

# Distribution of high-risk human papillomavirus genotypes in male attendees at a clinic for sexually transmitted infections in Northern China

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**Abstract. – OBJECTIVE:** High-risk (HR) human papillomavirus (HPV) is one of the major causes for most anal and penile cancers and oropharyngeal cancers in men, and vaccination against HPV is recommended for the prevention of these cancers. Data on HPV infection in Chinese men is still limited, which requires further investigation to guide vaccine development and assess the effectiveness of HPV vaccines. We thus studied the HR-HPV genotype distribution in HPV-infected men in Northern China.

**PATIENTS AND METHODS:** Genital specimens were obtained from male patients ( $\geq 18$  years old) at the clinic for sexually transmitted infections of the Shandong Provincial Hospital between January 2016 and December 2018. Samples were analyzed for 15 HR-HPV genotypes, and 2 low-risk HPV (LR-HPV) genotypes using a multiplex real-time quantitative polymerase chain reaction (qPCR) assay.

**RESULTS:** Of 1,163 participants enrolled, 426 patients were diagnosed as verruca acuminata (CA) and 737 were asymptomatic men. The overall prevalence of HPV infection was 42% (489/1,163), and 27.4% (319/1,163) were positive for HR-HPV. HPV 16 (5.2%, 61/1,163) was the most common HR genotype overall, followed by HPV 52 (4.6%, 54/1,163), 51 (4.3%, 50/1,163), 18 (4.1%, 48/1,163), and 39 (4.0%, 47/1,163). Genotypes 16, 52, 39, 51, and 18 were most prevalent in CA patients, and 16, 51, 18, 59, and 39 in asymptomatic men. Prevalence of genotypes 31, 33, and 45 covered by the 9-valent HPV prophylactic vaccine was low in the assessed region.

**CONCLUSIONS:** HPV 16, 52, 51, 18, 39, and 59 were the most common HR genotypes detected in men in Northern China. Importantly, HPV 39, 51, and 59 are not currently covered by either the 4-valent or 9-valent HPV vaccines.

*Key Words:*

Human papillomavirus, Genotype, Male.

## Introduction

Human papillomavirus (HPV) is the most common infection due to sexual transmission<sup>1,2</sup>. It is easily transmitted by skin-to-skin contact and affects approximately 11% of the world population. HPV infection is frequently transient in most cases, and the virus is effectively cleared by the immune system<sup>3</sup>, but sometimes it persists and results in genital benign lesions or even cancer<sup>4</sup>. It is now firmly believed that HPV is the etiologic agent of cervical cancer and a proportion of vulvar and vaginal cancers in women<sup>5</sup>. The prevalence in men is also high, particularly among men at high risk<sup>6,7</sup>. It is estimated that HPV is responsible for roughly one-third of penile cancers and up to 90% of anal cancers, which presents growing incidence in recent years<sup>8</sup>. In total, HPV accounts for around 5% of the worldwide cancer burden<sup>5</sup>.

More than 200 subtypes of HPV have been identified to date, of which approximately 40 cause infections of the genital tract. These are classified into two categories, low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV), according to the association with cervical cancer<sup>9</sup>. The LR-HPV or non-oncogenic HPV category includes HPV genotypes 6, 11, 40, 42, 44, 54, 61, 72, 81, and 89 that lead to genital warts and other benign lesions. The HR-HPV or oncogenic/carcinogenic HPV category comprises 12  $\alpha$ -mucosal HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and certain others (e.g., 68, 66, and 82), which are possibly carcinogenic. HPV genotypes in this category cause high-grade lesions and give rise to nearly all cervical cancers and a proportion of anal, vulvar, vaginal, and penile cancers<sup>5,10</sup>.

HPV genotype distribution varies by region and population<sup>11</sup>. HPV16 is the most common type detected in HPV-related cancers worldwide<sup>12</sup>, with less variation in the geographical distribution compared to other HPV types, followed by HPV18. HPV 52 and 58 are prevalent subtypes among women in Asia<sup>13,14</sup>. Considering that HPV strains exhibit different pathogenicity, current vaccines only confer protection against certain HPV types and specific regional variation in HPV types may impact on the effectiveness of vaccination. Therefore, HPV genotype distribution should be fully assessed to determine the prophylactic effect before the development and application of the vaccine.

