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Risk factors associated with human papillomavirus infection status in a Korean cohort

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SUMMARY

By surveying extensive epidemiological behavioural and sexual risk factors in a Korean twin cohort, risk factors for human papillomavirus (HPV) infection were investigated in South Korea. A total of 912 vaginal specimens were collected from the Healthy Twin Study, consisting of twins and their families. A range of epidemiological, behavioural, and sexual activity characteristics were evaluated using multivariate logistic regression analyses of family and twin relationships, adjusted to elucidate the risk factors for HPV infection. Of the various epidemiological characteristics, the possibility of extramarital affairs [odds ratio (OR) 2·48, 95% confidence interval (CI) 1·02–6·02] significantly increased the prevalence of HPV infection. Our multivariate regression analysis indicated that oral contraceptive use (OR 40·64, 95% CI 0·99–1670·7) and history of sexually transmitted disease (OR 2·56, 95% CI 0·93–7·10) were strongly associated with an increase in HPV infection. On the other hand, more frequent vaginal douching (OR 0·32, 95% CI 0·13–0·77) significantly decreased the prevalence of HPV infection. Our results suggested that HPV infection is associated with both biological and behavioural factors.

Key words: HPV infection, oral contraceptive, risk factor, sexual behaviour, twins.

INTRODUCTION

Uterine cervical cancer is the second most frequent cancer in women worldwide [1]. Human papillomavirus (HPV) infection is a recognized cause of cervical cancer [2]. HPV infection, which is common in women, is a critical event in a series of processes leading to cervical cancer. The lifetime risk of HPV infection has been estimated at up to 80% [3]. About 100 HPV genotypes have been characterized worldwide [4]. Infection with high-risk HPV genotypes, such as HPV-16 and HPV-18, is closely related to cervical cancer [5]. Vaccines targeting these high-risk HPV genotypes have been developed and have been reported to be effective for preventing HPV infection [6].

The overall prevalence of HPV worldwide is estimated to be about 10%, ranging from 1·4% to 25·6% [7]. A meta-analysis reported that the prevalence of HPV in women aged <25 years was significantly higher than that in older women [8]. In Korea, the prevalence of HPV infection was reported as 8·5–15·2% [7, 9–11]. Both incidence and mortality rates of uterine cervical cancer have decreased over the past 10 years [12]. However, the prevalence and

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associated risk factors for HPV infection in Korea have not yet been fully investigated.

The risk factors for HPV infection and development of cervical cancer are not identical, although there is some overlap. The well-established risk factors for cervical cancer are host genetics [13–15], viral genotype, various behavioural and environmental factors (such as oral contraceptive use, earlier sexual debut, smoking), and HIV infection [16–20]. By contrast, physical activity and antioxidant nutrients decrease the risk [21, 22]. With regard to HPV infection status, various sexual behaviours have been reported as strong determinants; however, the components of specific behavioural factors, such as multiple sexual partners, HIV infection, unprotected intercourse, vaccination, earlier sexual debut, multiple partners, marital status, bacterial vaginosis, and oral contraceptive use [18, 23–25], are not consistently associated in studies. It is not clear whether specific types of sexual behaviours have their own biological meaning or if they are proxies of overall behavioural patterns that may reflect characteristics of the measurement tools or populations. In particular, whether some risk factors for HPV infection, such as smoking, oral contraceptive use, and age, are proxies of sexual behaviours or are true risk factors remains controversial [26–28].

The objectives of this study were to examine the prevalence of HPV infection and its various genotypes in the Korean population, and to investigate the risk factors for HPV infection using comprehensive surveys that examined the lifestyle and reproductive and sexual behaviours of women and their partners.

METHODS

Study population and specimen preparation

All cervico-vaginal smear samples were collected from the female participants in the Healthy Twin Study in South Korea as described in a previous study [29]. A total of 912 samples were collected from participants who took the Papanicolaou smear tests between 2005 and 2009 at three participating hospitals in Korea: Samsung Medical Center, Seoul ($n=618$), Busan Paik Hospital, Busan ($n=226$), and Dankook University Hospital, Cheonan ($n=68$). Subjects were aged 25–79 years, and >95% of the women ($n=867$) had been married (728 were married at examination), 848 had given birth to a child, 421 were menopausal and 166 had used an oral contraceptive. Of the participants, there were 174 twin pairs [142 monozygotic (MZ) and 32 dizygotic (DZ) pairs], and 169 of their relatives. The characteristics of the 912 study subjects are summarized in Table 1.

