

## Genotype distribution characteristics of high-risk human papillomaviruses in women from Shanghai, China

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### SUMMARY

High-risk human papillomaviruses (HPVs) are highly prevalent worldwide, and HPV genotype distribution varies regionally. Molecular surveys of HPVs are important for effective HPV control and prevention. Fifteen high-risk HPV strains (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and six low-risk HPV strains (HPV6, 11, 42, 43, 44, CP8304) were detected by cervical cytology from 10 501 subjects. High-risk HPVs, low-risk HPVs, and both high- and low-risk HPVs were detected in 14·5%, 2·8%, and 2·4% of cases, respectively. Of 1782 subjects with high-risk HPV infection, 75·5%, 18·1%, and 6·4% were infected with one, two, and  $\geq 3$  strains of high-risk HPVs, respectively. HPV52, HPV16, and HPV58 were the top three most dominant high-risk HPV genotypes in our population with positivity rates of 23·0%, 17·7% and 16·9%, respectively. Multiple infection was common, with significantly higher co-infection rates of HPV58/HPV33 (12·9%) and HPV58/HPV52 (11·3%). Further data comparisons showed that HPV genotype distribution varied markedly between domestic and international regions. In conclusion, a monolithic vaccination strategy is obviously impractical, and regional HPV surveillance is essential to optimize current HPV control and prevention.

**Key words:** Human papilloma virus (HPV), molecular survey, viral strain distribution.

### INTRODUCTION

Approximately 10–15% of human cancers are caused by infection with high-risk human papillomaviruses (HPVs), Epstein–Barr virus, hepatitis B virus, human T-lymphotropic virus-I, hepatitis C virus, Kaposi's sarcoma herpesvirus, and Merkel cell polyomavirus [1]. HPV is a double-stranded DNA virus belonging to the papillomavirus family that is capable of infecting the basal cells of the stratified epithelium in humans [2]. HPVs are highly prevalent worldwide,

and high-risk HPV infections cause various cancers, including vaginal, vulvar, penile, oropharyngeal, anal, and cervical [3–6]. Cervical cancer, which is mainly categorized into squamous cell carcinoma and adenocarcinoma, occurs when abnormal cells of the cervix grow out of control, and is a leading cause of death in women with 0·5 million new cases and 275 000 deaths worldwide each year [7]. Significantly, almost all cases of cervical cancer are caused by long-lasting infections with certain types of human HPV [8].

The genome of HPVs consists of a circular DNA molecule of ~8000 base pairs subdivided into the early (E) region, upstream regulatory region (URR), and late (L) region [9, 10]. The E region encodes at least eight early viral proteins: E1, responsible for

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viral DNA replication; E2, responsible for activating the replication of viral DNA in synergy with E1 and transcriptional repression of E6 and E7 in the nucleus, and responsible for induction of apoptosis and genomic instability in the cytoplasm; E4, responsible for virion maturation; E5, responsible for stimulation of cell proliferation; E6, responsible for oncogenic protein promoting degradation of p53 by the proteasome; E7, responsible for oncogenic protein promoting degradation of retinoblastoma susceptibility protein; and E3 and E8, of no known functions [9, 10]. The L region encodes the major protein capsid protein L1 and the minor protein L2 to form the viral particle [9, 10]. To date, over 170 HPV genotypes have been identified [11], with types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 defined as high-risk, carcinogenic, sexually transmitted HPVs that may lead to the development of cervical cancer [12]. The diagnosis of HPV infection is based primarily on detection of viral DNA [12, 13]. Genotyping of HPV is mainly based on the unique sequence in the L1 region of the HPV genome [14]. HPV genotypes are not only disease-specific but also strain-specific within a certain disease or cancer [15–18]. HPV6, 11, 42, and 44 infections are correlated with anogenital warts [15]. HPV16 infection causes oropharyngeal cancer [16]. HPV16 and 18 are most prevalent in head and neck squamous cell carcinomas [17]. HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82 infections cause genital cancers [12, 18].

As HPVs are highly prevalent worldwide and genotypes differ among regions, molecular epidemiological survey of HPVs could provide background data regarding characteristic HPV genotype distribution and composition in a certain area and therefore support local vaccination strategies. For this purpose, the distribution characteristics of high-risk HPV genotypes were studied in women living in Shanghai.

