguyen Cuong Hung, Tran Vuong Thi, Sasagawa Toshiyuki, Saikawa Kunikazu, Lihana Raphael, Pham Hung Viet, Bi Xiuqiong, Ta Van Thanh, Pham Thuc Van, Ichimura Hiroshi
journal or publication title	Journal of Medical Virology
volume	85
number	6
page range	1069-1076
year	2013-06-01
URL	http://hdl.handle.net/2297/34675

doi: 10.1002/jmv.23566

E6 and E7 variants of human papillomavirus-16 and -52 in Japan, the Philippines, and Vietnam

Azumi Ishizaki[1], Kaori Matsushita[1], Huyen Thi Thanh Hoang[1,2,3], Dorothy M. Agdamag[1], Cuong Hung Nguyen[1,3], Vuong Thi Tran[1,3], Toshiyuki Sasagawa[4], Kunikazu Saikawa[5], Raphael Lihana[1], Hung Viet Pham[1], Xiuqiong Bi[1], Van Thanh Ta[2], Thuc Van Pham[3], and Hiroshi Ichimura[1]

[1] Department of Viral infection and International Health,

Graduate school of Medical Science, Kanazawa University, Kanazawa, Japan

[2] Hanoi Medical University, Ha Noi, Viet Nam

[3] Haiphong Medical University, Hai Phong, Viet Nam

[4] Department of Reproductive and Perinatal Medicine, Kanazawa Medical University, Kanazawa, Japan

[3] Department of Morpho-Functional Pathology, Graduate school of Medical Science, Kanazawa University, Kanazawa, Japan

Corresponding author: Hiroshi Ichimura, M.D., Ph.D.

Department of Viral infection and International Health, Kanazawa University,

Graduate school of Medical Science, Japan.

13-1, Takaramachi, Kanazawa, Ishikawa, 9208640, Japan

Tel: +81-76-265-2229

Fax: +81-76-234-4237

E-mail: ichimura@med.kanazawa- u.ac.jp

Running head: E6 and E7 variants of HPV-16 and HPV-52 in Asia

ABSTRACT

Human papillomavirus (HPV) has several intragenotypic variants with different geographical and ethnic distributions. This study aimed to elucidate the distribution patterns of E6 and E7 (E6/E7) intragenotypic variants of HPV type 16 (HPV-16), which is most common worldwide, and HPV-52, which is common in Asian countries such as Japan, the Philippines, and Vietnam. In previous studies, genomic DNA samples extracted from cervical swabs were collected from female sex workers in these three countries and found to be positive for HPV-16 or HPV-52. Samples were amplified further for their E6/E7 genes using type-specific primers and analyzed genetically. Seventy-nine HPV-16 E6/E7 genes were analyzed successfully and grouped into three lineages: European (Prototype), European (Asian), and African-2. The prevalences of HPV-16 European (Prototype)/European (Asian) lineages were 19.4%/80.6% (n=31) in Japan, 75.0%/20.8% (n=24) in the Philippines, and 0%/95.8% (n=24) in Vietnam. The 109 HPV-52 E6/E7 genes analyzed successfully were grouped into four lineages, A to D; the prevalences of lineages A/B/C/D were respectively 5.1%/ 92.3%/0%/2.6% in Japan (n=39), 34.4%/62.5%/0%/3.1% in the Philippines (n=32), and 15.8%/73.7%/7.9%/2.6% in Vietnam (n=38). The distribution patterns of HPV-16 and HPV -52 lineages in these countries differed significantly ($p<0.000001$ and $p=0.0048$, respectively). There was no significant relationship between abnormal cervical cytology and either HPV-16 E6/E7 lineages or specific amino acid mutations, such as E6 D25E, E6 L83V, and E7 N29S. Analysis of HPV-16 and HPV-52 E6/E7 genes can be a useful molecular–epidemiological tool to distinguish geographical diffusion routes of these HPV types in Asia.

Key words: intragenotypic variants, geographical diffusion route, cervical cancer

INTRODUCTION

Genital human papillomavirus (HPV) infection is one of the most common infections transmitted sexually worldwide [Herrero et al., 2005; de Sanjosé et al., 2007; Bruni et al., 2010]. Cervical cancer is the second most frequent cancer among women, with about 530,000 new cases and 250,000 deaths occurring globally every year [WHO/ICO, 2010]. HPV is indicated as a necessary factor for cervical cancer and also recognized as being associated with other cancers, such as anogenital and nasopharyngeal cancers [Bouvard et al., 2009; zur Hausen, 2009; Arbyn et al., 2011].

HPV belongs to the family Papillomaviridae. More than 100 HPV genotypes based on L1 gene sequences have been identified [Schiffman et al., 2010]. Of these, 13 genotypes such as HPV-16, -18, -31, -52, and -58 are known to cause cervical cancer and designated as high-risk HPV genotypes. HPV-16 and HPV-18 account for 70% of invasive cervical cancer cases worldwide [Muñoz et al., 2003; Bouvard et al., 2009; Schiffman et al., 2009], and HPV-16 is also most common among women with normal cytology [de Sanjosé et al., 2007; Bao et al., 2008; Bruni et al., 2010]. Although HPV-16 has been reported to be the most prevalent type in Asia except for Japan [de Sanjosé et al., 2007; Bruni et al., 2010; Tsao et al., 2010; Chen et al., 2011a; Konno et al., 2011], recent epidemiological surveys have shown that HPV-52 is most prevalent among female sex workers in Japan, the Philippines, and Vietnam, followed by HPV-16 [Miyashita et al., 2009; Matsushita et al., 2011; Hoang et al., 2013].

The oncogenic functions of E6 and E7 proteins especially for HPV-16 have been studied extensively [Zehbe et al., 2009; Ghittoni et al., 2010; Moody and Laimins, 2010; Jabbar et al., 2012; Mesplède et al., 2012]. Most cervical HPV infections are eliminated mechanically and/or by host immunity before generating any pathological changes [Nobbenhuis et al., 2001; Schiffman et al., 2007;

Kjær et al., 2010; Moscicki et al. ; 2010; Rodríguez et al., 2010An et al., 2011]. During chronic HPV infection, E6 and E7 genes of the high-risk HPV types are integrated into host chromosomes, and uncontrolled expression of E6 and E7 proteins is induced, followed by malignant transformation [Jeon et al., 1995; zur Hausen, 2002; DeFilippis et al., 2003].

