*Epidemiol. Infect.* (2017), **145**, 2838–2847. © Cambridge University Press 2017
doi:10.1017/S0950268817001698

# Genital HPV infection among heterosexual and homosexual male attendees of sexually transmitted diseases clinic in Beijing, China

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*Received 25 January 2017; Final revision 3 July 2017; Accepted 12 July 2017; first published online 8 August 2017*

## SUMMARY

Human papillomavirus (HPV) has been identified as etiologic agent of various cancers for both men and women. However, HPV vaccine has not been recommended for men in China by far. To provide more evidences to promote HPV vaccination among males at high-risk of infection, this study investigated genital HPV genotypes among male attendees of sexually transmitted disease (STD) clinic. Male attendees ($\geqslant$18 years old) were recruited from STD clinic of Beijing Ditan Hospital. Data on sociodemographic characteristics and self-reported sexual behaviors were collected based on questionnaire. Genital swab specimens were collected for HPV genotypes. Finally, a total of 198 eligible participants were included in the study. Nearly half of them were infected with at least one type of HPV. The prevalence of genital infection among participants with only heterosexual behaviors (50·91%, 56/110) was significantly higher than those with only homosexual behaviors (36·36%, 32/88) ($P < 0·001$). However, the distribution pattern of the most frequently observed HPV subtypes were found to be similar between these two subgroups. HPV31, HPV18, HPV16 and HPV58 were the most frequently identified high-risk types and HPV11, HPV6, HPV81 and HPV61 were the most frequently observed low-risk types. Our results, although need further verification by larger sample size, suggested that currently available HPV vaccines covered most prevalent HPV types observed in Chinese men. As HPV vaccine has been approved for application in females in China, molecular epidemiological studies and intervention studies among high-risk males should be promoted as well.

**Key words**: Genital HPV infection, heterosexual men, homosexual men, sexually transmitted diseases clinic.

## INTRODUCTION

Human papillomavirus (HPV) is a common skin and mucosa infection in worldwide. Persistent infection with some high-risk types of HPV has been identified as an etiologic agent of cervical cancer in women, penile cancer and anal cancer in men, and certain types of head and neck cancers in both men and women [1–5].

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Infection with some low-risk HPV types, such as HPV6 and HPV11, is associated with anogenital warts and mild dysplasia among both men and women [6–8]. Since Harald zur Hausen first described the connection between persistent infection with HPV and cervical cancer in 1977 [9], the idea of developing a vaccine against HPV infection and related diseases has been successfully achieved over the past decades. Currently, a quadrivalent vaccine targeting HPV6, 11, 16 and 18, a bivalent vaccine targeting HPV16 and 18 and a nine-valent vaccine targeting HPV31, 33, 45, 52 and 58 in addition to HPV 6, 11, 16 and 18 had been available and widely vaccinated among girls in some developed countries such as Australia and UK [10]. The Chinese Food and Drug Administration (CFDA) licensed the bivalent vaccine in mainland China until June, 2016. In consistence with most countries, HPV vaccines were not recommended for boys mainly because of little recognition of an emerging epidemic of HPV-associated cancers in men. However, it was estimated that 40·7% anus cancer and 50·1% penis cancer could attribute to HPV infection in men worldwide. In some areas, the incidence of all oropharyngeal cancers, which mainly occurred in male has surpassed that of cervical cancer in women for the first time in 2010 [11, 12]. Thus, more information on the prevalence and risk factors for acquisition of HPV infection and related diseased in men are urgently needed for evaluating the potential impact of prophylactic HPV vaccines. In the past 5 years, we conducted several molecular epidemiological studies to investigate the anal HPV infection in men who have sex with men (MSM), especially HIV-infected MSM, due to their high-risk sexual behaviors [13–15]. However, less attention has been poured on men who have sex with women (MSW). The current study aims to perform genital HPV genotyping among sexually transmitted disease (STD) clinic attendees, including both MSM and MSW, to understand the profile of HPV prevalence and to promote the research and the application of HPV vaccine among high-risk male populations in China.