## METHODS

### Study population

The Shanghai 7th Hospital is a general hospital located in northeastern Shanghai. Outpatients visiting our Department of Gynecology between January 2014 and May 2015 for cervical screening were included in this study. All participants were screened by clinical gynecological examination, cytology examination, and colposcopy examination. Age, clinical data, and HPV testing results were recorded. Inclusion criteria

were a sexual history, not currently pregnant, no history of total uterus or cervix resection, and agreement to undergo HPV testing. Patients that had visited a doctor 2 months previously and received cervical physical therapy and hormone treatment were excluded from the study. A total of 10 501 subjects (age: median 48; quartile range 39–55 years) were included. For Pap smears, cells were collected at the outer opening of the cervix at the transformation zone and then examined under a microscope to detect any abnormalities in the exfoliated cervical cells. Pap smears were interpreted by experienced pathologists in our hospital. Cytological diagnoses were evaluated according to the Bethesda System criteria [19]. At the same time, the cell samples were analysed to detect HPV DNA.

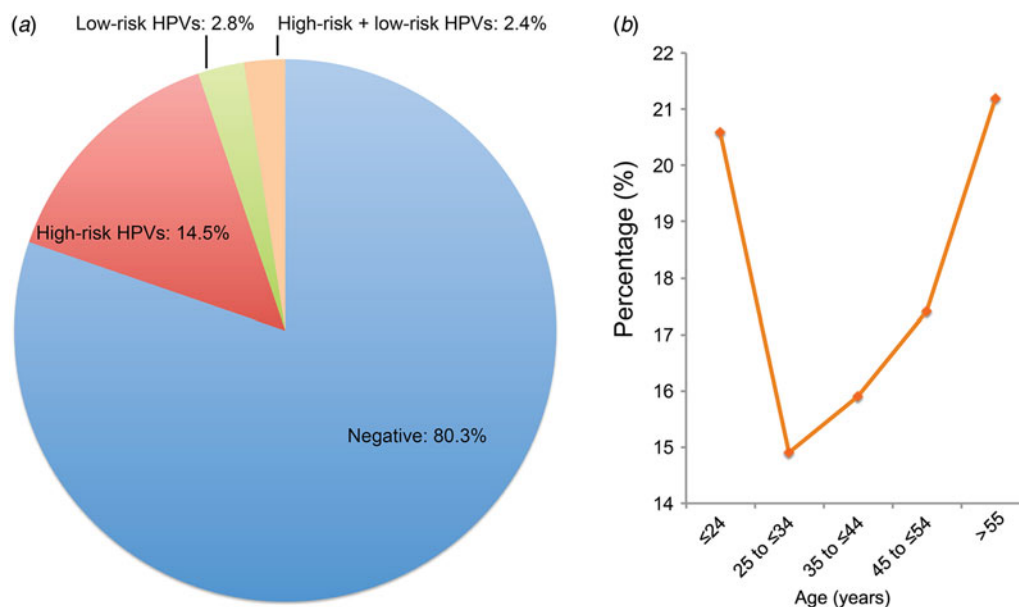
### Ethical standards

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. The Review Board of the Ethics Committee of Medical Research at Shanghai Seventh People's Hospital (Shanghai, China) approved the study protocols. Written informed consent was obtained from all patients according to the guidelines of the Chinese National Ethics Regulation Committee. All patients were informed of their rights to withdraw consent personally or via relatives, caregivers, or guardians.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

### HPV genotyping

HPV genotyping was performed using a commercial detection kit purchased from HybriBio (Chaozhou, China). The kit could detect and distinguish 15 high-risk HPV strains (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and six low-risk HPV strains (HPV6, 11, 42, 43, 44, CP8304) (81). The kit is based on polymerase chain reaction followed by HPV DNA microarray analyses. All detection procedures were guided by the protocol provided by manufacturers. Detected HPV strains were defined by genotype not by sub-genotype or quasi-species [20].



**Fig. 1.** Overall prevalence of human papillomavirus (HPV) and age-specific distribution. (a) Overall prevalence of high-risk and low-risk HPVs in 10501 subjects. (b) Distribution characteristics of high-risk and low-risk HPVs according to age stratification for 10501 subjects.

### Statistical analysis

Statistical analysis was performed using SPSS v. 13.0 (SPSS Inc., USA). Continuous variables are presented as means  $\pm$  standard deviation (s.d.) and categorical data are presented as numbers (percentages). Differences between groups were examined using the *t* test, one-way ANOVA,  $\chi^2$  test, or Fisher's exact probability test according to the characteristics of data distribution. The significance level  $\alpha$  was set at 0.05.