HPV diagnostics and genotyping

A liquid-based preparation method was used for cervico-vaginal samples, according to standard procedures (ThinPrep®, Hologic, USA and SurePath™, BD Diagnostics-TriPath, USA). Genomic DNA of cervico-vaginal samples was extracted using the Chemagic viral DNA/RNA kit according to the manufacturer's instructions (Chemagen, Germany). Amplification of partial sequences of HPV was performed using two primer sets, GP5+/GP6+ and PGMY09/PGMY11 [30, 31]. Amplified PCR products were first identified by electrophoresis. PCR amplicons of the expected sizes were sequenced for confirmation and subsequent genotyping. PCR amplicons were later sequenced by a commercial sequencing company (Cosmo Genetech, South Korea). Next, the sequences were searched using the NCBI BLAST program, and HPV infection status was determined. Samples detected by at least one of the two primer sets were considered positive, and those not detected by either primer set were scored as negative. HPV genotyping was determined through multiple alignments using ClustalW and phylogenetic analysis with a number of reference HPV genotypes using Molecular Evolutionary Genetics Analysis software v. 5.05 (MEGA 5.05, USA), as indicated in a previous study [32].

Collection of epidemiological and behavioural information

Adhering to the protocols of the Healthy Twin Study [29], epidemiological and behavioural information for each subject were collected from questionnaires and clinical tests. This information included demographics, lifestyle (smoking, alcohol use, physical activity, education, income), reproductive history (marriage, childbirth, abortion, oral contraceptive use, menopausal status), and other clinical information.

A self-administered questionnaire pertaining to the individual's sexual behavioural characteristics was taken in a private room prior to a Pap smear test, and confirmed by trained interviewers who explained the strict privacy protection policy. The sexual activity questionnaire asked about the subject's age at sexual debut, existence of multiple partners, method of

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Table 1. *Epidemiological characteristics of the study population as related to human papillomavirus (HPV) infection (n = 912)*

		HPV prevalence		
		High risk (%)*	Non-high risk (%)	Total (%)†
Age (years)	25–34 ($n=121$)	5 (4·1)	4 (3·3)	9 (7·4 ± 4·7)
	35–44 ($n=286$)	12 (4·2)	12 (4·2)	24 (8·4 ± 3·2)
	45–54 ($n=232$)	15 (6·5)	6 (2·6)	21 (9·1 ± 3·7)
	55–64 ($n=194$)	7 (3·6)	3 (1·5)	10 (5·2 ± 3·1)
	⩾65 ($n=79$)	5 (6·3)	3 (3·8)	8 (10·1 ± 6·6)
Zygosity	Monozygotic twin ($n=284$)	15 (5·3)	9 (3·2)	24 (8·5 ± 3·2)
	Dizygotic twin ($n=64$)	3 (4·7)	3 (4·7)	6 (9·4 ± 7·1)
	Individual ($n=564$)	26 (4·6)	16 (2·8)	42 (7·4 ± 2·2)
Marriage	Single ($n=45$)	4 (8·9)	1 (2·2)	5 (11·1 ± 9·2)
	Married ($n=728$)	31 (4·3)	23 (3·2)	54 (7·4 ± 1·9)
	Divorced, separated, or cohabiting ($n=139$)	9 (6·5)	4 (2·9)	13 (9·4 ± 4·9)
Education	⩽ Elementary school ($n=230$)	7 (3·0)	4 (1·7)	11 (4·8 ± 2·8)
	⩽ High school ($n=425$)	25 (5·9)	16 (3·8)	41 (9·6 ± 2·8)
	⩾ College ($n=257$)	12 (4·7)	8 (3·1)	20 (7·8 ± 3·3)
Occupation	Housewife ($n=433$)	23 (5·3)	19 (4·4)	42 (9·7 ± 2·8)
	Worker ($n=479$)	21 (4·4)	9 (1·9)	30 (6·3 ± 2·2)
Gave birth to a child	Yes ($n=848$)	40 (4·7)	27 (3·2)	67 (7·9 ± 1·8)
	No ($n=29$)	1 (3·5)	0	1 (3·5 ± 6·7)
	Missing ($n=35$)	3 (8·6)	1 (2·9)	4 (11·4 ± 10·5)
Oral contraceptive use	No ($n=733$)	34 (4·6)	16 (2·2)	50 (6·8 ± 1·8)
	Ever used ($n=157$)	7 (4·5)	10 (6·4)	17 (10·8 ± 4·9)
	Current use ($n=9$)	2 (22·2)	1 (11·1)	3 (33·3 ± 30·8)
	Missing ($n=13$)	1 (7·7)	1 (7·7)	2 (15·4 ± 19·6)
Menstruation	Menopause ($n=421$)	22 (5·2)	11 (2·6)	33 (7·8 ± 2·6)
	Pre-menopause ($n=482$)	22 (4·4)	17 (3·7)	39 (8·1 ± 2·4)
	Missing ($n=9$)	0	0	0
Smoking	Never smoker ($n=823$)	38 (4·6)	28 (3·4)	67 (8·1 ± 1·9)
	Ever smoker ($n=26$)	4 (15·4)	0	4 (15·4 ± 13·9)
	Current smoker ($n=59$)	2 (3·4)	0	2 (3·4 ± 4·6)
	Missing ($n=4$)	0	0	0
Alcohol intake	Non-drinker ($n=407$)	16 (3·9)	11 (2·7)	28 (6·9 ± 2·5)
	Ever drinker ($n=80$)	5 (6·3)	2 (2·5)	7 (8·8 ± 6·2)
	Current drinker ($n=424$)	23 (5·4)	15 (3·5)	38 (9·0 ± 2·7)
	Missing ($n=1$)	0	0	0