## METHODS

### Ethic statement

All procedures performed in studies involving human participants were in accordance with the Ethics Committees of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College (IPB-2014-7) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from each study participant before the interview and testing.

### Study population

The participants of the cross-sectional study were recruited between July 2015 and October 2016. Men attending a STD clinic in Beijing Ditan Hospital of the Capital Medical University, were consecutively invited to participate in the study. Beijing Ditan Hospital is a special hospital for infectious disease, it was one of the two designated hospital for HIV diagnosis and treatment in Beijing city. Eligibility criteria for being included in study were: (1) clients visited the STD clinic for diagnosis or treatment of STDs including but not limited to genital warts, (2) male aged 18 years older, (3) registered residents of Beijing city, (4) willingness to sign the informed consent, to complete the interview-based questionnaire and to provide genital swab specimens for HPV genotyping, (5) Only with heterosexual behaviors or only had homosexual behaviors. Individuals ever had sex with both men and women were excluded.

### Data collection

Data were collected using a questionnaire administered by trained interviewers in a private room. Each study participant was assigned a unique code that was used to link the questionnaire. Self-reported sociodemographic characteristics data (e.g., age, income, education, marriage status and history of STDs) were collected. Questionnaire on sexual behaviors was designed separately for participants with heterosexual behaviors and participants with homosexual behaviors. Age was calculated from date of birth. Income, education, marriage status, history of STDs and data on sexual behaviors were collected as category variables.

### Sample collection and laboratory tests

The sample collection was performed by trained and experienced physicians from STD clinic of Beijing Ditan Hospital. Physicians collected genital sample by rotating saline water moistened nylon flocked swab around the penile shaft, glans, coronal sulcus and scrotum for about 2 min. The swab was then

kept in 3 ml of sample transport medium for Hybribio 37 HPV GenoArray Diagnostic Kit (Chaozhou Hybribio Limited Corporation, Guangzhou, China). Hybribio 37 HPV GenoArray Diagnostic Kit Test is based on a flow-through hybridization and gene-chip method. The low-density gene chip was pre-fixed with 37 type-specific oligonucleotides and the genotype was analyzed using HybriMax (Chaozhou Hybribio Limited Corporation). The results were then evaluated by a colorimetric change on the chip under direct visualization. Blue–purple spots were recognized as HPV positive. This testing kit can detect 37 common types of HPV, including 23 oncogenic (16, 18, 26, 31, 33, 35,39, 45, 51, 52, 56, 58, 59, 66, 68, 82,83,53, 55, 34, 57, 69 and 71) and 14 non-oncogenic types (06, 11, 40, 42, 44, 54,61, 67, 70, 72, 73, 82 and 84). Study participants with genotyping positive for any HPV type were determined to be currently infected with HPV.

### Statistical analysis

Data for questionnaire responses and laboratory tests were entered independently by two study staff and verified with EpiData software (EpiData 3·1 for Windows, The EpiData Association Odense, Denmark). After cleaning, the data were then converted and analyzed using Statistical Analysis System (SAS 9·4 for Windows; SAS Institute Inc., NC, USA). Descriptive analyses were performed to compare socio-demographic characteristics and history of STDs between participants only with heterosexual behaviors and those only with homosexual behaviors. The Pearson's $\chi^2$ test was used to evaluate the different distribution of category variables. Multivariable logistic regression was employed to determine predictors of genital HPV infection. All variables with $P < 0{\cdot}05$ in the univariate analysis were all entered into the unconditional multiple logistic regression analyses and the associations was assessed with odds ratio (OR) and 95% confidence interval (CI). Age was fixed in the model. Cochran–Armitage tests were used to explore HPV positivity trends with a history of genital warts.