## RESULTS

### Overall prevalence of HPV

Data from a total of 10 501 outpatients were collected; 8430 (80.3%) were negative for HPV. The overall prevalence of HPV was 19.7% (2071 patients), 14.5% (1525 patients) of whom were infected with high-risk HPVs, 2.8% (289 patients) were infected with low-risk HPVs, and 2.4% (257 patients) were co-infected with both (Fig. 1a). The overall HPV prevalence rates were calculated for the following age groups:  $\leq 24$  years, 20.6%; 25–34 years, 14.9%; 35–44 years, 15.9%; 45–55 years, 17.4%; and  $> 55$  years, 21.2% (Fig. 1b). The prevalence ratio of HPV infection decreased from 20.6% in subjects aged  $< 24$  years to the lowest ratio of 14.9% in those aged 25–34 years, and then increased to 21.2% in subjects aged  $> 55$  years (Fig. 1b).

### Prevalence characteristic of high-risk HPVs

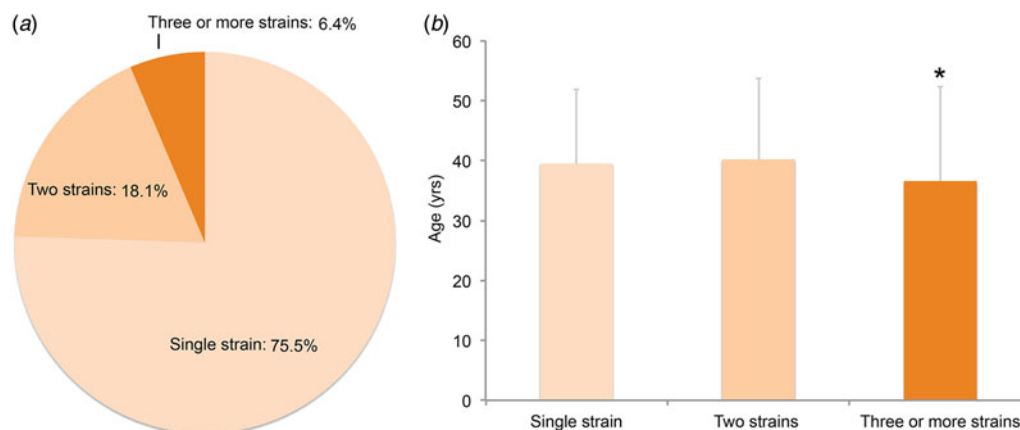
A total of 1782 subjects were infected with high-risk HPVs. Of these, 1346 (75.5%) subjects were infected with a single high-risk HPV strain, 322 (18.1%) subjects were infected with two high-risk strains, and 114 (6.4%) subjects were infected with  $\geq 3$  high-risk strains (Fig. 2a). The average ages of subjects infected with one, two, and  $\geq 3$  HPV strains were  $39.5 \pm 12.4$ ,  $40.2 \pm 13.6$ , and  $36.6 \pm 15.8$  years, respectively (Fig. 2b). The average age of subjects infected with  $\geq 3$  HPV strains was significantly younger than that of the other two groups (Fig. 2b).

### Distribution characteristics of high-risk HPV genotypes

As shown in Table 1, HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68 were detected in 17.7%, 6.3%, 7.5%, 10.4%, 2.2%, 9.3%, 2.1%, 9.3%, 23.0%, 10.8%, 3.1%, 16.9%, 2.7%, 4.9%, and 7.0% of the 1782 high-risk HPV-infected subjects, respectively. The top three most dominant HPV genotypes prevalent in our population were HPV52, HPV16, and HPV58.

### Characteristics of HPV co-infection

As described above, multiple HPV infection was common in this population. To determine the co-infection



**Fig. 2.** Prevalence and distribution characteristics of high-risk human papillomaviruses (HPVs). (a) Constituent ratios of single and multiple infection in 1782 high-risk HPV-infected subjects. (b) Average ages of subject groups infected by one, two, and  $\geq 3$  HPV strains. \*  $P < 0.05$  compared to the other two groups.

**Table 1.** Distribution characteristics of high-risk HPV genotypes ( $n = 1782$ )

Genotype	Number	(%)
HPV16	316	(17.7)
HPV18	112	(6.3)
HPV31	134	(7.5)
HPV33	185	(10.4)
HPV35	39	(2.2)
HPV39	165	(9.3)
HPV45	37	(2.1)
HPV51	165	(9.3)
HPV52	410	(23.0)
HPV53	193	(10.8)
HPV56	56	(3.1)
HPV58	302	(16.9)
HPV59	48	(2.7)
HPV66	88	(4.9)
HPV68	124	(7.0)

HPV, Human papillomavirus.