HPV-16 intragenotypes are classified into European and non-European lineages based largely on complete genome analyses [Smith et al., 2011]. In addition, HPV-16 intragenotypes based on E6 and E7 genes have been investigated and showed different global geographical distribution [Yamada et al., 1995; Yamada et al., 1997; Huertas-Salgado et al., 2011]. Some epidemiological studies have found that the HPV-16 non-European lineage is related more strongly to cervical cancer, but others found that the prevalent variants could differ by population [Chang et al., 2010; Huertas-Salgado et al., 2011; Lee et al., 2011; Tornesello et al., 2011; Zuna et al., 2011]. HPV-52 intragenotypes are categorized into four lineages, A to D, based on HPV-52 complete genome analysis [Chen et al., 2011b].

The association between E6 and E7 intragenotypic variations in other high-risk HPV types such as HPV-52 and cervical cancer is not understood well. In the current study, therefore, the E6 and E7 variations of HPV-16 and HPV-52, which circulate dominantly in Japan, the Philippines, and Vietnam, were investigated to elucidate the variant distribution patterns in these countries and to evaluate the association between intragenotypic variants and abnormal cervical cytology.

SUBJECTS AND METHODS

Subjects

In previous studies, genomic DNA was extracted from cervical swab samples collected from female sex workers who tested positive for HPV-16 (Japan: n=32; the Philippines: n=24; Vietnam: n=25) and/or HPV-52 (Japan: n=42; the Philippines: n=34; Vietnam: n＝39) [Miyashita et al., 2009; Matsushita et al., 2011; Hoang et al., 2013]. This DNA was analyzed further in the current work.

DNA amplification

The E6 and E7 genes of HPV-16 and HPV-52 were amplified with type-specific primers for HPV-16 E6 (5’-GAA ATC GGT TGA ACC GAA AC-3’ and 5’-GCA ATG TAG GTG TAT CTC CA-3’, nt 38 to 586 corresponding to the HPV-16 prototype; accession number: K02718, 549 bp); HPV-16 E7 (5’-GAC CGG TCG ATG TAT GTC TTG-3’ and 5’-CAT TAC ATC CCG TAC CCT CTT C-3’; nt 499 to 913, 415 bp); HPV-52 E6 (5’-GAA CAC AGT GTA GCT AAC GCA CG-3’ and 5’-TTG CTT TGT CTC CAC GCA TGA C-3’; nt 76 to 571 corresponding to the HPV-52 prototype; accession number: X74481, 496 bp) [Xin et al., 2001; Aho et al., 2003]; and HPV-52 E7 (5’-ACC TGT GAC CCA AGT GTA ACG TC-3’ and 5’-TCA AAC CAG CCT GTA CAT CCC T-3’; nt 530 to 919, 390 bp). The amplification was performed with AmpliTaq Gold (Applied Biosystems, Hammonton, NJ, USA) under the following conditions: one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 30 s; 50°C for HPV-16 E6, 53°C for HPV-16 E7, 60°C for HPV-52 E6, or 55°C for HPV-52 E7 for 30 s; and 72°C for 45 s, with a final extension at 72°C for 10 min.

Sequence analysis and determination of intragenotypic variants of HPV

The amplified products were sequenced directly and analyzed with an ABI PRISM 310 and/or a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with BigDye Terminator v1.1 (Applied Biosystems, Foster City, CA, USA). Obtained sequences were compared with the reference sequences of HPV-16 and -52 retrieved from GenBank. Multiple alignments were performed using ClustalW version 1.83 with minor manual adjustments. Phylogenic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates and visualized with the NJplot Win program. The HPV-16 E6 and E7 variants are categorized into European and non-European lineages [Huertas-Salgado et al., 2011; Smith et al., 2011]. The European lineage is classified further into European (prototype) and European (Asian) sublineages and their variants. The non-European lineage is subclassified into the African-1 and -2, Asian-American -1 and -2, and North American 1 sublineages. The HPV-52 E6 and E7 variants were categorized into four lineages: A, B, C, and D, [Chen et al., 2011b].

Analysis of cervical cytology

The E6 and E7 variants of HPV-16 and -52 were compared with the results of cervical cytology investigated in previous studies [Miyashita et al., 2009; Matsushita et al., 2011; Hoang et al., 2013]. A high-grade squamous intraepithelial lesion, or adenocarcinoma in situ was considered as abnormal cytology.

Statistical analysis

The Fisher's exact probability test or *Chi* square test was used for statistical analysis. P values obtained in all tests were considered to be significant when below 0.05..

RESULTS

HPV-16 E6 and E7 variants

A total of 79 HPV-16 strains (Japan: n=31; the Philippines: n=24; Vietnam: n=24) were analyzed successfully for both E6 and E7 regions (Figure 1A and Table I). Most of the HPV-16 strains belonged to the European lineage (97.5%), and only two strains, one each from the Philippines and Vietnam, belonged to the African-2 sublineage in the non- European lineage (2.5%). The prevalences of European (Prototype) and European (Asian) sublineages were respectively 19.4% and 80.6% in Japan, 75.0% and 20.8% in the Philippines, and 0% and 95.8% in Vietnam. The distribution patterns of HPV-16 intragenotypic variants differed significantly among these three countries ($p<0.000001$). An amino acid mutation of E6, E113D, was found in both European (Prototype) and European (Asian) sublineages, but mostly in Japanese strains (92.3%, n=14), and not at all in the Philippine strains ($p=0.000015$). The HPV-16 European (Prototype) sublineage with E6 L83V and E113D and E7 L28F (n=4) and the European (Asian) sublineage with E7 N29S without any mutation in E6 (n=8) were found only in Japanese strains. The HPV-16 European (Asian) sublineage with E6 D25E and without any mutation in E7 was found only in Vietnamese strains (n=3). The HPV-16 strains with E7 N29S, which was observed in European (Prototype), European (Asian), and African-2 sublineages, were more prevalent in Japanese (48.1%, n=52) and Vietnamese strains (40.4%) than in the Philippine strains (11.5%) ($p=0.000004$).

HPV-52 E6 and E7 variants

A total of 109 HPV-52 strains (Japan: n=39; the Philippines: n=32; Vietnam: n=38) were successfully analyzed for both E6 and E7 regions (Figure 1B and Table II). Phylogenetic analysis revealed that the HPV-52 strains were grouped into four lineages, A through D. The prevalences of lineages A, B, C, and D were respectively 5.1%, 92.3%, 0%, and 2.6% in Japan; 34.4%, 62.5%, 0%, and 3.1% in the Philippines; and 15.8%, 73.7%, 7.9%, and 2.6% in Vietnam. The distribution pattern of HPV-52 intragenetypic variants differed significantly among these three countries (p=0.0048). Lineage C was found only among Vietnamese strains (n=3).