* Samples in which at least one high-risk HPV genotype was detected.
† Prevalence and 95% confidence intervals are presented.

contraception, hygiene habits after sexual intercourse, frequency of vaginal douching, number of lifetime sexual partners, history of sexually transmitted disease (STD), and details about their male partner (education level, circumcised or not, and possibility of an extramarital affair).

Informed consent was obtained from each participant. Prior to the study, the research protocols and informed consent forms were reviewed and approved by the Institutional Review Board of Seoul Samsung Hospital, Busan Paik Hospital, and Seoul National University School of Public Health (no. 144-2011-07-11).

Statistical methods

For univariate analysis, odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for each risk factor. For the multivariate model, the generalized estimating equation (GEE) model was applied, in which genetic correlations between families and twin pairs were treated as random effects. To confirm the risk factors for HPV infection, factors reaching the significance level ($P<0·1$) were included in the multivariate logistic regression models. Univariate regression analysis was performed with all samples ($n=912$). Due to missing data, multivariate logistic

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regression analysis was performed with a smaller sample size ($n=653$). To further dissect genetic and non-genetic associations, a co-twin control study involving HPV-discordant MZ twin pairs was performed. Using conditional logistic regression, HPV-infected and unaffected pairs were compared. As MZ twins are genetically identical, significant risk factors replicated in the co-twin control study indicate non-genetic cause of disease. All statistical analyses were performed using SAS software, v. 9.2 (SAS Institute Inc., USA).

RESULTS

The prevalence of HPV

The prevalence of HPV is summarized in Table 1. Of 912 tested specimens, 72 (7·9%) samples were positive for HPV infection. The prevalence of HPV-infected subjects was slightly increased in the 45–54 years age group. The prevalence of HPV was highest in those aged $\geqslant 65$ years and lowest in those aged 55–64 years. The prevalence of HPV before and after menopause was similar. Married women, who were the majority group in this study, had a HPV prevalence of 7·4%, which was lower than that in single women (11·1%). The prevalence of HPV in twins (MZ 8·5%, DZ 9·4%) was slightly higher than in singletons (7·4%). The prevalence of HPV in never smokers was 8·1%, which was higher than in current smokers (3·4%), but lower than in ever smokers (15·4%). The prevalence of HPV in non-drinkers (6·9%) was lower than in ever drinkers (8·8%) and in current drinkers (9·0%). In addition, women who had used oral contraceptives showed a higher HPV prevalence (10·8%), particularly those currently taking an oral contraceptive (33·3%).

Identification of HPV genotypes

A phylogenetic tree (Fig. 1) shows the identified HPV genotypes in various reference HPV genotypes. Seventy-two positive samples were categorized into 12 high-risk genotypes (HPV genotypes 16, 18, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66), five low-risk genotypes (HPV genotypes 32, 42, 43, 54, 70), and seven HPV-X (risk-undetermined HPV genotypes) (HPV genotypes 30, 67, 74, 81, 84, 90, 102). In total, 82 HPV genotypes were detected in 72 positive samples. Of these, 63 samples hosted a single HPV genotype and nine were infected by multiple HPV genotypes (double HPV, $n=8$ samples; triple HPV, $n=1$ sample). Of the nine multiple HPV samples, high-risk HPV was detected in seven, and two were infected with low-risk HPV and HPV-X. Of the identified HPV genotypes, HPV-16 was the most prevalent, followed by HPV-18.

Association between HPV prevalence and epidemiological characteristics

Table 2 summarizes the HPV-positivity rate and ORs based on the questionnaires. Table 1 shows the distributions of HPV infection for high-risk and non-high-risk HPV genotypes. In further statistical analysis, the risks of HPV infection were estimated with combined data due to the small number of HPV-positive subjects. The prevalence of HPV was positively associated with oral contraceptives ($P=0·007$), method of contraception ($P=0·012$), and possibility of a partner's extramarital affair ($P=0·044$).