Since there is no cure for HPV at present, vaccination against HPV has become the most important strategy to reduce infection rates. The initial recommendation for HPV vaccination of female adolescents was issued in 2006, and by the end of 2017, 71 countries worldwide had approved the use of HPV vaccines<sup>15</sup>. Three prophylactic HPV vaccines are commercially available: a bivalent vaccine (GlaxoSmithKline, Warren, NJ, USA) targeting HPV16 and 18; a 4-valent vaccine (Merck, Branchburg, NJ, USA) targeting HPV6, 11, 16, and 18; and a 9-valent vaccine (Merck, Branchburg, NJ, USA) targeting HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58<sup>16,17</sup>. Studies<sup>2,18,19</sup> show that these vaccines are very safe and highly effective in preventing HPV-associated diseases.

Due to the inadequate awareness of sexually transmitted infections (STIs), as well as HPV vaccination is still not included in the national immunization program of mainland China, China is facing up to the increasing threat of HPV infection and its adverse consequences<sup>20</sup>. Recently, the three available vaccines to prevent HPV infections have been approved but not yet recommended for males. In the present study, we investigated the HR-HPV genotype distribution in male patients diagnosed in STI clinic in Northern China.

## Patients and Methods

### Study Participants

This was a cross-sectional study involving sexually active men in the STI clinic of Shandong Provincial Hospital for an HPV test from January 2016 to December 2018. Participants were excluded if they once received an HPV vaccine.

### Ethical Statement

Informed consents were obtained from all participants. The investigation was approved by the Ethics Committee of Shandong Provincial Hospital, ensuring confidentiality and anonymity.

### Clinical Sampling and HPV DNA Extraction, Detection, and Typing

Genital specimens were obtained from the coronal sulcus, glans penis, penile shaft, scrotum, and urethral using dacron swabs, which were subsequently combined into one sample. DNA from the sample was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. HPV DNA testing was carried out using the High-Risk Human Papillomavirus (HPV) Genotyping Real Time PCR Kit (Liferiver, Shanghai, China). The kit contains a specific ready-to-use system for the detection of 15 high-risk HPV types: 15 HR-HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 82) and 2 LR-HPVs (6 and 11) by polymerase chain reaction with a real-time PCR system (Applied Biosystems, Waltham, MA, USA).

### Statistical Analysis

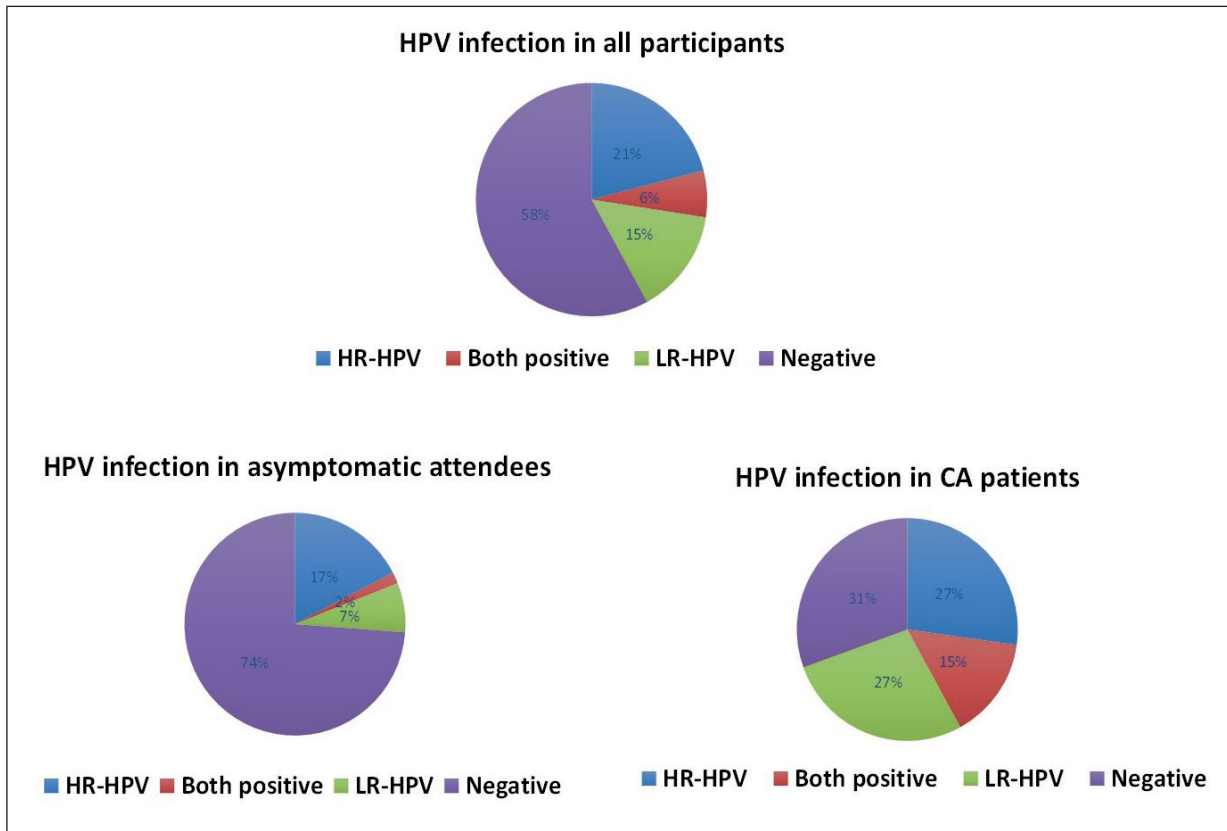
All statistical analyses were performed using SPSS 17.0 (IBM, Armonk, NY, USA). Age-specific distribution was compared between HR-HPV positive men and HR-HPV negative men using Chi-square test and Fisher exact tests. The threshold of significance was set at  $p < 0.05$  (two-tailed) and  $p < 0.01$  was taken as highly significant.