Distributions of intragenotypic variants in abnormal cervical cytology

The cases with abnormal cervical cytology were found in those infected with HPV-16 European (Prototype) (2/24, 8.3%) and European (Asian) (4/53, 7.5%) sublineages, and in those infected with HPV-52 lineages A (0/19, 0%) and B (4/84, 4.8%) (Tables I and II). There was no significant association between abnormal cervical cytology and intragenotypic variants of HPV-16 (p=1.000) and HPV-52 (p=1.000). The cases with abnormal cervical cytology were found in those infected with HPV-16 (n=79) with the amino acid mutation of E6 D25E (4/42, 9.5%) and without the mutation (2/37, 5.4%); with the amino acid mutation of E6 L83V (0/14, 0%) and without the mutation (6/65, 9.2%); and with the amino acid mutation of E7 N29S (4/52, 7.7%) and without the mutation (2/27, 7.4%) (Table I). There was no significant association between abnormal cervical cytology and the specific amino acid mutations of HPV-16 E6 D25E (p=0.617), L83V (p=0.237) and E7 N29S (p=0.964). The cases with abnormal cervical cytology were found in those infected with HPV-52 (n=109) with the amino acid mutation of E6 K93R (4/84, 4.8%) and without the mutation (0/25, 0%)(Table II). There

was no significant association between abnormal cervical cytology and the amino acid mutation of HPV-52 E6 K93R (p=0.572). Only one case of adenocarcinoma in situ, which was positive for HPV-52 lineage B, was found in Japan. Therefore, the relationship between intragenotypic variation and cancer could not be analyzed in the current study.

Sequence data

The sequences described in this report have been deposited in GenBank/EMBL/DDBJ under accession numbers AB663688 to AB664063.

DISCUSSION

The E6 and E7 intragenotypic variants of HPV-16 and HPV-52 showed significant differences in their distribution patterns among Japan, the Philippines, and Vietnam, even though a similar HPV genotype distribution profile based on L1 regions has been observed in these countries [Miyashita et al., 2009; Matsushita et al., 2011; Hoang et al., 2013]. Although the HPV-16 European lineage was more prevalent than the non-European lineage, the proportion of European sublineages differed among these three countries; the prevalence of the HPV-16 European (Asian) sublineage was higher in Japan and Vietnam than in the Philippines, and no European (Prototype) sublineage was found in Vietnam. The proportion of European (Prototype) and European (Asian) sublineages in Japan (19.4% and 80.6%, respectively) was similar to that in China and Korea while the proportion in the Philippines (75.0% and 20.8%, respectively) was similar to that in Australia and New Caledonia [Lee et al., 2011; Tornesello et al., 2011]. The HPV-52 lineage B showed a significantly higher prevalence in Japan (92.3%) compared to the Philippines (62.5%) and Vietnam (73.7%). The HPV-52 lineage B is prevalent in Asian countries such as China (100% of the HPV-52 strains isolated) and Taiwan (88.2%), but less so in Canada (13.0%), and undetectable in other countries [Xin et al., 2001; Aho et al., 2003; Chang et al., 2010; Ding et al., 2010]. The HPV-52 lineage C was found only in Vietnam in the current study but has been reported from Asian countries such as China (21.1%) and Taiwan (10.9%), and rarely from other areas of the world [Aho et al., 2003; Calleja-Macias et al., 2005; Raiol et al., 2009; Ding et al., 2010; Chang et al., 2010; Chen et al., 2011b].

The E6 and E7 amino acid mutations found in the HPV-16 European sublineage and HPV-52 lineage isolated from Japan, the Philippines, and Vietnam showed distinct differences in their

distribution patterns, as well. Most of the HPV-16 variants with E6 E113D were found in Japan (92.3%) in the current study. This variant has been identified also in other East Asian countries, such as China (8.0 to 14.5% of all HPV-16 strains isolated in the reports), Korea (3.5 to 5.4%), and Hong Kong (3.1%), but very seldom in other areas [Chan et al., 2002; de Boer et al., 2004; Tornesello et al., 2004; Choi et al., 2007; Qiu et al., 2007; Lurchachaiwong et al., 2009 ; Lee et al., 2011]. The HPV-16 European (Prototype) variant with E6 L83V, D113D, and E7 L28F was found only in Japan in the current study and has been reported in Thailand [Lurchachaiwong et al., 2009]. The HPV-16 variant with E7 N29S, one of the essential mutations for the European (Asian) sublineages, was identified in 80.6% of Japanese HPV-16 strains (n=31). This variant is prevalent mainly in East Asia in China (70.2%), Korea (53.2 to 73.0%), and Hong Kong (58.0%), followed by Indonesia (22.7%), Thailand (14.3%), and India (1.7 to 37.8%) [Chan et al., 2002; Tornesello et al., 2004; Chopjitt et al., 2009; Lurchachaiwong et al., 2009; Vrtačnik Bokal et al., 2010; Lee et al., 2011]. The European (Asian) sublineage with E6 D25E was found only in Vietnam in the current study. This variant is rare and has been reported previously only in Southeast Asia (5.7%), China (1.8%), Japan (1.2%), and Hong Kong (0.8%) [Huertas-Salgado et al., 2011; Sun et al., 2012].

The distribution patterns of the E6 and E7 intragenotypic variants and specific amino acid mutations of HPV-16 and HPV-52 found in Japan, the Philippines, and Vietnam in the current study and those reported in previous studies suggest the possible association of HPV strains between Japan and the East Asian continent, and between the Philippines and Oceania. Because HPV is considered to have spread globally along with human migration and because human gene polymorphisms are the main driving force for HPV evolution [Calleja-Macias et al., 2005; Bernard et al., 2006; Sun et al.,

2012], these findings may confirm the relationship between the geographical routes of HPV diffusion and distinctive human migration in Asia [Stoneking and Delfin, 2010].

Some specific amino acid mutations in HPV-16 E6 have been reported to be associated with a greater capacity for carcinogenesis. The European (Asian) sublineage characterized by D25E [Sun et al., 2012], European (Prototype) sublineage with L83V, non-European lineage with Q14H/H78Y/L83V (corresponding to the African-1 and -2, Asian-American-1 and -2, and North American 1 sublineages) [Lizano et al., 2009; Zehbe et al., 2009; Richard et al., 2010; Schiffman et al., 2010; Chansaenroj et al., 2012], and HPV-16 E7 N29S mutations are considered to be related to the development of cervical cancer in Asian populations [Chan et al., 2002; Choi et al., 2007; Lee et al., 2011; Chansaenroj et al., 2012]. However, no significant correlation between abnormal cervical cytology and intragenotypic variations of either HPV-16 or -52 sublineages or between abnormal cervical cytology and the specific amino acid mutations at HPV-16 E6 D25E, E6 L83V, and E7 N29S was observed in the current study. This absence could be due to the small number of study subjects or the small number of cervical cancer cases and abnormal cervical cytology. Further analysis for HPV E6 and E7 intragenotypic variants among cervical cancer patients is needed to elucidate the real risk of their carcinogenesis.