Percentages for individuals co-infected by  $\geq 2$  high-risk HPV strains were calculated respectively.

status, the co-infection rates of the three most dominant high-risk HPVs were calculated. As shown in Table 2, only 60.0% of cases positive for HPV genotype 52 were mono-infections, and the rates of HPV52 co-infection with HPV16, 18, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59, 66, and 68 were 5.4%, 4.9%, 4.4%, 4.1%, 0.5%, 6.8%, 1.0%, 4.4%, 6.6%, 2.7%, 8.3%, 1.7%, 2.7%, and 4.4%, respectively. Of HPV16 cases, only 67.7% were mono-infections, and the rates of HPV16 co-infection with HPV18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68 were 3.8%,

**Table 2.** Characteristics of HPV co-infection

Genotype	HPV52	HPV16	HPV58	<i>P</i>
	( <i>N</i> = 410)	( <i>N</i> = 316)	( <i>N</i> = 302)	
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	
HPV16	22 (5.4)	214 (67.7)	21 (7.0)	0.4272
HPV18	20 (4.9)	12 (3.8)	11 (3.6)	0.6603
HPV31	18 (4.4)	15 (4.7)	14 (4.6)	0.9704
HPV33	17 (4.1)	12 (3.8)	39 (12.9)	<b>&lt;0.001</b>
HPV35	2 (0.5)	0 (0)	5 (1.6)	1.0000
HPV39	28 (6.8)	13 (4.1)	19 (6.3)	0.2780
HPV45	4 (1.0)	2 (0.6)	4 (1.3)	1.0000
HPV51	18 (4.4)	16 (5.1)	12 (4.0)	0.8025
HPV52	245 (60.0)	22 (7.0)	34 (11.3)	<b>0.0425</b>
HPV53	27 (6.6)	10 (3.2)	14 (4.6)	0.1038
HPV56	11 (2.7)	5 (1.6)	7 (2.3)	0.6065
HPV58	34 (8.3)	22 (7.0)	162 (53.6)	0.3009
HPV59	7 (1.7)	7 (2.2)	8 (2.6)	0.6873
HPV66	11 (2.7)	9 (2.8)	12 (4.0)	0.5857
HPV68	18 (4.4)	5 (1.6)	6 (2.0)	<b>0.0446</b>

HPV, Human papillomavirus.

Differences in co-infection were examined by  $\chi^2$  test or Fisher's exact probability test according to the characteristics of the data distribution.

4.7%, 3.8%, 0%, 4.1%, 0.6%, 5.1%, 7.0%, 3.2%, 1.6%, 7.0%, 2.2%, 2.8%, and 1.6%, respectively (Table 2). Only 53.6% of cases positive for HPV58 were mono-infections, and the rates of HPV58 co-infected with HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 59, 66, and 68 were 7.0%, 3.6%, 4.6%, 12.9%, 1.6%, 6.3%, 1.3%, 4.0%, 11.3%, 4.6%, 2.3%, 2.6%, 4.0%, and 2.0%, respectively (Table 2). The greatest number of high-risk HPV strains detected in a single subject was seven; a 55-year-old woman was

simultaneously co-infected with HPV31, 35, 52, 53, 39, 66, and 68. HPV58/HPV33 and HPV58/HPV52 showed significantly higher co-infection rates, suggesting that HPV58 is the most frequent co-infection high-risk HPV in this population (Table 2). HPV58/HPV68 and HPV16/HPV68 showed significantly lower co-infection rates, suggesting that HPV68 may be the rarest co-infection strain in our population (Table 2).

### High-risk HPV genotype distribution differs regionally

To determine the differences in HPV genotype distributions, we reviewed recent representative reports from the National Center for Biotechnology Information. In high-risk HPV-infected subjects, the three most dominant high-risk HPV strains (HPV16, 52, 58) were the same in six studies performed in Chinese populations, although the ratios varied between studies, while HPV53 was among the three most dominant strains instead of HPV52 in one study performed in Harbin (Table 3). However, the order from high to low varied widely. In the present study, the order was HPV52, HPV16, and HPV58 in order of decreasing prevalence (Table 3). Furthermore, other genotype distributions also differed regionally in China (Table 3). Clifford *et al.* [26] reported a distribution tendency worldwide, with HPV16, 31, and 51 representing the three most dominant HPV genotypes (Table 3). Of these, only HPV16 is among the three most dominant strains in China. In the general population included in our study, the prevalence rates of HPV52, 16, and 58 were 3.9%, 3.0%, and 2.9%, respectively (Table 3). Data from India, Northern Ireland and the USA varied widely, except for HPV16 which remained a dominant strain worldwide [27–30]. The genotype distribution differed regionally worldwide.