## Results

### Prevalence of Genital HPV Infection

A total of 1,163 men were tested for HPV infection in this study. The median age was 35 years (range: 18-67 years), with 94.4% (1,086/1,163) aged between 20 and 49 years, 3.4% (39/1,163) under 20 years, and 2.2% (8/1,163) over 50 years. The overall HPV infection rate was 42% (489/1,163), and 27.4% (319/1,163) were positive for HR-HPV. The distribution of HPV infection in all participants, CA patients, and asymptomatic men was shown in Figure 1 A-C.

Multiple HR-HPV types were detected in 7.6% (88/1,163) of all tested men, accounting for 18% (88/489) of all HPV-positive men, and 27.6% (88/319) of HR-HPV-positive men. The infection rate with multiple HR-HPV types was 13.7% (58/426) in CA patients and 15.5% (30/193) in asymptomatic HPV-infected men.



**Figure 1.** Genital human papilloma virus (HPV) infection rates. HPV infection rates by risk category were shown for all study participants (A), for asymptomatic men only (B), and for CA patients only (C). Negative: negative for both high-risk HPV (HR-HPV) and low-risk (LR-HPV, HPV 6 and 11); HR-HPV: positive for HR-HPV only, LR-HPV: positive for LR-HPV (HPV 6 and 11) only; both positive: positive for both HR-HPV and LR-HPV (HPV 6 and 11).

### Age-Specific Prevalence of HR-HPV Infection

The HPV infection rate was assessed according to the age distribution of study participants and the data were shown in Figure 2. 18- to 24-year-old participants represent high risk group for HR-HPV, or LR-HPV (HPV6 and 11) (Figure 2A). Nevertheless, the rates of total HPV (Figure 2A), HR-HPV, or LR-HPV infection among all participants (Figure 2A), asymptomatic men (Figure 2B) or CA patients (Figure 2C) showed no significant difference regarding the age ( $p > 0.05$ ).