In conclusion, this report is the first regarding genetic variations in E6 and E7 genes of HPV-16 and HPV-52 in Japan, the Philippines, and Vietnam. The E6 and E7 intragenotypic variant distributions of HPV-16 and HPV-52 differed significantly among these three countries, although similar HPV genotypes profiles based on L1 regions were observed. The fact that distribution patterns of European (Prototype) and European (Asian) sublineages among Japanese strains were not similar to

those of the Philippines but were similar to those in China and Korea may suggest human migration and HPV diffusion routes in Japan that are distinct from those in the Philippines. Thus, E6 and E7 intragenotypic variant analysis for HPV-16 and HPV-52 can be a useful epidemiological marker to investigate HPV diffusion routes in Asia. Further analysis for E6 and E7 genes of HPV-16 and HPV-52 isolated from cervical cancer patients would be necessary to understand the real risk of their intragenotypic variants for carcinogenesis in Asian countries.

ACKNOWLEDGMENTS

The authors are grateful to all of the study participants. For this study, the first author was awarded the prize for encouragement from the Japanese Association for Infectious Disease, Central Japan Branch, in November 2011.

1

2

REFERENCES

Aho J, Hankins C, Tremblay C, Lang F, Forest P, Pourreaux K, Rouah F, Coutlée F, Group CWsHS. 2003. Molecular analysis of human papillomavirus type 52 isolates detected in the genital tract of human immunodeficiency virus-seropositive and -seronegative women. J Infect Dis 188:1517-1527.

An HJ, Sung JM, Park AR, Song KJ, Lee YN, Kim YT, Cha YJ, Kang S, Cho NH. 2011. Prospective evaluation of longitudinal changes in human papillomavirus genotype and phylogenetic clade associated with cervical disease progression. Gynecol Oncol 120:284-290.

Arbyn M, Castellsagué X, de Sanjosé S, Bruni L, Saraiya M, Bray F, Ferlay J. 2011. Worldwide burden of cervical cancer in 2008. Ann Oncol 22:2675-2686.

Bao YP, Li N, Smith JS, Qiao YL, members A. 2008. Human papillomavirus type distribution in women from Asia: a meta-analysis. Int J Gynecol Cancer 18:71-79.

Bernard HU, Calleja-Macias IE, Dunn ST. 2006. Genome variation of human papillomavirus types: phylogenetic and medical implications. Int J Cancer 118:1071-1076.

Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V, Group WIAfRoCMW. 2009. A review of human carcinogens--Part B: biological agents. Lancet Oncol 10:321-322.

Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. 2010. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 202:1789-1799.

23 Calleja-Macias IE, Villa LL, Prado JC, Kalantari M, Allan B, Williamson AL, Chung LP, Collins RJ,
24 Zuna RE, Dunn ST, Chu TY, Cubie HA, Cuschieri K, von Knebel-Doeberitz M, Martins CR,
25 Sanchez GI, Bosch FX, Munoz N, Bernard HU. 2005. Worldwide genomic diversity of the
26 high-risk human papillomavirus types 31, 35, 52, and 58, four close relatives of human
27 papillomavirus type 16. J Virol 79:13630-13640.
28 Chan PK, Lam CW, Cheung TH, Li WW, Lo KW, Chan MY, Cheung JL, Xu LY, Cheng AF. 2002.
29 Human papillomavirus type 16 intratypic variant infection and risk for cervical neoplasia in
30 southern China. J Infect Dis 186:696-700.
31 Chang YJ, Chen HC, Lee BH, You SL, Lin CY, Pan MH, Chou YC, Hsieh CY, Chen YM, Cheng YJ,
32 Chen CJ, Group CHS. 2011. Unique variants of human papillomavirus genotypes 52 and 58
33 and risk of cervical neoplasia. Int J Cancer 129:965-973.
34 Chansaenroj J, Theamboonlers A, Junyangdikul P, Swangvaree S, Karalak A, Poovorawan Y. 2012.
35 Whole genome analysis of human papillomavirus type 16 multiple infection in cervical cancer
36 patients. Asian Pac J Cancer Prev 13:599-606.
37 Chen HC, You SL, Hsieh CY, Schiffman M, Lin CY, Pan MH, Chou YC, Liaw KL, Hsing AW, Chen
38 CJ, Group C-HS. 2011a. Prevalence of genotype-specific human papillomavirus infection and
39 cervical neoplasia in Taiwan: a community-based survey of 10,602 women. Int J Cancer
40 128:1192-1203.
41 Chen Z, Schiffman M, Herrero R, Desalle R, Anastos K, Segondy M, Sahasrabuddhe VV, Gravitt PE,
42 Hsing AW, Burk RD. 2011b. Evolution and Taxonomic Classification of Human

Papillomavirus 16 (HPV16)-Related Variant Genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67. PLoS One 6:e20183.

Choi BS, Kim SS, Yun H, Jang DH, Lee JS. 2007. Distinctive distribution of HPV16 E6 D25E and E7 N29S intratypic Asian variants in Korean commercial sex workers. J Med Virol 79:426-430.

Chopjitt P, Ekalaksananan T, Pientong C, Kongyingyoes B, Kleebkaow P, Charoensri N. 2009. Prevalence of human papillomavirus type 16 and its variants in abnormal squamous cervical cells in Northeast Thailand. Int J Infect Dis 13:212-219.

de Boer M, Peters L, Aziz M, Siregar B, Cornain S, Vrede M, Jordanova E, Kolkman-Uljee S, Fleuren G. 2004. Human papillomavirus type 16 E6, E7, and L1 variants in cervical cancer in Indonesia, Suriname, and The Netherlands. Gynecol Oncol 94:488-494.

de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch F. 2007. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 7:453-459.

DeFilippis RA, Goodwin EC, Wu L, DiMaio D. 2003. Endogenous human papillomavirus E6 and E7 proteins differentially regulate proliferation, senescence, and apoptosis in HeLa cervical carcinoma cells. J Virol 77:1551-1563.

Ding T, Wang X, Ye F, Cheng X, Ma D, Lu W, Xie X. 2010. Distribution of human papillomavirus 58 and 52 E6/E7 variants in cervical neoplasia in Chinese women. Gynecol Oncol 119:436-443.