## RESULTS

### Subject recruitment and characteristics

In total, 198 eligible participates were included in the study with a mean age of 33 (ranged 18–66). The distributions of the major characteristics of the study participants were shown in Table 1. Around a half of them received >12 years education (college or higher) (53·03%, 105/198), had an income more than 5000RMB per month (51·52%, 102/198) and had never smoked (52·53%, 104/198). Two third of them had never married (62·12%, 123/198) and only a minority of participants were circumcised (17·68%, 35/198). In addition, 60·91% (120/197), 52·02% (103/198) and 44·95% (89/198) reported a history of genital warts, a history of HIV and a history of other STDs, respectively.

There are 110 subjects were self-reported with only heterosexual behaviors and 88 with only homosexual behaviors. Age, income and a history of genital warts were found distributed evenly among the two subgroups. While education level, marriage status, smoking status, circumcision status, a history of HIV and a history of other STDs other than genital warts and HIV-1 were found distributed significantly different. For those only with homosexual behaviors, majority of them (94·32%, 83/88) were unmarried and 81·82% (72/88) of them had a history of HIV-1. While for participants only with heterosexual behaviors, half of them (52·73%, 58/110) had history of STDs other than genital warts and HIV-1.

### Genital HPV genotyping

Genital swabs were successfully collected and detected for each of the study participant. As shown in Table 2, 44·44% (88/198) of them were positive for at least one of the targeted 37 HPV types. Prevalence of infection with any type of HPV was higher among participants only with heterosexual behaviors (50·91%, 56/110), as compared with those only with homosexual behaviors (36·36%, 32/88) ($P < 0{\cdot}05$). Among the participants, HPV31 (6·06%, 12/198), HPV18 (4·55%, 9/198), HPV16 (4·55%, 9/198) and HPV58 (3·41%, 6/198) were found to be the most frequently identified high-risk types. HPV11 (18·69%, 37/198), HPV6 (11·03%, 27/198), HPV81 (3·54%, 7/198), HPV61 (3·03%, 6/198) were found to be the most frequently identified low-risk types. The prevalence of HPV6 was found to be significantly higher among heterosexual participants (19·09%, 21/110) as compared with homosexual participants (6·82%, 6/88). In addition, among 88 HPV positives, 47 were infected with only one genotype and 41 were infected with multiple genotypes. Individuals infected with multiple genotypes were almost composed of individuals with a history of genital warts (more details please refer to Supplementary Fig. S1).

Table 1. *Characteristics of the study population by sexual behaviors*

| Variable | Total[a] ($n = 198$) (%) | Sexual behaviors: Only heterosexual behaviors ($n = 110$) (%) | Sexual behaviors: Only homosexual behaviors ($n = 88$) (%) | *P* value |
|---|---|---|---|---|
| Age | | | | |
| ⩽29 years | 81 (40·91) | 37 (33·64) | 44 (50·00) | 0·057 |
| 30–39 years | 76 (38·38) | 46 (41·82) | 30 (39·47) | |
| ⩾40 years | 41 (20·71) | 27 (24·55) | 14 (15·91) | |
| Education | | | | |
| ⩽12 years | 93 (46·97) | 61 (55·45) | 32 (36·36) | 0·008 |
| >12 years | 105 (53·03) | 49 (44·55) | 56 (63·64) | |
| Marriage status | | | | |
| Never unmarried | 123 (62·12) | 40 (36·36) | 83 (94·32) | <0·001 |
| Current married/ever married | 75 (37·88) | 70 (63·64) | 5 (5·68) | |
| Income (RMB) | | | | |
| ⩽5000 | 96 (48·48) | 54 (49·09) | 42 (47·73) | 0·849 |
| >5000 | 102 (51·52) | 56 (50·91) | 46 (52·27) | |
| Smoking status | | | | |
| Never smoked | 104 (52·53) | 67 (60·91) | 37 (42·05) | 0·008 |
| Current smoked/ever smoked | 94 (47·47) | 43 (39·09) | 51 (57·95) | |
| Circumcised | | | | |
| No | 163 (82·32) | 83 (75·45) | 80 (90·91) | 0·005 |
| Yes | 35 (17·68) | 27 (24·55) | 8 (9·09) | |
| With prior or current genital warts | | | | |
| No | 77 (30·09) | 39 (35·45) | 38 (43·68) | 0·240 |
| Yes | 120 (60·91) | 71 (64·55) | 49 (56·32) | |
| With HIV-1 infection | | | | |
| No | 95 (47·98) | 79 (71·82) | 16 (18·18) | <0·001 |
| Yes | 103 (52·02) | 31 (28·18) | 72 (81·82) | |
| With prior or current STDs other than genital warts and HIV-1 | | | | |
| No | 109 (55·05) | 52 (47·27) | 57 (64·77) | 0·014 |
| Yes | 89 (44·95) | 58 (52·73) | 31 (35·23) | |