Subjects with >12 years' education had higher HPV infection rates than those with $\leqslant 6$ years (OR 2·11, 95% CI 1·06–4·18). The risk of HPV infection in those currently taking oral contraceptives was higher than in those who had never taken oral contraceptives (OR 6·83, 95% CI 1·66–28·13). The use of condoms was associated with HPV infection ($P=0·012$). Oral contraceptives in combination with other methods, such as the intra-uterine loop, female sterilization, vasectomy, or the rhythm method, increased the risk of HPV infection to a greater extent than did use of condoms (Table 2; OR 8·20, 95% CI 1·80–37·33). The likelihood of a partner's extramarital affair (OR 2·42, 95% CI 1·18–4·95), and history of STD (OR 2·37, 95% CI 1·00–5·59), were also associated with higher rates of HPV infection in the univariate analyses.

Table 3 shows the risk factors for HPV infection related to host history and hygiene behaviour determined by multivariate logistic regression analysis. Of the tested covariates, level of education (OR 3·16, 95% CI 1·25–7·97) and the possibility of a partner's extramarital affair (OR 2·48, 95% CI 1·02–6·02) remained significant. The use of various contraceptive methods, such as oral contraceptives, contraceptive suppositories, intra-uterine loops, female sterilization, vasectomy, and the rhythm method, but not the use of condoms alone, significantly increased the risk of HPV infection (OR 6·26, 95% CI 1·36–28·93). Interestingly, the use of condoms together with other contraceptive methods (OR 5·81, 95% CI 1·06–32·03) also increased the risk of HPV infection. In addition, women who cleansed with a douche more frequently

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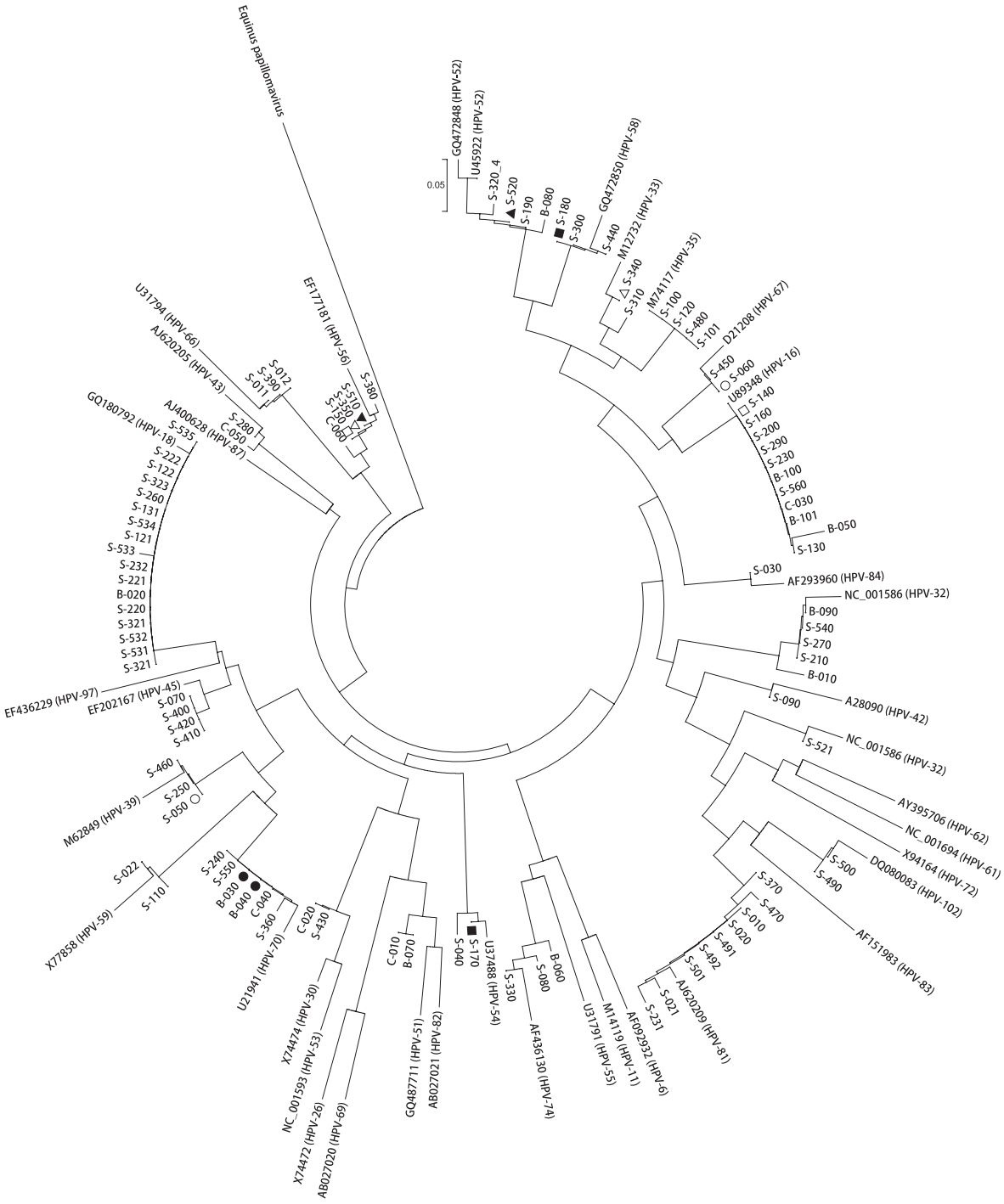