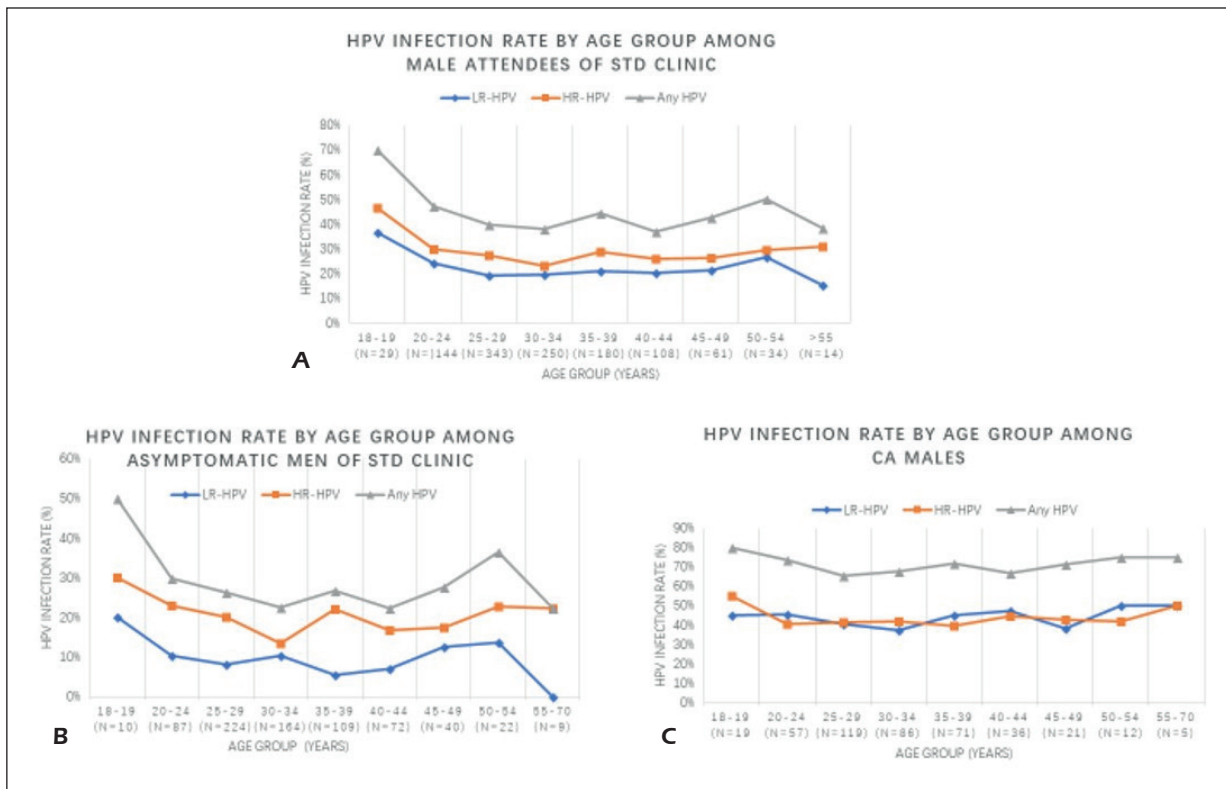
### Distribution of HPV Genotypes

The most common genotype overall was HPV16 with a rate of 5.2% (61/1,163) among all participants, accounting for 19.1% (61/319) of all HR-HPV-positive men (Figure 3A). HPV 52 was the second most frequently detected genotype (54/319, 16.9%), followed by HPV 51 (50/319, 15.7%), HPV 18 (48/319, 15.1%), and HPV 39 (47/319, 14.7%). HPV 51 and 39

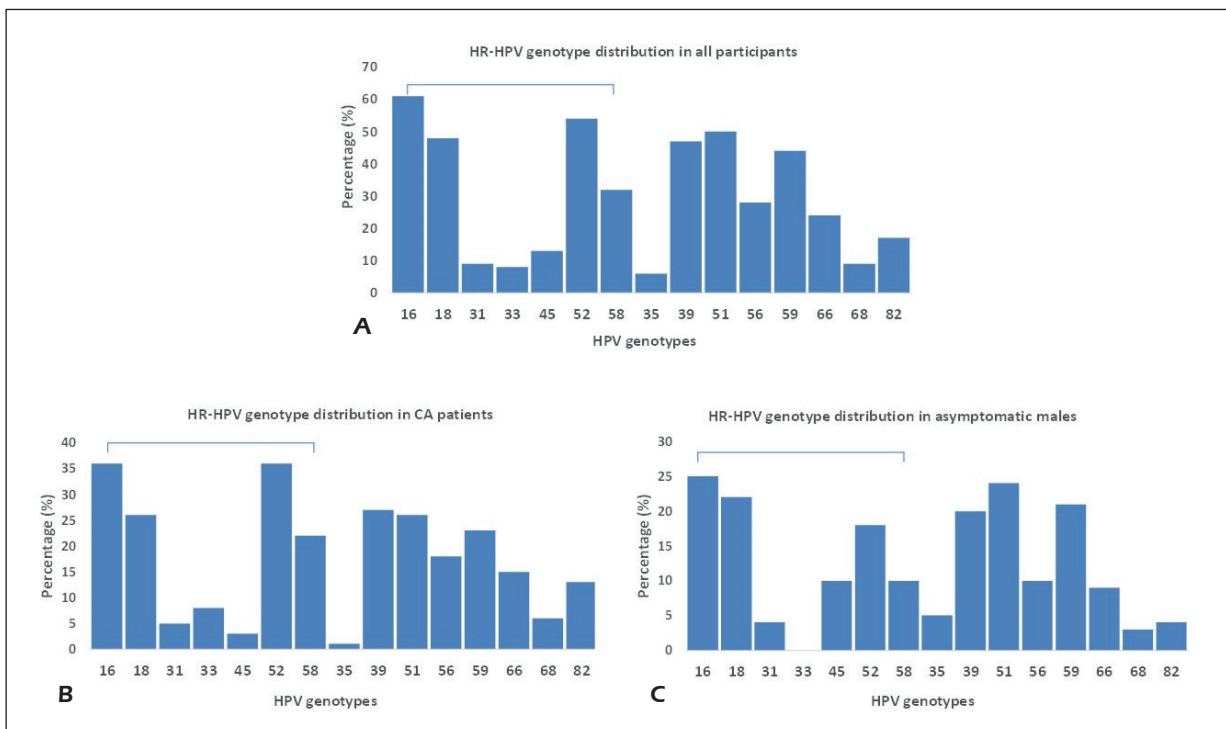
however were not covered by the protective range of 9-valent vaccine (Figure 3A). Other HR-HPV genotypes detected were HPV 59, 58, 56, 66, and 82 (Figure 3A). The prevalence of genotypes HPV 31 (9/319, 2.8%), 33 (8/319, 2.5%), and 45 (13/319, 4.1%), which are covered by the vaccine, was low in our study.