STDs, sexual transmitted diseases.
[a] Sum might not always be in total because of missing data.

### Risk factors for genital HPV infection

The associations of genital HPV infection with potential risk factors among homosexual and heterosexual participants were shown in Tables 3 and 4, respectively. For homosexual participants, those not tend to take receptive sexual behavior were more likely to be infected with genital HPV with an adjusted OR of 2·58 (0·87–7·66), but it did not reach statistical significant. Individuals with a history of genital warts were more likely to be HPV genotyping positive with an adjusted OR of 3·11 (95% CI 1·12–8·64). For heterosexual participants who received >12 years education (college or higher), had a history of genital warts, with HIV infection and had sexual partners with a history of genital warts were associated with increased risk of genital HPV infection. Multiple logistic regression analysis showed an adjusted OR of 3·26 (95% CI 1·31–8·14) for >12 years education, 10·05 (95% CI 3·11–32·05) for individuals with a history of genital warts and 10·09 (95% CI 1·50–67·93) for individuals with sexual partners with a history of genital warts.

In order to explore the accumulated effect of genital warts on HPV infection, in heterosexual behaviours, individuals with a history of genital warts and had sexual partners with a history of genital warts were stratified into four levels (Both of the participants and their partners without a history of genital warts, only participants with a history of genital warts, only their partners with a history of genital warts and both of the participants and their partners with a history of genital warts.) When considering history of genital warts for both participants and their partners, the highest HPV positivity (100%, 5/5) were observed for those both reported a history of genital warts (*P* for