Fig. 1. Phylogenetic analysis of human papillomavirus (HPV) in the study population. Equinus papillomavirus is a HPV outgroup. Samples are expressed using the initial character with given numbers. S, C, and B represent the sample collection regions of Seoul, Cheonan and Busan, respectively. Solid symbols represent a monozygotic twin, open symbols represent a family member. Each shape represents a single sample.

than every other day were more protected against HPV than those who douched $\geqslant$1–2 times per week (OR 0·32, 95% CI 0·13–0·77). Current use of oral contraceptives (OR 40·64, 95% CI 0·99–1670·7) and history of STD (OR 2·56, 95% CI 0·93–7·10) increased the risk of HPV infection; however, the associations were not statistically significant, possibly due to the smaller sample size.

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Table 2. *Summary of odd ratios of host characteristics for human papillomavirus (HPV) prevalence estimated by univariate logistic regression analysis (n=912)*

Factor	Category	HPV positive (%)*	OR	95% CI	*P* value†
Age (years)	⩽34 (*n*=121)	9 (7·4)	1		0·541
	35–44 (*n*=286)	24 (8·4)	1·14	0·51–2·53	
	45–54 (*n*=232)	21 (9·1)	1·24	0·55–2·80	
	55–64 (*n*=194)	10 (5·2)	0·68	0·27–1·72	
	⩾65 (*n*=79)	8 (10·1)	1·40	0·52–3·80	
Marriage	Married (*n*=728)	54 (7·4)	1		0·288
	Single, divorced and other‖ (*n*=184)	18 (9·8)	1·35	0·77–2·37	
Education	⩽ Elementary school (*n*=228)	11 (4·8)	1		0·177
	High school (*n*=425)	41 (9·7)	2·11	1·06–4·18	
	⩾ College (*n*=257)	20 (7·8)	1·67	0·78–3·55	
	Missing (*n*=2)	0	—	—	
No. of children	No (*n*=27)	1 (3·7)	1		0·674
	1 (*n*=107)	10 (9·4)	2·68	0·33–21·90	
	2 (*n*=416)	29 (7·0)	1·95	0·26–14·87	
	⩾ 3 (*n*=328)	28 (8·5)	2·42	0·32–18·55	
	Missing (*n*=34)	4 (11·8)	3·47	0·36–32·99	
Oral contraceptive use	Never (*n*=733)	50 (6·8)	1		0·007
	Ever used (*n*=157)	17 (10·8	1·66	0·93–2·96	
	Current use (*n*=9)	3 (33·3)	6·83	1·66–28·13	
	Missing (*n*=13)	2 (15·4)	2·48	0·54–11·51	
Menopausal status	Pre-menopause (*n*=482)	39 (8·1)	1		0·671
	Menopause (*n*=421)	33 (7·8)	0·97	0·60–1·57	
	Missing (*n*=9)	0	—	—	
Smoking	Never smoker (*n*=823)	66 (8·0)	1		0·260
	Ever smoker (*n*=26)	4 (15·4)	2·09	0·70–6·23	
	Current smoker (*n*=59)	2 (3·4)	0·40	0·10–1·69	
	Missing (*n*=4)	0	—	—	
Alcohol intake	Non-drinker (*n*=407)	27 (6·6)	1		0·632
	Ever drinker (*n*=80)	7 (8·8)	1·35	0·57–3·22	
	Current drinker (*n*=424)	38 (9·0)	1·39	0·83–2·32	
	Missing (*n*=1)	0	—	—	
Duration of marriage (years)	<10 (*n*=124)	8 (6·5)	1		0·600
	⩽19 (*n*=230)	17 (7·4)	1·16	0·49–2·76	
	⩽29 (*n*=224)	22 (9·8)	1·58	0·68–3·66	
	⩽39 (*n*=160)	9 (5·6)	0·86	0·32–2·31	
	⩾40 (*n*=101)	8 (7·9)	1·25	0·45–3·45	
	Missing (*n*=73)	8 (11·0)	1·79	0·64–4·98	
Method of contraception	Condom (*n*=84)	2 (2·4)			0·012
	Condom + other‡ (*n*=96)	7 (7·3)	3·23	0·65–15·97	
	Oral contraceptive + other‡ (*n*=84)	14 (16·7)	8·20	1·80–37·33	
	Other‡ (*n*=596)	44 (7·4)	3·27	0·78–13·74	
	Not used (*n*=52)	5 (5·6)	4·36	0·81–23·37	
Douching	1–2 times per week (*n*=92)	11 (12·0)	1		0·638
	⩾ Every other day (*n*=739)	54 (7·3)	0·58	0·29–1·16	
	1–2 times a month (*n*=13)	1 (7·7)	0·61	0·07–5·19	
	No (*n*=21)	2 (9·5)	0·76	0·16–3·79	
	Missing (*n*=47)	4 (8·5)	0·69	0·21–2·28	
Hygiene habits after sexual intercourse	By douche or shower (*n*=578)	48 (8·3)	1		0·129
	Using paper or tissue (*n*=185)	11 (6·0)	0·70	0·36–1·37	
	No sanitation (*n*=44)	7 (15·9)	2·09	0·88–4·94	
	Missing (*n*=105)	6 (5·7)	0·67	0·28–1·61	
Circumcision of partner	Yes (*n*=154)	10 (6·5)	1		0·471
	No (*n*=546)	48 (8·8)	1·39	0·69–2·81	
	Missing (*n*=212)	14 (6·6)	1·02	0·29–2·69	