HPV16 was also the most common genotype detected in asymptomatic participants alone, with a positive rate of 3.4% (25/737), accounting for 12.9% (25/193) of HPV-positive and 17.8% (25/140) of HR-HPV-positive asymptomatic men (Figure 3B). The next most common HPV genotypes in asymptomatic participants were HPV 51, 18, 59, and 39 (Figure 3B).

In CA patients, HPV16 also led the list of most common genotypes with a positive rate of 8.6% (36/426), accounting for 12.1% (36/296) of HPV-positive and 20.1% (36/179) of HR-HPV-positive CA patients, followed by HPV 52, 39, 51, and 18 (Figure 3C).



**Figure 2.** Human papilloma virus (HPV) infection rates by age group. The HPV infection rate was assessed according to the age distribution of all participants (A), asymptomatic men (B) and CA patients (C). No significant differences in total HPV, high-risk HPV (HR-HPV), or low-risk HPV (LR-HPV) infection rates were detected between the age groups of all participants, asymptomatic men, or CA patients ( $p>0.05$ ).



**Figure 3.** Distribution of high-risk human papilloma virus (HR-HPV) genotypes. HR-HPV genotypes are shown for all participants (A), for asymptomatic men only (B), and for CA patients only (C).

## Discussion

HPV infection has been widely studied in women worldwide and HR-HPV was identified as the cause of cervical cancer which occurs primarily (85%) in developing countries<sup>21</sup>. Vaccines against certain HR-HPV types have been developed and proven effective in the prevention of HPV infection<sup>16,22</sup>. It is estimated that the 9-valent vaccine could have prevented 80% of HPV infections, over 90% of cervical and anal cancers, and a proportion of penile, vulvar, vaginal, and oropharyngeal cancers caused by certain HPV types<sup>5</sup>.

Characterization of male genital HPV infection is essential to the prevention of genital cancer in men and consequently HPV infection in women, which then in turn would reduce incidence rates of cervical cancer. Nonetheless, most studies<sup>13,23</sup> on HPV infection in China were conducted in women and only few studies<sup>24,25</sup> in men. In the present study, we assessed the prevalence and genotype distribution of genital HPV in sexually active men in our clinic. HPV genotype distribution would be influenced by a widespread immunization program with HPV vaccines<sup>23,26</sup>, but the participants in our study had never been vaccinated against HPV. Therefore, our results provide primary information on HPV genotype-specific prevalence in a high-risk cohort of sexually active men in Northern China. HPV prevalence varies widely among different regions<sup>27</sup>, with differences of more than five-fold observed across the world<sup>4</sup>. The worldwide prevalence of HPV infection in women without cervical abnormalities is 11-12%<sup>4</sup>, and that of HR-HPV infection among women with normal histology is 11.2%<sup>4</sup>. We identified a prevalence of 26.1% for HR-HPV in asymptomatic men attending our STI clinic, which is lower than that reported in studies from Western countries but higher than that reported in other studies<sup>28,29</sup>. These discrepancies may reflect differences in sexual preferences between countries, HPV types tested, techniques used for HPV detection, and the diversity of specimens. The actual HPV prevalence in Northern China may differ from that detected in our study, as men in our STI clinic may not be representative for the general, healthy population. Most of our study participants exhibited high-risk sexual behavior, including commercial sex, multiple sex partners, MSM, and female partners with HR-HPV infection.