Ghittoni R, Accardi R, Hasan U, Gheit T, Sylla B, Tommasino M. 2010. The biological properties of E6 and E7 oncoproteins from human papillomaviruses. Virus Genes 40:1-13.

63 Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, Alfaro M, Sherman ME,
64 Wacholder S, Chen S, Rodriguez AC, Burk RD. 2005. Epidemiologic profile of type-specific
65 human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis
66 191:1796-1807.
67 Hoang HT, Ishizaki A, Nguyen CH, Tran VT, Matsushita K, Saikawa K, Hosaka N, Pham HV, Bi X,
68 Ta VT, Van Pham T, Ichimura H. 2013. Infection with high-risk HPV types among female sex
69 workers in northern Vietnam. J Med Virol 85:288-294..
70 Huertas-Salgado A, Martín-Gámez DC, Moreno P, Murillo R, Bravo MM, Villa L, Molano M. 2011.
71 E6 molecular variants of human papillomavirus (HPV) type 16: an updated and unified
72 criterion for clustering and nomenclature. Virology 410:201-215.
73 Jabbar SF, Park S, Schweizer J, Berard-Bergery M, Pitot HC, Lee D, Lambert PF. 2012. Cervical
74 cancers require the continuous expression of the human papillomavirus type 16 e7 oncoprotein
75 even in the presence of the viral e6 oncoprotein. Cancer Res 72:4008-4016.
76 Jeon S, Allen-Hoffmann BL, Lambert PF. 1995. Integration of human papillomavirus type 16 into the
77 human genome correlates with a selective growth advantage of cells. J Virol 69:2989-2997.
78 Kjær SK, Frederiksen K, Munk C, Iftner T. 2010. Long-term absolute risk of cervical intraepithelial
79 neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J
80 Natl Cancer Inst 102:1478-1488.
81 Konno R, Tamura S, Dobbelaere K, Yoshikawa H. 2011. Prevalence and type distribution of human
82 papillomavirus in healthy Japanese women aged 20 to 25 years old enrolled in a clinical study.
83 Cancer Sci 102:877-882.

84 Lee CW, Bae JH, Lee SJ, Ho EM, Lee IH, Park YG, Park JS. 2011. Distribution of human
85 papillomavirus type 16 E6 and E7 gene variants in the progression of cervical dysplasia in
86 Korean women. J Obstet Gynaecol Res 37:1320-1326.
87 Lizano M, Berumen J, García-Carrancá A. 2009. HPV-related carcinogenesis: basic concepts, viral
88 types and variants. Arch Med Res 40:428-434.
89 Lurchachaiwong W, Junyangdikul P, Payungporn S, Chansaenroj J, Sampathanukul P, Tresukosol D,
90 Termrungruanglert W, Theamboonlers A, Poovorawan Y. 2009. Entire genome
91 characterization of human papillomavirus type 16 from infected Thai women with different
92 cytological findings. Virus Genes 39:30-38.
93 Matsushita K, Sasagawa T, Miyashita M, Ishizaki A, Morishita A, Hosaka N, Saikawa K, Hoshina S,
94 Bi X, Ichimura H. 2011. Oral and cervical human papillomavirus infection among female sex
95 workers in Japan. Jpn J Infect Dis 64:34-39.
96 Mesplède T, Gagnon D, Bergeron-Labrecque F, Azar I, Sénéchal H, Coutlée F, Archambault J. 2012.
97 p53 degradation activity, expression, and subcellular localization of E6 proteins from 29 human
98 papillomavirus genotypes. J Virol 86:94-107.
99 Miyashita M, Agdamag D, Sasagawa T, Matsushita K, Salud L, Salud C, Saikawa K, Leano P,
100 Pagcaliwagan T, Acuna J, Ishizaki A, Kageyama S, Ichimura H. 2009. High-risk HPV types in
101 lesions of the uterine cervix of female commercial sex workers in the Philippines. J Med Virol
102 81:545-551.
103 Moody CA, Laimins LA. 2010. Human papillomavirus oncoproteins: pathways to transformation. Nat
104 Rev Cancer 10:550-560.

105 Moscicki AB, Ma Y, Wibbelsman C, Darragh TM, Powers A, Farhat S, Shiboski S. 2010. Rate of and
106 risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women.
107 Obstet Gynecol 116:1373-1380.
108 Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ,
109 Group IAfRoCMCCS. 2003. Epidemiologic classification of human papillomavirus types
110 associated with cervical cancer. N Engl J Med 348:518-527.
111 Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Voorhorst FJ, Bezemer PD,
112 Verheijen RH, Meijer CJ. 2001. Cytological regression and clearance of high-risk human
113 papillomavirus in women with an abnormal cervical smear. Lancet 358:1782-1783.
114 Qiu AD, Wu EQ, Yu XH, Jiang CL, Jin YH, Wu YG, Chen Y, Shan YM, Zhang GN, Fan Y, Zha X,
115 Kong W. 2007. HPV prevalence, E6 sequence variation and physical state of HPV16 isolates
116 from patients with cervical cancer in Sichuan, China. Gynecol Oncol 104:77-85.
117 Raiol T, Wyant PS, de Amorim RM, Cerqueira DM, Milanezi NG, Brígido MeM, Sichero L, Martins
118 CR. 2009. Genetic variability and phylogeny of the high-risk HPV-31, -33, -35, -52, and -58 in
119 central Brazil. J Med Virol 81:685-692.
120 Richard C, Lanner C, Naryzhny SN, Sherman L, Lee H, Lambert PF, Zehbe I. 2010. The
121 immortalizing and transforming ability of two common human papillomavirus 16 E6 variants
122 with different prevalences in cervical cancer. Oncogene 29:3435-3445.
123 Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, Solomon D, Guillén D,
124 Alfaro M, Morales J, Hutchinson M, Katki H, Cheung L, Wacholder S, Burk RD. 2010.