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Table 2 (*cont.*)

Factor	Category	HPV positive (%)*	OR	95% CI	*P* value†
Possibility of partner's extramarital affair	Not likely ($n=379$)	20 (5·3)	1		0·044
	Maybe ($n=86$)	5 (5·8)	1·11	0·40–3·04	
	Likely ($n=118$)	14 (11·9)	2·42	1·18–4·95	
	Have no idea ($n=184$)	21 (11·4)	2·31	1·22–4·39	
	Missing ($n=145$)	12 (8·3)	1·62	0·77–3·40	
Age at sexual debut (years)§	$\leqslant 19$ ($n=36$)	2 (5·6)	1		0·740
	$\leqslant 21$ ($n=75$)	4 (5·3)	0·96	0·17–5·49	
	$\leqslant 23$ ($n=148$)	15 (10·1)	1·92	0·42–8·79	
	$\geqslant 24$ ($n=578$)	45 (7·79)	1·44	0·33–6·17	
	Missing ($n=75$)	6 (8·0)	1·48	0·28–7·71	
No. of lifetime sexual partners	1 ($n=664$)	48 (7·2)	1		0·475
	$\geqslant 2$ ($n=186$)	18 (9·7)	1·38	0·78–2·43	
	Missing ($n=62$)	6 (10·0)	1·38	0·56–3·35	
History of sexually transmitted disease (STD)¶	No ($n=665$)	48 (7·2)	1		0·168
	Yes ($n=45$)	7 (15·6)	2·37	1·00–5·59	
	Have no idea ($n=22$)	3 (13·6)	2·03	0·58–7·10	
	Missing ($n=180$)	14 (7·8)	1·08	0·58–2·01	

OR, Odds ratio; CI, confidence interval.
* Both high-risk HPV, low-risk HPV, and HPV-X.
† As determined by χ^2 test.
‡ Including contraceptive infection, contraceptive suppository, intra-uterine loop, female sterilization, vasectomy, and rhythm method.
§ Age when sexually active more than once per month.
¶ Sexually transmitted disease not including cystitis or urethritis.
‖ Separation and cohabitation.

Co-twin–control analysis on the risk factors of HPV infection

Of the 142 pairs of MZ twins enrolled in this study, we identified 18 pairs discordant for HPV infection; these subjects were selected for additional co-twin control analysis. Oral contraceptive use was not found to be a significant risk factor in this analysis (Table 4).

DISCUSSION

In this study, the genetic correlation between zygosity was adjusted for as a random effect, and non-genetic risk factors for HPV infection were determined. Therefore, the genetic correlation between zygosity and HPV infection was not high. However, not all women infected with HPV develop cervical neoplasia; therefore, genetic and other factors are likely to play important roles in disease progression. The effect of genetic factors on HPV infection has not been sufficiently explained, and few studies reported that HLA-associated genes were significantly associated with HPV infection [15, 33]. A recent study reported that 37% and 14% of cervical smear abnormalities between MZ twins and first-degree relatives, respectively, could be explained by genetic factors [34].