When we analyzed HR-HPV distribution in different age groups, we discovered that the prevalence of HPV infection among all participants,

CA patients, and asymptomatic men in our STI clinic did not significantly differ between the age groups. A similar distribution has been reported for men by Giuliano et al<sup>30</sup>, but most studies in women showed that the infection rate peaks at below 30 years of age, and the age-specific HR-HPV prevalence significantly changes with age.

The HR-HPV genotype distribution shows diversity across different countries and populations. A meta-analysis of 1 million women with normal cytology demonstrated that the 5 most common genotypes worldwide were HPV 16, 18, 52, 31, and 58<sup>31</sup>, while another meta-analysis identified HPV 16, 18, 33, 45, and 31 as the most prevalent genotypes in cervical cancer worldwide<sup>30</sup>. In contrast, HPV 52 and 58 are the most frequently detected genotypes other than HPV 16 in both healthy women and women with cervical lesions in Asia<sup>13,14</sup>. Two studies<sup>32,33</sup> analyzed the HPV genotype distribution in China and identified HPV 16, 58, 51, 39, and 52 as the most common HR-HPV genotypes causing male genital warts detected in Shanghai, and HPV 52, 16, 81, and 58 as those in the Guangdong province. HPV16 is the most common high-risk genotype of all HPV infections (cervical and non-cervical) worldwide, while the prevalence of other genotypes varies greatly between different regions and populations. HPV 31 and 33 are commonly detected in genital lesions in European and North American patients, while HPV52 and HPV58 are more prevalent in Asia<sup>13</sup>.

We detected a high prevalence of vaccine-targeted HPV genotypes in our study, which is attributed to the fact that men in this region are typically not getting vaccinated against HPV. For example, HPV 16, 18, and 52 could be prevented with the 4-valent or 9-valent HPV vaccines. HPV 31, 33, and 45, which are also targeted by the vaccines and are highly prevalent in most developed countries, exhibited a low prevalence in our study. In turn, the prevalence of HPV 39, 51, and 59 was high in this region of China but these genotypes are not covered by the new 9-valent HPV vaccine and hence could not have been prevented by vaccination, which indicated that a new HPV vaccine should be considered to cover HPV genotypes with a high prevalence in this region. Also, our data provide new fundamental data for the further development of microbicide for the population with genital HPV-infection as recently reported to accelerate the normal clearance of genital HPV infection<sup>34</sup>.

Certain differences in HPV genotype distribution were identified between CA patients and asymptomatic participants. HPV 52 and 58, the predominant subtypes in cervical cancers in Asia, were common in CA patients but not in asymptomatic men assessed in our study. Furthermore, a higher proportion of asymptomatic patients (72.5%, 140/193) were positive for HR-HPV compared to verruca acuminata (CA) patients (42%) which is primarily caused by LR-HPV. This indicates that asymptomatic men pose a higher risk of transmitting HR-HPV than CA patients do. These results are limited by the fact that only two LR-HPV types were detected in our study, which means that the total number of asymptomatic HPV-infected men determined might be lower than the actual value, subsequently resulting in a higher proportion of HR-HPV-positive men in the asymptomatic patient group. Nevertheless, the LR-HPV genotypes 6 and 11 typically account for over 90% of all genital warts. Therefore, we likely covered most LR-HPV infections with our assessment. The limitation is that the percentage of homosexuals between these participants has not been identified due to the privacy policy and the distribution of high-risk human papillomavirus genotypes in homosexuals requires to be further investigated on condition that the special informed consents were obtained.

## Conclusions

We identified a comparatively high prevalence of HR-HPV infections among both CA patients and asymptomatic men in our STI clinic in Northern China. Not all of the HPV genotypes that were most frequently detected in our study are covered by the 9-valent vaccine. Although some of these infections are transient and might be cleared by the immune system, they may result in continuous virus transmission to the sexual partners. Therefore, the continuous monitoring of HPV-positive men is warranted in our region.

## Acknowledgements

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## Conflict of Interests

The authors declare that they have no conflict of interests.

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