125 Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia
126 grade 2/3: critical role of duration of infection. J Natl Cancer Inst 102:315-324.
127 Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. 2007. Human papillomavirus and
128 cervical cancer. Lancet 370:890-907.
129 Schiffman M, Clifford G, Buonaguro F. 2009. Classification of weakly carcinogenic human
130 papillomavirus types: addressing the limits of epidemiology at the borderline. Infect Agent
131 Cancer 4:8.
132 Schiffman M, Rodriguez A, Chen Z, Wacholder S, Herrero R, Hildesheim A, Desalle R, Befano B, Yu
133 K, Safaeian M, Sherman M, Morales J, Guillen D, Alfaro M, Hutchinson M, Solomon D,
134 Castle P, Burk R. 2010. A population-based prospective study of carcinogenic human
135 papillomavirus variant lineages, viral persistence, and cervical neoplasia. Cancer Res
136 70:3159-3169.
137 Smith B, Chen Z, Reimers L, van Doorslaer K, Schiffman M, Desalle R, Herrero R, Yu K, Wacholder
138 S, Wang T, Burk RD. 2011. Sequence Imputation of HPV16 Genomes for Genetic Association
139 Studies. PLoS One 6:e21375.
140 Stoneking M, Delfin F. 2010. The human genetic history of East Asia: weaving a complex tapestry.
141 Curr Biol 20:R188-193.
142 Sun M, Gao L, Liu Y, Zhao Y, Wang X, Pan Y, Ning T, Cai H, Yang H, Zhai W, Ke Y. 2012. Whole
143 genome sequencing and evolutionary analysis of human papillomavirus type 16 in central
144 China. PLoS One 7:e36577.

145 Tornesello ML, Duraturo ML, Salatiello I, Buonaguro L, Losito S, Botti G, Stellato G, Greggi S,
146 Piccoli R, Pilotti S, Stefanon B, De Palo G, Franceschi S, Buonaguro FM. 2004. Analysis of
147 human papillomavirus type-16 variants in Italian women with cervical intraepithelial neoplasia
148 and cervical cancer. J Med Virol 74:117-126.
149 Tornesello ML, Losito S, Benincasa G, Fulciniti F, Botti G, Greggi S, Buonaguro L, Buonaguro FM.
150 2011. Human papillomavirus (HPV) genotypes and HPV16 variants and risk of
151 adenocarcinoma and squamous cell carcinoma of the cervix. Gynecol Oncol 121:32-42.
152 Tsao KC, Huang CG, Kuo YB, Chang TC, Sun CF, Chang CA, Yang SL, Chan EC. 2010. Prevalence
153 of human papillomavirus genotypes in northern Taiwanese women. J Med Virol
154 82:1739-1745.
155 Vrtačnik Bokal E, Kocjan BJ, Poljak M, Bogovac Z, Jančar N. 2010. Genomic variants of human
156 papillomavirus genotypes 16, 18, and 33 in women with cervical cancer in Slovenia. J Obstet
157 Gynaecol Res 36:1204-1213.
158 WHO/ICO. 2010. Human Papillomavirus and Related Cancers in World. Summary Report 2010.
159 WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre).
160 Xin C, Matsumoto K, Yoshikawa H, Yasugi T, Onda T, Nakagawa S, Yamada M, Nozawa S, Sekiya S,
161 Hirai Y, Shiromizu K, Fujii T, Taketani Y. 2001. Analysis of E6 variants of human
162 papillomavirus type 33, 52 and 58 in Japanese women with cervical intraepithelial
163 neoplasia/cervical cancer in relation to their oncogenic potential. Cancer Lett 170:19-24.

Yamada T, Wheeler CM, Halpern AL, Stewart AC, Hildesheim A, Jenison SA. 1995. Human papillomavirus type 16 variant lineages in United States populations characterized by nucleotide sequence analysis of the E6, L2, and L1 coding segments. J Virol 69:7743-7753.

Yamada T, Manos MM, Peto J, Greer CE, Munoz N, Bosch FX, Wheeler CM. 1997. Human papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. J Virol 71(3):2463-2472.Zehbe I, Richard C, DeCarlo C, Shai A, Lambert P, Lichtig H, Tommasino M, Sherman L. 2009. Human papillomavirus 16 E6 variants differ in their dysregulation of human keratinocyte differentiation and apoptosis. Virology 383:69-77.

Zuna RE, Tuller E, Wentzensen N, Mathews C, Allen RA, Shanesmith R, Dunn ST, Gold MA, Wang SS, Walker J, Schiffman M. 2011. HPV16 variant lineage, clinical stage, and survival in women with invasive cervical cancer. Infect Agent Cancer 6:19.

zur Hausen H. 2002. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2:342-350.

zur Hausen H. 2009. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 384:260-265.

FIGURE LEGENDS

Figure 1. Phylogenetic analysis of HPV-16 and HPV-52 strains isolated from Japan, the Philippines, and Vietnam based on E6 and E7 sequences. (A) Phylogenetic analysis of 79 HPV-16 strains based on E6 and E7 sequences (776 bp) from Japan, the Philippines, and Vietnam. HPV-16 strains are classified into European and non-European lineages. The European lineage is classified further into European (Prototype) and European (Asian) sublineages. (B) Phylogenetic analysis of 109 HPV-52 strains based on E6 and E7 sequences (751 bp) from Japan, the Philippines, and Vietnam. HPV-52 was classified into four groups: lineages A, B, C, and D. Closed circles: strains from Japan; triangles: strains from the Philippines; open squares: strains from Vietnam. Bootstrap values greater than 700 are shown.

Table I. Intratypic variations of HPV-16 E6 and E7 nucleotide and their associated amino acid positions with the result of abnormal cytology.

	Country N (Total 79)			E6 nucleotide positions and their variants																							E7 nucleotide positions and their variants									Amino acid positions and their variants		Abnormal cytology[b] N (Total 6)		
Group[a]	J 31	P 24	V 24	83	93	94	95	109	132	143	145	178	241	245	256	257	262	286	289	335	350	361	403	442	525	532	645	647	663	666	732	789	795	843	846	E6 region	E7 region	J 2	P 3	V 1
European (Prototype)	1	7	0	A	A	G	A	T	G	C	G	T	T	C	C	A	A	T	A	C	T	A	A	A	G	A	A	A	G	G	T	T	T	T	T			0	2	0
	0	1	0	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			0	0	0
	0	1	0	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R48W		0	0	0
	1	5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L83V[c]		0	0	0
	0	1	0	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L83V		0	0	0
	0	2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	g	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L83V		0	0	0
	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I52V L83V		0	0	0
	4	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	C	-	-	C	-	-	-	-	-	-	-	-	L83V E113D	L28F	0	0	0
European (Asian)	5	5	14	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	c	D25E[c]	N29S[c]	1	1	1
	1	0	0	-	-	-	-	-	-	-	-	G	-	-	t	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	c	D25E	N29S	0	0	0
	1	0	0	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	G	-	-	-	-	-	-	c	D25E	N29S	0	0	0
	0	0	5	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	c	c	D25E	N29S	0	0	0
	1	0	0	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	G	-	-	-	-	-	-	c	D25E R141T	N29S	0	0	0
	8	0	0	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	G	-	-	-	-	-	-	c	D25E E113D	N29S	1	0	0
	1	0	1	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	G	a	-	-	-	-	-	c	D25E E113D	N29S	0	0	0
	6	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-		N29S	0	0	0
	1	0	0	-	-	-	-	-	-	-	-	-	g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-		N29S	0	0	0
	1	0	0	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	c		N29S	0	0	0
	0	0	3	-	-	a	g	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	a	-	-	-	-	-	D25E		0	0	0
African type-2	0	1	1	-	-	-	-	c	T	G	T	-	-	-	-	-	-	a	g	T	-	-	g	-	-	-	-	G	-	-	-	c	g	-	-	R10I Q14D H78Y	N29S	0	0	0

J: Japan; P: the Philippines; V: Vietnam. Capital letters in nucleotide columns indicate the variant accompanied by amino acid changes.