The HPV prevalence in this study (7·9%, 72/912) is similar to that reported elsewhere [7, 8]. South Korea belongs to the HPV intermediate-prevalence region, which was previously reported to have a HPV prevalence of 8·5% [9]. However, the prevalence of HPV in different age groups was distinct from that in other regions [8]. Another study reported that women aged <25 years had the highest HPV prevalence, with a continuous decline in infection rate as age increased to 35–44 years. In contrast, the prevalence increased in those aged $\geqslant 45$ years, except in Asia where the prevalence continued to decline [8]. In this study, all subjects were aged $\geqslant 25$ years, with most (98·6%) HPV-infected individuals aged >30 years. Interestingly, subjects aged <35 years had a prevalence of HPV equivalent to that of the $\geqslant 65$ years group, with the highest prevalence seen in the 45–54 years age group. This observation marks a clear contrast between this and other studies, where prevalence generally peaked in late teens and early twenties followed by a sharp decline thereafter. Difference in social behaviour patterns and other biological factors (host

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Table 3. *Identification of risk factors for human papillomavirus (HPV) infection related to host history and hygiene behaviour by multivariate logistic regression analysis (n = 653)*

Factor	Category	HPV positive (%)*	OR†	95% CI
Age (years)	⩽34 (*n* = 69)	5 (7·3)	1	
	35–44 (*n* = 214)	19 (8·9)	0·96	0·32–2·88
	45–54 (*n* = 185)	15 (8·1)	1·04	0·31–3·47
	55–64 (*n* = 135)	7 (5·2)	0·61	0·16–2·37
	⩾65 (*n* = 50)	5 (10·0)	1·62	0·38–6·99
Oral contraceptive use	Never (*n* = 533)	36 (6·8)	1	
	Ever used (*n* = 114)	12 (10·5)	1·56	0·68–3·57
	Current use (*n* = 6)	3 (50·0)	40·64	0·99–1670·7
Education	⩽ Elementary school (*n* = 165)	8 (4·9)	1	
	⩽ High school (*n* = 310)	33 (10·7)	3·16	1·25–7·97
	⩾ College (*n* = 178)	10 (5·6)	2·04	0·62–6·71
Method of contraception	Condom (*n* = 69)	1 (1·5)	1	
	Condom + other§ (*n* = 87)	7 (8·1)	5·81	1·06–32·03
	Oral contraceptive (*n* = 22)	2 (9·1)	2·14	0·14–33·36
	Oral contraceptive + other§ (*n* = 41)	7 (17·1)	7·56	0·79–72·40
	Other methods§ (*n* = 434)	34 (7·8)	6·26	1·36–28·93
Douche	1–2 times a week (*n* = 63)	10 (15·9)	1	
	⩾ Every other day (*n* = 570)	39 (6·8)	0·32	0·13–0·77
	1–2 times a month (*n* = 6)	1 (16·7)	1·79	0·13–24·38
	No (*n* = 14)	1 (7·1)	0·33	0·02–6·52
Possibility of partner's extramarital affair	Not likely (*n* = 324)	15 (4·6)	1	
	Maybe (*n* = 222)	23 (10·4)	2·48	1·02–6·02
	Likely (*n* = 107)	13 (12·2)	2·07	0·99–4·33
History of STD‡	No (*n* = 595)	42 (7·1)	1	
	Yes (*n* = 40)	7 (17·5)	2·56	0·93–7·10
	Have no idea (*n* = 18)	2 (11·1)	1·20	0·24–6·14

OR, Odds ratio; CI, confidence interval.
* Both high-risk HPV, low-risk HPV, and HPV-X.
† Adjusted genetic correlation between family and zygosity as random effect and other fixed effects.
‡ Sexually transmitted disease not including cystitis or urethritis.
§ Including contraceptive infection, contraceptive suppository, intra-uterine loop, female sterilization, vasectomy, and rhythm method.

genetics, hormone levels, vaginal microbiome, etc.) unique to different geographical regions could be related to the prevalence in different age groups.

The data in Figure 1 indicate that the HPV genotypes identified in this study were diverse. HPV-16 and HPV-18 were the most prevalent, which is consistent with previous studies [7, 8]. With the exception of one sample that was HPV negative, HPV-16, -18, -35, -42, -45, -59, and -90 were each detected in one of the remaining seven samples. In addition, HPV-42 (low-risk) and HPV-90 (HPV-X) were detected in two cervical intraepithelial neoplasia (CIN) samples. The former CIN sample may have harboured another high-risk HPV. HPV-90, which was not classified into any risk group in previous studies, is now suspected to be a high-risk HPV [35].

STDs are known co-risk factors that contribute to another infection. For example, HIV infection is a risk factor for HPV infection. In addition, the incidence of STDs greatly increases the risk of HPV infection [25, 36]. Primary protection from virus transmission through proper hygiene is important for prevention of HPV infection. In this study, the significant associations of condom use and frequent vaginal douching with decreased risk of HPV infection support that principle. Therefore, at present, low-risk sexual behaviour and hygiene could be recommended to prevent HPV infection and eventually, cervical cancer. A number of recent studies in various countries and settings reported that a greater number of sexual partners, HIV infection, and condom use were associated with HPV infection [37–39].

