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Distribution of Human Papillomavirus Type 58 Variants in Progression of Cervical Dysplasia in Korean Women

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This cross-sectional study examined the distribution of HPV 58 sequence variation in Korean women for the first time. Among 1,750 Korean women, 53 women were positive for HPV 58 single infection, of whom 26 were without disease, 20 were with cervical intraepithelial neoplasia (CIN) 1, and 7 with CIN 2 or 3. Altogether, 36 different nucleotide sequence variations were identified with the L1, 20 within E2, 5 within E6, and 10 within E7. Further studies on variants of oncogenic HPVs are necessary, particularly for the purpose of developing more predictive HPV detection methods.

Keywords: Human papillomavirus (HPV), cervical intraepithelial neoplasia (CIN), HPV variants

High-risk human papillomavirus (HPV) is necessary for the development of cervical cancer and their precursor, cervical intraepithelial neoplasia (CIN). HPV 16 and 18 account for about 70% of all cervical cancers worldwide, whereas HPV 58 infections occur in only 2% [1, 3–8, 13]. The relative importance of other oncogenic HPV types differs by region, and HPV 58 is particularly prevalent in northeastern Asian and Korean women [1].

Generally, two HPV isolates are classified as different types when the nucleotide sequences of their L1 open reading frames (ORFs) differ by more than 10%, whereas isolates differing by 2–10% are considered as subtypes, and those differing by less than 2% are considered as variants [8, 15]. Most HPV variant analyses have focused on HPV 16 and 18, the most prevalent oncogenic types worldwide [2, 9, 10, 12, 16]. In this study, we aimed to analyze the sequence variations of HPV 58, the third most common genotype in Korean women.

This analysis was part of a prospective study for a hospital-based cervical cancer screening program conducted between January 2002 and July 2006. Samples from cervical cytology were collected during clinical examination. When either gynecological examination or colposcopy revealed suspicious cervical lesions, cervical biopsies were performed.

HPV genotyping was performed using the HPV DNA oligonucleotide chip (Mygene Co., Seoul, Korea), which consisted of 22 type-specific probes, including 15 in the high-risk group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 69) and 7 in the low-risk group (6, 11, 34, 40, 42, 43, and 44).

The ORFs including E2, E6, E7, and L1 of HPV 58 were amplified based on specific primers designed using the HPV 58 prototype (GenBank Accession No. D90400). The conditions of the polymerase chain reaction (PCR) amplification were 95°C for 10 min, followed by 35 cycles at 94°C for 1 min, annealing 1 min for each gene target and 72°C for 1.5 min, and a final extension for 8 min. The expected PCR product sizes were checked by gel electrophoresis and visualized under UV transillumination. PCR products were cleaned with MicroSpin SR-400 (GE Healthcare, Waukesha, WI, U.S.A.).

The sequences of the three regions of interest were obtained by PCR-based cycle sequencing using BigDye Terminator v. 3.1 (Applied Biosystems, Foster City, CA, U.S.A.). The sequencing primers used for each region and PCR condition are shown in Table 1. Sequence reactions were performed on the 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). The sequences of the above gene regions were aligned by the computer software SeqScape v.2.5. (Applied Biosystems, Foster City, CA,

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Table 1. Primer sequences and PCR conditions

Gene region	Primer name	Primer sequence	Position	Annealing temperature (°C)	Product size
E2	HPV58-E2-out-1	5'-TGATGCAAATGGTAATCCAG-3'	2643–2662		
	HPV58-E2-out-2	5'-AACACCAGCAACCAAGCATA-3'	3998–3979C	59	1,356 bp
	HPV58-E2-in-3	5'-TGCAAATGGTAATCCAGTGT-3'	2646–2665		
	HPV58-E2-in-4	5'-AGAAATAGATAGCACCAATGGC-3'	3975–3955C	60	1,330 bp
E6/E7	HPV58E6-out-1	5'-CGAAAACGGTCTGACCGAAA-3'	42–61		
	HPV58E7-out-2	5'-TATCGTCTGCTGTTTCGTCC-3'	1009–990C	57	968 bp
	HPV58E6-in-3	5'-GACCGAAACCGGTGCATATA-3'	54–73		
	HPV58E7-in-4	5'-ACCGCTTCTACCTCAAACCA-3'	950–931C	57	901 bp
L1	HPV58L1-outer-1	5'-CCTCTTGTGTCATTGGAACC-3'	5456–5475		
	HPV58L1-outer-2	5'-TAGGGCAATTTAGGGACAGC-3'	7376–7357C	60	1,921 bp
	HPV58L1-inner-3	5'-GGTCCAGACATTGCATCTTC-3'	5477–5496		
	HPV58L1-inner-4	5'-AGAAACAGGAAACTGACAAGGAC-3'	7286–7263	60	1,809 bp

U.S.A.). The HPV 58 prototype (GenBank Accession No. D90400) was used as the reference for identifying sequence variations. Distributions of HPV 58 variations with respect to disease severity were examined by the exact Mantel-Haenszel's linear trend test (P_{trend}) and Fisher's exact test (P). A P-value less than 0.05 was considered significant.

