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

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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, Hammonon, 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. Phylogenetic 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 intragenotypic 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.

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180

181 **FIGURE LEGENDS**

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

190

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

Group ^a	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)																						
	J	P	V	8	9	9	1	1	1	1	2	2	2	2	2	2	2	3	3	3	4	4	5	5	6	6	6	7	7	7	8	8	E6 region				E7 region				J	P	V								
	31	24	24	3	3	4	5	9	2	3	5	8	1	5	6	7	2	6	9	5	0	1	3	2	5	2	5	7	3	6	2	9	5	3	6									2	3	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	R48W								0	2	0					
	0	1	0	-	-	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-									0	0	0						
	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	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	-	-	-	-	-	-	-	-	-					L83V E113D				L28F			0	0	0
European (Asian)	5	5	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	D25E ^c				N29S ^c				1	1	1					
	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	t	-	-	-	-	-	-	-	-	-	D25E				N29S				0	0	0			
	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	D25E				N29S				0	0	0			
	0	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	D25E				N29S				0	0	0			
	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	D25E				R141T				0	0	0			
	8	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	D25E				E113D				1	0	0			
	1	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	D25E				E113D				0	0	0			
	6	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					N29S				0	0	0				
	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	g	-	-	-	-	-	-	-	-	-					N29S				0	0	0				
	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	c	-	-	-	-	-	-	-	-	-					N29S				0	0	0				
	0	0	3	-	-	a	g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	D25E								0	0	0				
African type-2	0	1	1	-	-	-	-	c	T	G	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	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.

^aThe prevalence of the intratypic variations among three countries was significantly different ($P < 0.000001$).

^bThe prevalence of the abnormal cytology among different intratypic variations was not different ($P = 1.000$).

^cNo 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			E6 region	E7 region	J	P	V			
	39	32	38	1 2 2 2 3 3 3 3 4 4 5	5 5 6 7 7 7 7 7 7 7 8 8								
				7 0 3 5 5 5 7 7 2 6 3	7 9 6 0 0 2 2 3 4 5 6 0 4								
				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	H24D	Y59C				0 0 0		
	0	1	0	- - - - -	- - - - - t - - -								0 0 0
	0	1	0	G - - - - -	- - - - - - - - -								0 0 0
	0	1	0	- - - - -	- - - - - G - - - g -								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		

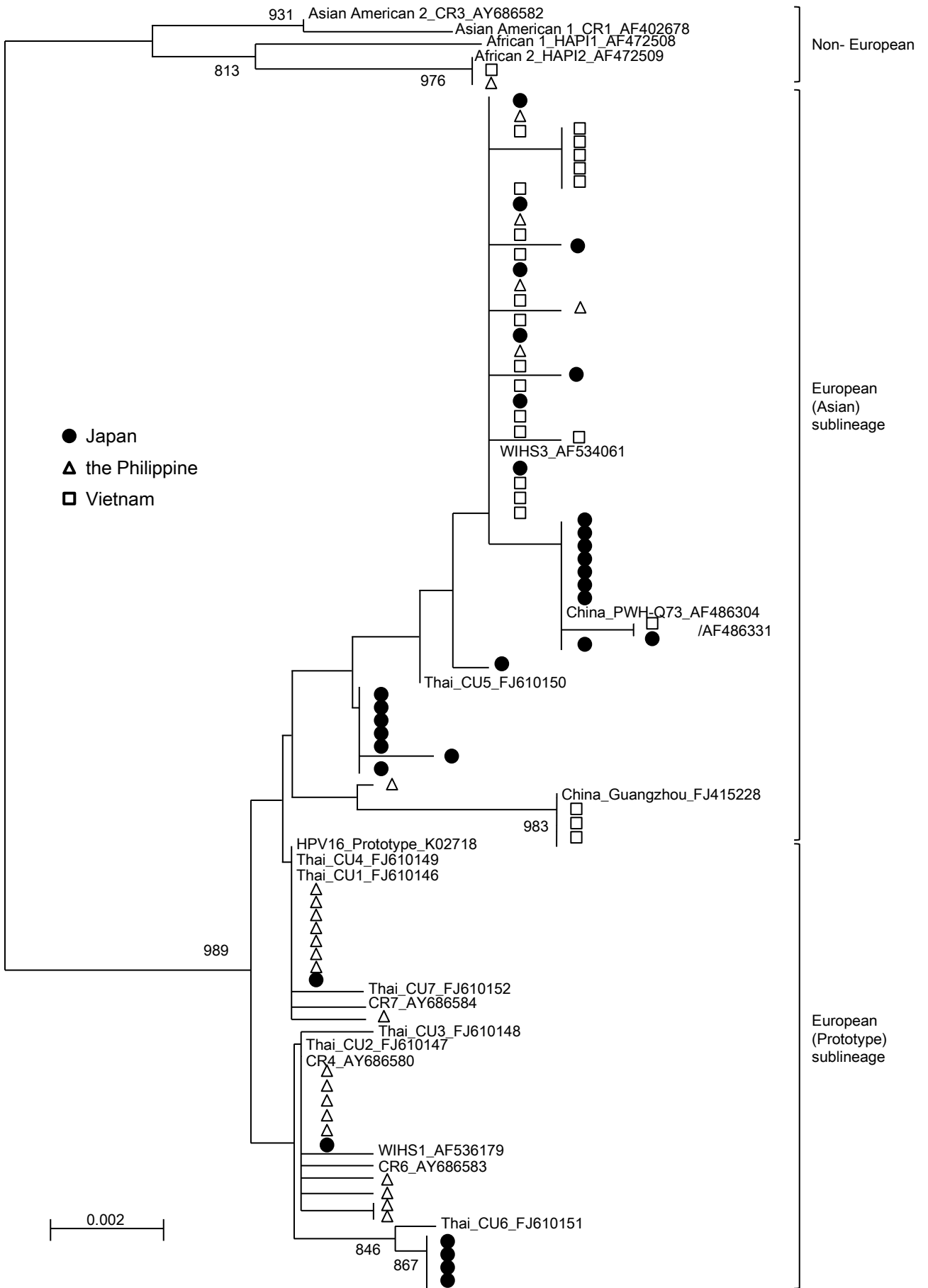
J: Japan; P: the Philippines; V: Vietnam. Capital letters in nucleotide columns indicate the variant accompanied by amino acid changes

^aThe prevalence of the intratypic variations among three countries was significantly different ($P = 0.0048$).

^bAdenocarcinoma in situ.

^cThe prevalence of the abnormal cytology among different intratypic variations was not significant. ($P = 1.000$)

^dNo significant relationship between abnormal cervical cytology and a specific amino acid mutation, HPV-52 E6 K93R, was observed ($P = 0.572$).



- Japan
- ▲ the Philippine
- Vietnam