Table 2. *Genital HPV genotyping by sexual behaviors*

| HPV type | Total ($n$ = 198) (%) | Sexual behaviors | | $P$ value |
|---|---|---|---|---|
| | | Only heterosexual behaviors ($n$ = 110) (%) | Only homosexual behaviors ($n$ = 88) (%) | |
| Any type | 88 (44·44) | 56 (50·91) | 32 (36·36) | 0·041 |
| High risk | | | | |
| HPV 31 | 12 (6·06) | 6 (5·45) | 6 (6·82) | 0·690 |
| HPV 18 | 9 (4·55) | 4 (3·64) | 5 (5·68) | 0·514 |
| HPV 16 | 9 (4·55) | 6 (5·45) | 3 (3·41) | 0·734 |
| HPV 58 | 6 (3·03) | 3 (2·73) | 3 (3·41) | 1·000 |
| HPV 39 | 5 (2·53) | 2 (1·82) | 3 (3·41) | 0·657 |
| HPV 66 | 4 (2·02) | 1 (0·91) | 3 (3·41) | 0·325 |
| HPV 53 | 3 (1·52) | 2 (1·82) | 1 (1·14) | 1·000 |
| HPV 59 | 3 (1·52) | 3 (2·73) | 0 (0·00) | / |
| HPV 51 | 3 (1·52) | 0 (0·00) | 3 (3·41) | / |
| HPV 45 | 3 (1·52) | 0 (0·00) | 3 (3·41) | / |
| HPV 68 | 2 (1·01) | 1 (0·91) | 1 (1·14) | 1·000 |
| HPV 34 | 2 (1·01) | 2 (1·82) | 0 (0·00) | / |
| HPV 52 | 2 (1·01) | 1 (0·91) | 1 (1·14) | 1·000 |
| HPV 82 | 1 (0·51) | 0 (0·00) | 1 (1·14) | / |
| HPV 33 | 1 (0·51) | 0 (0·00) | 1 (1·14) | / |
| HPV 26 | 1 (0·38) | 1 (0·91) | 0 (0·00) | / |
| Low risk | | | | |
| HPV 11 | 37 (18·69) | 22 (20·00) | 15 (17·05) | 0·596 |
| HPV 6 | 27 (11·03) | 21 (19·09) | 6 (6·82) | 0·012 |
| HPV 81 | 7 (3·54) | 5 (4·55) | 2 (2·27) | 0·465 |
| HPV 61 | 6 (3·03) | 3 (2·73) | 3 (3·41) | 1·000 |
| HPV 84 | 4 (2·02) | 1 (0·91) | 3 (3·41) | 0·325 |
| HPV 44 | 4 (2·02) | 1 (0·91) | 3 (3·41) | 0·325 |
| HPV 54 | 2 (1·01) | 2 (1·82) | 0 (0·00) | / |
| HPV 40 | 1 (0·51) | 0 | 1 (1·14) | / |
| HPV 70 | 1 (0·51) | 1 (0·91) | 0 | / |
| HPV 73 | 1 (0·51) | 1 (0·91) | 0 | / |
| Single type | 47 (23·74) | 33 (30·00) | 14 (15·91) | 0·021 |
| With only one high risk[a] | 16 (8·08) | 13 (11·82) | 3 (3·41) | 0·052 |
| With only one low risk[b] | 31 (15·66) | 20 (18·18) | 11 (12·50) | 0·371 |
| Multiple types | 41 (20·71) | 23 (20·91) | 18 (20·45) | 0·938 |
| With multiple high risk[c] | 8 (4·04) | 2 (1·82) | 6 (6·82) | 0·094 |
| With multiple low risk[d] | 7 (3·54) | 6 (5·45) | 1 (1·14) | 0·119 |
| With both high and low risk[e] | 26 (13·13) | 15 (13·64) | 11 (12·50) | 0·986 |

HPV, human papillomavirus.
[a] With only one high risk, including HPV6, HPV11 and HPV81.
[b] With only one low risk, including HPV18, HPV16, HPV59, HPV39, HPV58, HPV31, HPV66, HPV53 and HPV26.
[c] With multiple high risk, including HPV18, HPV16, HPV61, HPV39, HPV58, HPV31, HPV51, HPV33 and HPV45.
[d] With multiple low risk, including HPV6, HPV11, HPV73, HPV 84, HPV54 and HPV81.
[e] With both high and low risk, including HPV6, HPV11, HPV40, HPV44, HPV70, HPV81, HPV54, HPV84, HPV16, HPV18, HPV31, HPV34, HPV39, HPV45, HPV51, HPV52, HPV53, HPV59, HPV61, HPV66, HPV68 and HPV82.

trend < 0·001) (more detailed information please refer to Supplementary Fig. S2).

## DISCUSSION

To our knowledge, this is the first molecular epidemiological study addressing in genital HPV infection among heterosexual and homosexual male attendees of STD clinic in China. The prevalence of infection with any type of HPV was found to be significantly higher among participants with only heterosexual behaviors (50·91%) as compared with those with only homosexual behaviors (36·36%). Meaningfully, the distribution pattern of the most frequently