### DISCUSSION

Cervical cancer is estimated to affect about half a million women each year, of whom 80% live in developing countries [31]. Virtually all cases of cervical cancer are caused by HPV infection [1–3]. Well-organized programmes of regular gynaecological screening and treatment of precancerous lesions have been very effective for preventing squamous cervical cancer but have had less impact on adenocarcinoma [31]. Fortunately, HPV vaccines were licensed for use in several countries in 2006, and adenocarcinoma is

now expected to be controlled by HPV vaccines [32]. However, currently available HPV vaccines are not region-specific. That is, current vaccination strategies do not adequately take into consideration the distribution and prevalence characteristics of HPV genotypes. Both the quadrivalent and bivalent vaccines protect against HPV types 16 and 18, which cause 70% of cervical cancers; the quadrivalent vaccine also protects against HPV types 6 and 11, which cause 90% of genital wart cases [33]. Although it is difficult to map the HPV genotype distribution and prevalence, large amounts of data indicate that HPV genotype distribution varies largely across regions [21–30]. Thus, regional HPV surveillance is essential for effective vaccination and HPV-related cancer control and prevention. In this study, we found that 17.3% of outpatients undergoing cervical screening were infected with high-risk HPVs. Of these subjects, 24.5% were infected with  $\geq 2$  HPV strains, and the average age of subjects infected with  $\geq 3$  HPV strains was significantly younger than those of other groups. The three most dominant HPV genotypes prevalent in our population were HPV52, HPV16, and HPV58. Of the three most dominant HPV strains, only 60.0% of subjects infected with HPV52, 67.7% of subjects infected with HPV16, and 53.6% of subjects infected with HPV58 were mono-infections, with the remainder being co-infected with other high-risk HPVs. HPV58/HPV33 (12.9%) and HPV58/HPV52 (11.3%) showed significantly higher co-infection rates than other combinations. HPV genotype distribution varied markedly between domestic and international regions, while HPV16 remained among the three most dominant strains worldwide. Our data emphasize the complexity of HPV genotype distribution and the importance of local HPV surveys for vaccination strategies.

The prevalence rates of high-risk HPVs according to age are somewhat confusing, with 20.6%, 14.9%, 15.9%, 17.4%, and 21.2% of subjects in the  $\leq 24$ , 25–34, 35–44, 45–55, and  $>55$  years age groups, respectively. The majority of women who attended for cervical screening were aged 25–34 years. In contrast, women with cervical diseases who visited our department spontaneously, belonged primarily to the other age groups. This might explain why the lowest HPV positivity rate was seen in the population aged 25–34 years. Although we could not give a scientific explanation for the age-specific prevalence rates, our data are similar to the distribution tendencies in central and South America and Europe reported in a meta-analysis [30]. Thus, our data may represent the real