[a]The prevalence of the intratypic variations among three countries was significantly different ($P < 0.000001$).

[b]The prevalence of the abnormal cytology among different intratypic variations was not different ($P = 1.000$)

[c]No significant relationship between abnormal cervical cytology and specific amino acid mutations was observed; HPV-16 E6 D25E, $P = 0.617$; L83V, $P = 0.237$; HPV-16 E7 N29S, $P = 0.964$.

Table II. Intratypic variation of HPV-52 E6 and E7 nucleotide and their associated amino acids positions with the result of abnormal cytology.

Group[a]	Country N (Total 109)			E6 nucleotide positions and their variants											E7 nucleotide positions and their variants												Amino acid positions and their variants		Abnormal cytology[c] N (Total 4)		
	J	P	V	1	2	2	2	3	3	3	3	4	4	5	5	5	6	7	7	7	7	7	7	7	7	8	8	E6 region	E7 region	J	P	V
				7	0	3	5	5	5	7	7	2	6	3	7	9	6	0	0	2	2	3	4	5	6	0	4					
	39	32	38	1	0	7	1	0	6	8	9	5	7	0	3	5	2	6	7	7	8	3	2	1	6	1	8			2	2	0
Lineage A	2	8	6	C	C	C	A	G	G	A	A	G	C	A	T	C	C	A	G	T	A	C	G	C	C	A	T			0	0	0
	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	-	-	-			0	0	0
	0	1	0	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H24D		0	0	0
	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	g	-			Y59C	0	0	0
Lineage B	32	14	23	-	-	-	-	t	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	t	-	g	-	K93R[d]		1[b]	2	0
	1	3	3	-	-	-	-	t	a	-	G	-	-	-	-	-	-	-	-	-	-	-	-	t	-	g	-	K93R		0	0	0
	0	2	0	-	-	-	-	t	-	-	G	-	-	-	-	t	-	-	-	-	-	-	-	t	-	g	-	K93R		0	0	0
	0	1	0	-	-	-	g	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K93R		0	0	0
	2	0	1	-	-	-	-	t	-	G	G	-	-	-	-	-	-	-	-	-	-	-	-	t	-	g	-	K93G		1	0	0
	1	0	0	-	-	-	-	t	-	C	G	-	A	-	-	-	-	-	-	-	-	-	-	t	-	g	-	K93R	N122K	0	0	0
	0	0	1	-	-	-	-	t	-	-	G	-	-	-	-	-	-	-	-	-	-	-	A	t	-	g		K93R	L99R	0	0	0
Lineage C	0	0	3	-	-	-	-	t	-	-	-	-	-	g	a	-	T	G	A	G	-	T	A	-	-	g	G		T37I S52D Y59D H61Y D64N L99R	0	0	0
Lineage D	1	1	1	-	t	t	-	t	-	-	-	t	-	-	c	-	-	-	-	-	-	-	-	A	g	-			H72N	0	0	0

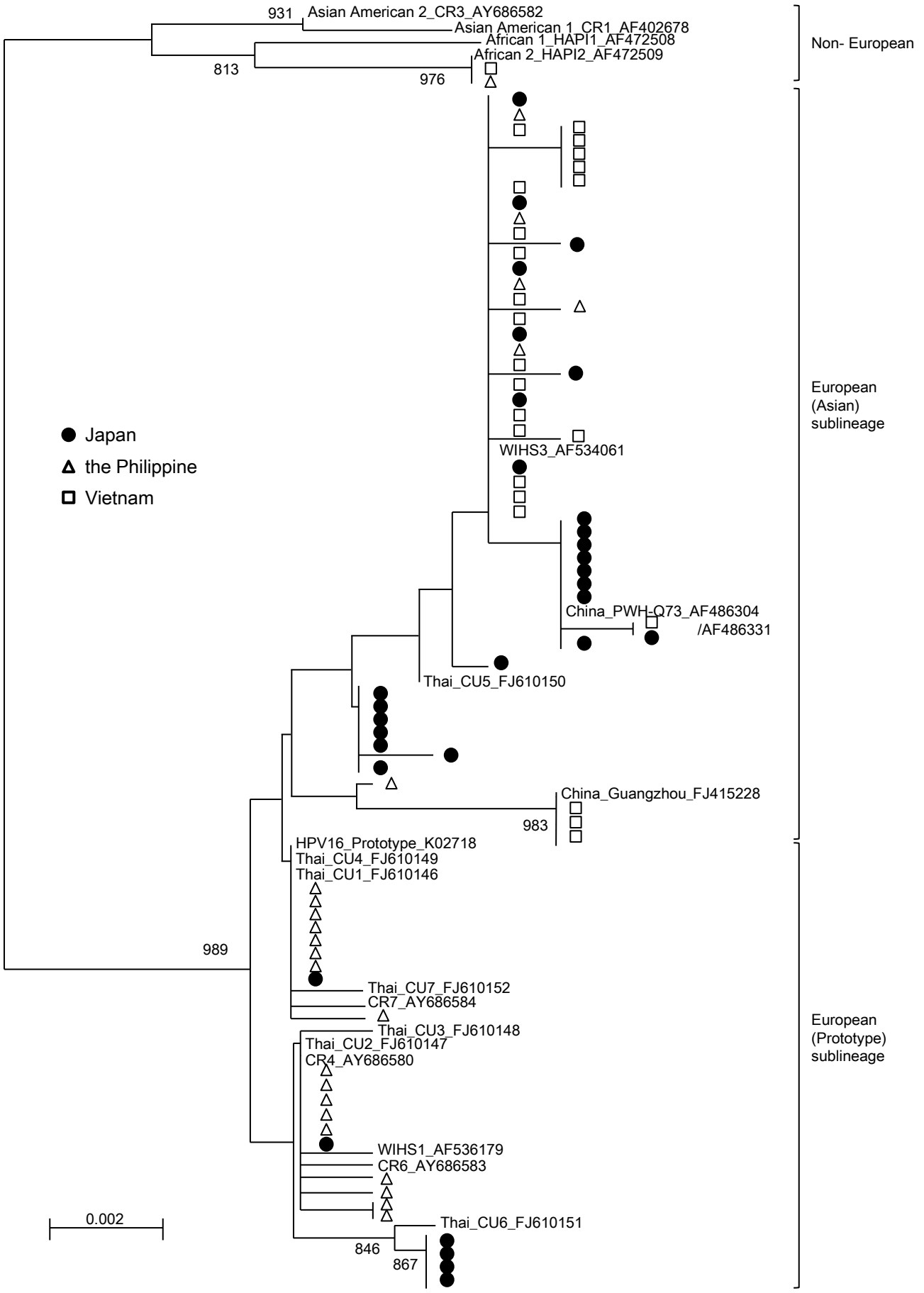
J: Japan; P: the Philippines; V: Vietnam. Capital letters in nucleotide columns indicate the variant accompanied by amino acid changes