Table 3. Potential risk factors associated with genital HPV infection among participants with only homosexual behaviors

| Variables | Prevalence n/N[a](%) | *P* value | Adjusted OR | 95% CI[b] |
|---|---|---|---|---|
| Age | | | | |
| ⩽29 years | 14/44 (31·82) | 0·468 | Ref. | |
| 30–39 years | 11/30 (36·67) | | 0·87 | 0·30–2·55 |
| ⩾40 years | 7/14 (50·00) | | 1·93 | 0·57–10·44 |
| Education | | | | |
| ⩽12 years | 11/32 (34·38) | 0·769 | | |
| >12 years | 21/56 (37·50) | | | |
| Income (RMB) | | | | |
| ⩽5000 | 16/42 (38·10) | 0·747 | | |
| >5000 | 16/46 (34·78) | | | |
| Smoking status | | | | |
| Never smoked | 14/37 (37·84) | 0·807 | | |
| Current smoked/ever smoked | 18/51 (35·29) | | | |
| Age at first homosexual behavior | | | | |
| <21 years | 22/54 (40·74) | 0·281 | | |
| ⩾21 years | 10/34 (29·41) | | | |
| Have regular homosexual partner | | | | |
| No | 14/43 (32·56) | 0·554 | | |
| Yes | 17/44 (38·64) | | | |
| Have causal homosexual partner | | | | |
| No | 19/53 (35·85) | 0·473 | | |
| Yes | 6/22 (27·27) | | | |
| Ever had multiple partner sex in the past year | | | | |
| No | 28/77 (36·36) | 1·000 | | |
| Yes | 3/10 (30·00) | | | |
| Ever had paid sex with me in the past year | | | | |
| No | 30/85 (35·29) | 1·000 | | |
| Yes | 1/2 (50·00) | | | |
| Condom use during sex in the past 6 months | | | | |
| Always | 26/76 (34·21) | 0·291 | | |
| Sometimes/never | 6/12 (50·00) | | | |
| Role of homosexual behavior | | | | |
| Only receptive | 6/29 (20·69) | **0·037** | Ref. | |
| Receptive or insertive | 24/55 (43·64) | | 2·58 | 0·87–7·66 |
| Circumcised | | | | |
| No | 28/80 (35·00) | 0·450 | | |
| Yes | 4/8 (50·00) | | | |
| With prior or current genital warts | | | | |
| No | 9/38 (23·68) | **0·026** | **Ref.** | |
| Yes | 23/49 (36·59) | | **3·11** | **1·12–8·64** |
| With HIV-1 infection | | | | |
| No | 5/16 (31·25) | 0·638 | | |
| Yes | 27/72 (37·50) | | | |
| With prior or current STDs other than genital warts and HIV-1 | | | | |
| No | 18/57 (31·58) | 0·206 | | |
| Yes | 14/31(45·16) | | | |

HPV, human papillomavirus; STDs, sexual transmitted diseases.
[a] Sum might not always be in total because of missing data.
[b] Variables with $P < 0·05$ (two-side test) in the univariate analysis and age were all entered into the unconditional multiple logistic regression analyses to assess the associations. Age was fixed in the model.

observed HPV subtypes were similar between the two subgroups. Association analysis suggested a history of genital warts were significantly associated with increased risk of genital HPV positive in both groups. In addition, those with higher education level (>12 years) and those ever had sexual partners with a history of genital warts were more likely to be infected with HPV among heterosexual participants.

Table 4. Risk factors associated with genital HPV infection among participants with only heterosexual behaviors