A total of 1,750 Korean women aged 15–75 years were included, with informed consent from each patient. Four hundred seventy-three patients were HPV DNA positive, and 78 tested positive for HPV 58. To exclude the possibility that multiple HPV infections may confound the results of the analysis, a total of 53 women (median age, 42 years; range, 15–71 years) with HPV 58 single infection were included. Of these, 26 patients had normal cytology, 20 had CIN 1, and 7 had CIN 2 or 3.

At the nucleotide level, 36 variants of L1, 20 variants of E2, 5 variants of E6, and 10 variants of E7 were identified in a total of 44 patients (83.0%). HPV 58 L1 gene sequence variants were identified in 40 samples (75.5%). The most frequent sequence variations were those at nucleotide positions 6014 (A to C, leucine→phenylalanine [L150F]) and 6539 (A to G, isoleucine→methionine [I325M]). HPV 58 E2 gene sequence variants were identified in 39 samples (73.6%). The most common sequence variations were those at nucleotide positions 3550 (T to C), 3587 (T to G, serine→alanine [S279A]), and 3596 (G to T, valine→leucine [V282L]). HPV 58 E6 and E7 gene variants were identified in a total of 43 samples (81.1%).

Of the HPV 58 gene variants identified, 28 resulted in missense mutations: 12 in L1, 7 in E2, 3 in E6, and 6 in E7. The most prevalent amino acid changes were in L1 (L5F, L150F, and I325M), E2 (S279A and V282L), and E7 (T20I and G63S). E2 S279A and E2 V282L missense mutations were consistently found in association, suggesting a possible linkage between the two mutations. Based on grouping of variants according to cervical neoplasia severity (Tables 2 and 3), there was no significant association.

Table 2. Sequencing data of HPV 58 L1 classified by severity of cervical neoplasia

Nucleotide sequence variation			Amino acid change	Diagnosis			P_{trend}	P
Position	Reference	Variant		Normal	CIN 1	CIN 2 or 3		
5579	A	C	L5F	3	8	3	0.1344	0.3181
5747	T	C	-	3	8	3	0.1344	0.3181
5798	C	T	-	1	2	0	0.7797	1.0000
6014	A	C	L150F	14	19	5	0.6398	0.1346
6416	A	G	-	10	10	3	0.4027	0.6813
6434	T	C	-	10	10	3	0.4027	0.6813
6539	A	G	I325M	13	19	6	0.1069	0.2692
6560	A	G	-	1	3	0	0.9398	0.6351
6641	G	A	-	10	8	5	0.9360	0.1556
6827	C	A	-	3	8	3	0.1344	0.3181

CIN, cervical intraepithelial neoplasia.
P_{trend}, Mantel-Haenszel's linear trend test.
P, Fisher's exact test.

Table 3. Sequencing data of HPV 58 E2, E6, and E7 classified by severity of cervical neoplasia

	Nucleotide sequence variation			Amino acid change	Diagnosis			P_{trend}	P
	Position	Reference	Variant		Normal	CIN 1	CIN 2 or 3		
E2	2935	A	C	-	10	9	3	0.2532	0.5338
	2953	A	C	-	1	2	0	0.7399	1.0000
	3445	C	G	-	13	18	6	0.7399	0.1941
	3550	T	C	-	12	18	6	0.3819	0.1204
	3587	T	G	S279A	14	18	6	0.6882	0.2962
	3596	G	T	V282L	14	18	6	0.6882	0.2962
	3685	A	G	-	2	6	3	0.1172	0.2619
E6	307	C	T	-	18	19	6	0.3096	1.0000
E7	632	C	T	T201I	5	8	3	0.2231	0.5201
	694	G	A	G41R	11	11	3	0.7901	1.0000
	744	T	G	-	18	19	6	0.3096	1.0000
	756	T	C	-	1	2	0	0.9228	1.0000
	760	G	A	G63S	6	8	3	0.3720	0.7596
	761	G	A	G63D	11	11	3	0.7901	1.0000

CIN, cervical intraepithelial neoplasia.
P_{trend}, Mantel-Haenszel's linear trend test.
P, Fisher's exact test.

This study represents the first analysis of HPV 58 variants in a cohort of Korean women. Because this was a cross-sectional study, any estimation of risk should be interpreted with caution. A previous study of HPV 16 in Korean women indicated a relationship between one HPV 16 E7 variant (N29S) and invasive cervical cancer [14]. However, in other more recent Korean studies, there was no significant relationship between the risk of cervical disease progression and HPV 16 variants [4, 11]. The lower prevalence of oncogenic HPVs other than HPV 16 and 18 makes further variant analyses more problematic.

The distribution of HPV variants appears to be related to geographic regions and differences in oncogenic potential [2, 9, 10, 12, 16]. The diversity of oncogenic potential of high-risk HPV variants may be generated by alteration of coding or regulatory elements within the genomes [2, 9, 12]. Despite the current understanding of the roles of individual HPV proteins, the detailed mechanisms used by specific HPV variants to increase oncogenic potential are not well understood and warrant further investigation on a larger scale.

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