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Table 4. *Analysis of possible risk factors for human papillomavirus (HPV) infection in monozygotic twins (n=36)*

Factor	Category	HPV positive (%)*	*P* value†
Oral contraceptive use	Never (*n*=29)	14 (48·3)	1·000
	Ever used (*n*=7)	4 (57·1)	
Method of contraception	Condom (*n*=4)	2 (50·0)	1·000
	Other methods§ (*n*=31)	15 (48·4)	
Douche	1–2 times a week (*n*=24)	10 (41·7)	0·479
	⩾ Every other day (*n*=8)	5 (62·5)	
	⩽ 1–2 times a week (*n*=3)	2 (66·7)	
Possibility of partner's extramarital affair	Not likely (*n*=21)	9 (42·9)	0·532
	Maybe (*n*=8)	5 (62·5)	
	Likely (*n*=3)	2 (66·7)	
History of STD‡	No (*n*=28)	14 (50·0)	1·000
	Yes (*n*=2)	1 (50·0)	

* Both high-risk HPV, low-risk HPV, and HPV-X.
† As determined by the χ^2 test.
‡ Sexually transmitted disease not including cystitis or urethritis.
§ Including contraceptive infection, contraceptive suppository, intra-uterine loop, female sterilization, vasectomy, and rhythm method.

An interesting finding in this study was the association of HPV infection with current use of oral contraceptives. The prevalence of HPV in those using oral contraceptives at present (33·3%) was higher than those in the past (10·8%) and those who had never used (6·8%) ($P=0·007$). Therefore, it is likely that currently taking an oral contraceptive is associated with both HPV infection and cervical cancer [40]. Long-term use of oral contraceptive has previously been shown to be associated with an increased the risk of developing cervical cancer [18]. However, the implications of this association remain controversial. Oral contraceptive use may be indicative of women who are more sexually active than those not using oral contraceptives and, therefore, not an independent risk factor for infection. In this study, we analysed monozygotic twins discordant for HPV, allowing us to control for both genetic and other possible confounding factors. When only HPV discordant twin pairs were considered, oral contraceptive use and other behavioural factors were not significantly associated with HPV infection. However, the significance of these results was limited due to small sample sizes ($n=36$). Furthermore, a recent study found that hormone levels strongly affected the composition of the vaginal microbiome, and that these changes were significantly associated with HPV infection [41]. These results suggest that oral contraceptive use may be an independent risk factor for HPV infection due to its ability to alter the vaginal microbiome. Therefore, both sexual behaviours and biological factors (e.g. differences in microbiome composition) may be responsible for the association between oral contraceptive use and HPV infection. Further research is necessary to fully examine the effects of these factors on HPV susceptibility.

It is also possible that other contraceptive methods, such as condom use, are negatively associated with both HPV infection and oral contraceptive use. In this study, risks of two factors were consistent in the univariate and multivariate regression analyses. Both analyses strongly indicated the association between oral contraceptive use and HPV infection. In previous studies, smoking was identified as a risk factor for cervical cancer [42, 43]. However, smoking itself was not identified as a risk factor for HPV infection in our study (OR 0·40, 95% CI 0·10–1·69).

HPV infection was significantly associated with douche cleansing (OR 0·35, 95% CI 0·15–0·81) and the possibility of a partner's extramarital affair (OR 2·36, 95% CI 1·06–5·27) in multivariate analyses (Table 3). Therefore, we have confirmed that douche cleansing and the possibility of a partner's extramarital affair are significant risk factors for HPV infection and are representative factors related to host history and hygiene behaviour. The health risk or benefit of vaginal douching has been debated for decades [44]. Frequent douching, at least monthly, increased the prevalence of *Chlamydia* [45], and in another study, douching within the last year was associated with chlamydial infection [46]. Moreover, one study found that douching during the last 90 days increased the prevalence of HPV infection [47]. Although

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moderate frequency of douching; i.e. 1–2 times per week is generally recommended to maintain a healthy vaginal microbial milieu dominated by *Lactobacillus* species [48], our findings suggest that more frequent douching (more than every other day) may protect against HPV infection; this has also been reported in a previous study [49]. Whether more frequent douche cleansing is protective against other STDs, including HPV, requires more data.

In conclusion, diverse HPV genotypes were identified in this study. A number of risk factors, including vaginal douching, the possibility of a partner's extramarital affair, and oral contraceptive use, were significantly associated with HPV infection in our study setting. This study provides information useful for identification of the risk factors for HPV infection and subsequent cervical cancer.

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DECLARATION OF INTEREST

None.

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