| Variables | Prevalence n/N[a](%) | *P* value | Adjusted OR | 95% CI[b] |
|---|---|---|---|---|
| Age | | | | |
| ⩽29 years | 17/37 (45·95) | 0·731 | Ref. | |
| 30–39 years | 24/46 (52·17) | | 0·86 | 0·31–2·42 |
| ⩾40 years | 15/27 (55·56) | | 3·71 | 1·00–13·74 |
| Education | | | | |
| ⩽12 years | 24/61 (39·34) | 0·006 | Ref. | |
| >12 years | 32/49 (65·31) | | 3·26 | 1·31–8·14 |
| Marriage status | | | | |
| Never unmarried | 22/40 (55·00) | 0·517 | | |
| Current married/ever married | 34/70 (48·57) | | | |
| Income (RMB) | | | | |
| ⩽5000 | 23/54 (42·59) | 0·087 | | |
| >5000 | 33/56 (58·93) | | | |
| Smoking status | | | | |
| Never smoked | 35/67 (52·24) | 0·728 | | |
| Current smoked/ever smoked | 21/43 (48·84) | | | |
| Age at first sexual behavior | | | | |
| <21 years | 24/43 (55·81) | 0·410 | | |
| ⩾21 years | 32/67 (47·76) | | | |
| Have regular heterosexual partner | | | | |
| No | 10/22 (45·45) | 0·531 | | |
| Yes | 45/85 (52·94) | | | |
| Have causal heterosexual partner | | | | |
| No | 43/83 (51·81) | 0·331 | | |
| Yes | 11/17 (64·71) | | | |
| Ever had multiple partner sex in the past year | | | | |
| No | 52/103 (50·49) | 0·441 | | |
| Yes | 4/6 (66·67) | | | |
| Ever had paid sex with women in the past year | | | | |
| No | 50/97 (51·55) | 0·850 | | |
| Yes | 6/11 (54·55) | | | |
| Condom use during sex in the past 6 months | | | | |
| Sometimes/never | 24/47 (51·06) | 0·978 | | |
| Always | 32/63 (50·79) | | | |
| Circumcised | | | | |
| No | 41/83 (49·40) | 0·578 | | |
| Yes | 15/27 (55·56) | | | |
| With prior or current genital warts | | | | |
| No | 11/39 (28·21) | 0·004 | Ref. | |
| Yes | 45/71 (63·38) | | 10·05 | 3·11–32·05 |
| With HIV-1 infection | | | | |
| No | 45/79 (56·96) | 0·043 | Ref. | |
| Yes | 11/31 (35·48) | | 0·50 | 0·18–1·38 |
| With prior or current STDs other than genital warts and HIV-1 | | | | |
| No | 25/52 (48·08) | 0·573 | | |
| Yes | 31/58 (53·45) | | | |
| Partners ever been diagnosed with genital warts | | | | |
| No | 47/99 (47·47) | 0·030 | Ref. | |
| Yes | 9/11 (81·82) | | 10·09 | 1·50–67·93 |

HPV, human papillomavirus; STDs, sexual transmitted diseases.

[a] Sum might not always be in total because of missing data.

[b] Variables with $P < 0{\cdot}05$ (two-side test) in the univariate analysis and age were all entered into the unconditional multiple logistic regression analyses to assess the associations. Age was fixed in the model.

A Meta-analysis including 12 studies summarized the prevalence of genital HPV infection among STD attendees in Europe, HPV prevalence was found to be ranged from 13·2% to 53·9% [16]. Sample size, HPV detection method and sampling sites were suggested to account for the observed heterogeneity. In China, a study reported a genital HPV infection rate of 17·5% in 2236 males in the general population from Henan province. It was much lower than our observation in our study population from STD clinic, which indicated STD clinic attendees might at higher risk of HPV infection due to the higher exposure associated with common high-risk behaviors of STDs [17].

In the past several years, our group mainly investigated the prevalence of anal HPV infection among MSM in China, the average infection frequency for any HPV type was 62·10% [13, 15]. In the current study, the genital HPV genotypes were investigated and 36·4% of homosexual participants were reported to be infected. Different sampling sites (anal/genital swab specimens) might account for the discrepant HPV positivity. As anal sex is commonly associated with male homosexuality, physical damage caused by delicate anus and rectal tissues can expose its participants to high number of infectious microorganisms and increased the risk of passing on STDs [18]. However, the most frequently identified subtypes in genital swab specimens were consistent with the findings in anal swab specimens. It indicated the distribution of HPV genotypes among MSM populations might not be related to their roles in anal sex.