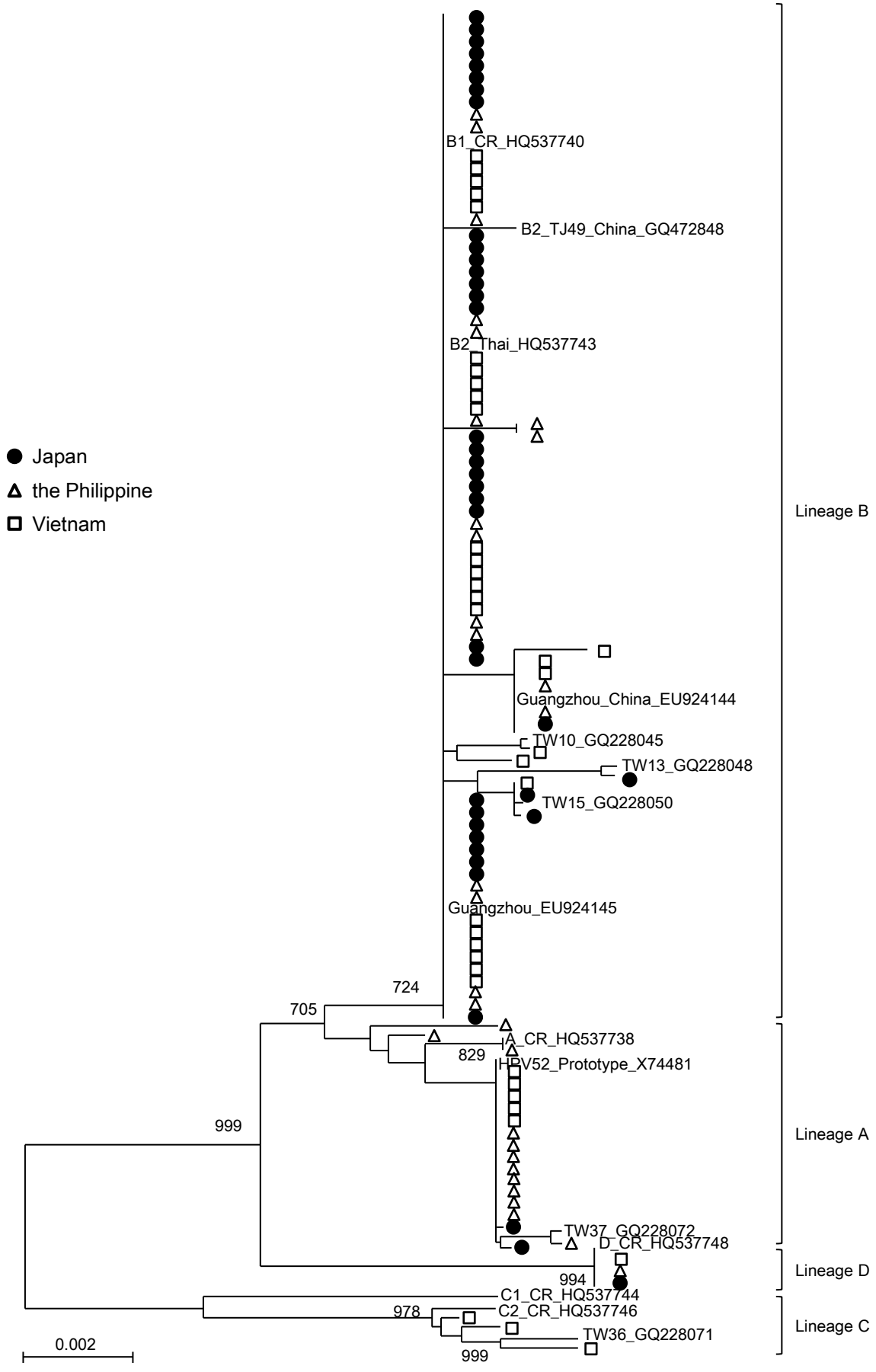
[a]The prevalence of the intratypic variations among three countries was significantly different ($P = 0.0048$).

[b]Adenocarcinoma in situ.

[c]The prevalence ot the abnormal cytology among different intratypic variations was not significant. ($P = 1.000$)

[d]No significant relationship between abnormal cervical cytology and a specific amino acid mutation, HPV-52 E6 K93R, was observed ($P = 0.572$).


931
Asian American 2_CR3_AY686582
Asian American 1_CR1_AF402678
African 1_HAPI1_AF472508
African 2_HAPI2_AF472509
813
976
Non- European
European
(Asian)
sublineage
Japan
the Philippine
Vietnam
WIHS3_AF534061
China_PWH-Q73_AF486304
/AF486331
Thai_CU5_FJ610150
China_Guangzhou_FJ415228
983
HPV16_Prototype_K02718
Thai_CU4_FJ610149
Thai_CU1_FJ610146
989
Thai_CU7_FJ610152
CR7_AY686584
Thai_CU3_FJ610148
Thai_CU2_FJ610147
CR4_AY686580
WIHS1_AF536179
CR6_AY686583
0.002
Thai_CU6_FJ610151
846
867
European
(Prototype)
sublineage


Japan
the Philippine
Vietnam
B1_CR_HQ537740
B2_TJ49_China_GQ472848
B2_Thai_HQ537743
Guangzhou_China_EU924144
TW10_GQ228045
TW13_GQ228048
TW15_GQ228050
Guangzhou_EU924145
724
705
A_CR_HQ537738
829
HPV52_Prototype_X74481
999
TW37_GQ228072
D_CR_HQ537748
994
C1_CR_HQ537744
978
C2_CR_HQ537746
TW36_GQ228071
999
0.002
Lineage B
Lineage A
Lineage D
Lineage C