Table 3. Summary of related reports

Source	High-risk HPV genotypes																Region	Population	Number
	16	18	31	33	35	39	45	51	52	53	56	58	59	66	68				
Ratios in high-risk HPV-infected subjects																			
Our data	<b>17.7</b>	6.3	7.5	10.4	2.2	9.3	2.1	9.3	<b>23.0</b>	10.8	3.1	<b>16.9</b>	2.7	4.9	7.0	Shanghai	Outpatient	1782	
Sun <i>et al.</i> [21]	<b>30.3</b>	5.1	4.9	7.5	2.2	4.9	1.3	2.1	7.0	<b>9.1</b>	5.2	<b>12.6</b>	2.8	4.4	8.0	Harbin	Outpatient	988	
Hong <i>et al.</i> [22]	<b>11.8</b>	5.1	5.1	10.1	2.8	3.9	1.7	2.8	<b>17.4</b>	4.5	6.2	<b>14.6</b>	2.2	2.8	6.7	Ningbo	Community	178	
Zhi <i>et al.</i> [23]	<b>22.2</b>	0.8	4.8	6.3	3.4	0.9	1.2	2.7	<b>12.1</b>	4.1	8.6	<b>13.5</b>	4.0	7.6	5.2	Zhengzhou	Outpatient	3690	
Xue <i>et al.</i> [24]	<b>26.2</b>	7.5	6.5	7.6	1.5	3.7	1.5	1.0	<b>19.4</b>	13.3	1.8	<b>13.8</b>	2.4	5.0	6.3	Liaoning	Physical examination	598	
Bi <i>et al.</i> [25]	<b>21.4</b>	4.1	3.9	3.4	3.7	2.0	1.5	5.2	<b>11.9</b>	8.7	5.5	<b>10.5</b>	4.2	6.2	5.4	Qingdao	Outpatient	1768	
Clifford <i>et al.</i> [26]	<b>26.6</b>	8.6	<b>11.7</b>	7.6	5.9	7.8	4.9	<b>10.9</b>	8.8	10.1	9.7	8.5	6.0	8.5	–	Worldwide	Low-grade squamous intraepithelial lesions	5910	
Ratios in general population																			
Our data	<b>3.0</b>	1.1	1.3	1.8	0.4	1.6	0.4	1.6	<b>3.9</b>	1.8	0.5	<b>2.9</b>	0.5	0.8	1.2	Shanghai	Outpatient	10 501	
Bhatla <i>et al.</i> [27]	<b>3.2</b>	<b>0.7</b>	0.5	0.6	0.5	0.5	0.2	0.3	<b>0.7</b>	–	<b>0.7</b>	0.3	0.5	–	0.0	India	Heath women	1048–3061	
Anderson <i>et al.</i> [28]	<b>3.2</b>	1.2	<b>1.8</b>	1.2	0.5	1.2	1.0	<b>1.6</b>	1.5	–	1.0	0.8	<b>1.6</b>	1.3	0.5	Northern Ireland	Normal cervical cytology	5068	
Wheeler <i>et al.</i> [29]	<b>7.4</b>	2.3	2.9	0.9	1.0	3.3	2.2	<b>3.0</b>	2.9	<b>4.9</b>	2.4	2.5	2.1	2.3	2.1	United States	Cervical screening	4007	
de Sanjosé <i>et al.</i> [30]	<b>2.5</b>	<b>0.9</b>	<b>0.7</b>	–	–	–	–	–	0.6	–	–	0.6	–	–	–	Worldwide	Normal cervical cytology	~15 764	

HPV, Human papillomavirus.

Data are presented as percentage (%). The three most dominant genotypes are shown in bold.

age-specific distribution characteristics that may be determined by bearing, female hormone dynamics, sex, and age of the host. On the other hand, our data also indicate that the average ages of subjects infected by one, two, and  $\geq 3$  HPV strains were  $39.5 \pm 12.4$ ,  $40.2 \pm 13.6$ , and  $36.6 \pm 15.8$  years, respectively. The average age of subjects infected by  $\geq 3$  HPV strains was significantly lower than that of the other two groups. As HPV is a sexually transmitted disease, the relatively high frequency of sexual intercourse and greater number of sexual partners might explain why multiple infections are more frequent in younger women. With regard to multiple infections, few studies have investigated the natural history of multiple HPV infection, how and when these HPV strains infect the same host, the impact of multiple infections on cervical cancer, and any tendency for an affinity between HPVs in co-infecting the same host. These questions are also important for HPV virological studies. However, these issues are outside the scope of this epidemiological study.

Further comparative analysis showed that HPV16, 52, and 58 are the three most dominant high-risk HPV strains in five Chinese reports, although the order of prevalence rates from high to low varied widely and other genotype distributions also differed regionally in China [21–25]. Other data from India, Northern Ireland, the USA and integrated data varied except for HPV16, which remained a dominant strain worldwide [26–30]. Although all data showed that HPV16 is a dominant strain worldwide, the highest percentage of HPV16 infection was only 26.2% in high-risk HPV-infected subjects, and therefore the genotype distribution differed regionally worldwide. Again, our report emphasizes the complexity of HPV genotype distribution and the importance of local HPV surveys.

In this study, we detected only 15 high-risk HPV strains (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and six low-risk HPV strains (HPV6, 11, 42, 43, 44, CP8304), and we could not determine the distribution characteristics of other HPVs in women living in Shanghai. HPV genotype distribution is known to differ with pathological grade. We are in the process of analysing these data regarding HPV infection of different pathological grades and will report the results in the near future.

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#### DECLARATION OF INTEREST

None.

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