Although the prevalence of HPV6 was found to be significantly higher among heterosexual males compared with homosexual males, the most frequently identified HPV types were comparable between the two subgroups. In addition, a Meta-analysis determining HPV type-distribution in the cervix of Chinese women found the priority HPV types were HPV16, HPV18, HPV52 and HPV58 [19]. HPV16, HPV18 and HPV58 were consistently observed to be the most frequently observed types in our heterosexual male participants. But it was worthy to notice that, different from the findings of women, HPV31 showed highest prevalence rate in our study population. Therefore, vaccines cover more HPV types should be considered for using in both men and women. Fortunately, the latest nine-valent vaccine indeed covered the predominant HPV subtypes discussed here. However, there is still a long way to go to promote the application of HPV vaccine for different subgroup populations in China as the vaccine has not been approved by CFDA yet.

Male circumcision has been reported to be associated with a reduced risk of penile HPV infection. As stated in a cohort study, removal of the foreskin could minimize the probability of viral entry by markedly decreasing both the size of the surface area vulnerable to HPV and the likelihood of mucosal trauma during intercourse [20]. However, such a logical association was not observed in our study participants, small number of participants with circumcision limited study power to disclose such a relation might be a major explanation. Individuals with higher level of education showed a higher prevalence of genital HPV infection among males with only heterosexual behaviors. The distributions of sexual behavior patterns, marriage status and a history of genital warts were not found to be different with respect to different education levels in our study population. Therefore, the observed high HPV infection rate in the individuals with higher education level might be explained by their higher willingness to pursue health care. Expectedly, for heterosexual participants, both participants and their partners had a history of genital warts were more likely to be HPV genotyping test positive (100%) as compared those without history of genital warts (30·77%). Furthermore, a lower prevalence was found among participants with a history of genital warts (60·61%) as compared with those their partners with a history of genital warts (66·67%) (Supplementary Fig. S2). HPV natural history might account for the phenomena as several studies had demonstrated higher rates of HPV transmission from females to males as compared with from males to females for heterosexual partners [21–23]. Therefore, the implement of HPV vaccination would not only protect from cervical cancer for women but also establish herd immunity for the whole population. Our study could not provide more detailed information to support such an assumption, further observational or interventional studies with more specific objectives are needed.

When interpreting the results, several limitations should be kept in mind. First, self-reported history of STDs might have introduced some bias due to misclassification of exposure into our estimates. Second, study results could not be generalized to general male population because our study participants were enrolled form a STD clinic as high-risk subgroup population for HPV infection. Third, the collected data on the potential risk factors were not

comprehensive. For example, the information on the number of sex partners and on the diagnosis time for those reported a history of genital warts was not collected in the present study, which limited to provide more evidences for risk factor analysis [24].

In summary, our study investigated genital HPV infection among high-risk males from STD clinic. Higher HPV infection rate was found among participants only with heterosexual behaviors than those only with homosexual behaviors. However, type-specific HPV distribution patterns were not significantly different between these two groups and most prevalent HPV types observed in our study population could be mostly covered by the available HPV vaccines. Considering HPV prophylactic vaccine has recently been approved for females, molecular epidemiological studies and intervention studies among males at high-risk for HPV infection and related disease should be promoted in China as well.

## SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817001698

## ACKNOWLEDGEMENTS

We thank physicians from STD clinic of Beijing Ditan Hospital of the Capital Medical University for their great efforts on study participants' enrollment and sample collection. The work was supported by The Beijing Nova program (Grant number Z121107002512073) and The Sanming Project of Medicine in Shenzhen (Grant number GCZX2015043015340574).

## ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the Ethics Committees of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College (IPB-2014-7) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## DECLARATION OF INTEREST

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

LG and WHL conceived and designed the experiments; HWY and JL collected the genital swab samples;

ZL, HJL and MFL performed the experiments; HNX and HJL analyzed the data; and LG and HNX wrote the paper.

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