

## RESEARCH ARTICLE

# The Prevalence and Genotype of Human Papillomavirus from Patients with Genital Warts in Eastern Guangdong Province

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## Abstract

**Background:** Low-risk human papillomavirus (LR-HPV) infection is the main cause of genital warts. LR-HPV genotypes 6 and 11 are associated with genital warts, but there have only been a few published studies about the genotype-specific prevalence of HPV in genital warts in China. The objective of our study was to assess the prevalence of HPV genotypes for clinical cases involving both men and women and to evaluate the potential benefit of a quadrivalent (genotypes 6, 11, 16, and 18) HPV vaccine in eastern Guangdong province of China. **Materials and Methods:** A total of 696 eligible patients with genital warts were enrolled during the period Aug 2009 through Oct 2014. Specimens were collected from genital warts, the HPV GenoArray test was used for HPV detection and genotyping, which could detect 21 HPV genotypes, including genotypes 6, 11, 16, and 18. **Results:** Among the 696 cases, 675 samples were successfully genotyped. The median age of patients was 32.1 years (range, 16-67 years). The most prevalent genotypes were HPV-6 (285/675, 42.2%), HPV-11 (265/675, 39.3%), HPV-52 (52/675, 7.7%), HPV-16 (51/675, 7.56%), HPV-81 (50/675, 7.40%) and HPV-58 (37/675, 5.48%). Low-risk genotypes predominated, with a prevalence of 96.59%. The cumulative prevalence of genotypes 6 and 11 was 78.7% (531/675), the cumulative prevalence of genotypes 16 and 18 was 11.6% (78/675), and the cumulative prevalence of genotypes 6, 11, 16, and 18 was 82.5% (557/675). **Conclusions:** Our results provide strong evidence that, in eastern Guangdong, different from Western countries, the most prevalent low risk HPV genotypes in patients with genital warts are 6, 11 and 81. The quadrivalent HPV vaccine could prevent 82.5% of genital warts in eastern Guangdong.

**Keywords:** Human papillomavirus - genital warts – prevalence - China

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## Introduction

Cervical cancer is the second most common cancer in females worldwide, including China (Chen et al., 2012). Molecular epidemiological studies clearly indicate that certain types of human papillomavirus (HPV) are the principal cause of cervical cancer (Walboomers et al., 1999; de Villiers et al., 2004; Chen et al., 2012). Over 120 different types of HPVs have been identified, and approximately one-third of these types specifically infect the anogenital tract (de Villiers et al., 2004). Genital HPV types have been subdivided into low risk (LR) types which are the cause of external genital warts, and high risk (HR) types which have high oncogenic potential for progression to cervical cancer (de Villiers et al., 2004). Prevalence of HPV infection has been estimated to be about 17.7%, varying between 6.52% and 20% depending on geography of China (Chen et al., 2012; Zhao et al., 2012). It is estimated that most females will be infected with HPV in their lifetime. Fortunately, HR-HPV infections

typically last for 12-18 months and are eventually cleared completely by the immune system (Richardson et al., 2003). Approximately 10% of women fail to clear HPV infections, resulting in long-term persistent infections (Bodily et al., 2001). Persistence of HR-HPV infection is the single most important risk factor in the development of cervical intraepithelial neoplasia (CIN) (Walboomers et al., 1999; Bodily et al., 2001).

Specifically, high risk types 16 and 18 are the most common HPV types found in cervical cancers and account for about 70% of cervical cancers (Chen et al., 2012; Zhao et al., 2012). HPV-6 and HPV-11 are the most commonly detected LR-HPV genotypes in genital warts (Cremin et al., 2012; Aubin et al., 2008; Johnson et al., 2012). Up to date, several vaccines have been developed to prevent HPV infection. Gardasil is a quadrivalent vaccine containing four genotypes, namely 16, 18, 6 and 11; and Cervarix (GSK) is a bivalent vaccine containing 16 and 18. In addition to providing excellent protection against the HPV types contained in the vaccine, Gardasil has

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also demonstrated significant cross-protection to other oncogenic types in both naïve and previously infected women (Mc Cormack et al., 2011).

At present in eastern Guangdong province of China, cervical lesions are screened by DNA-based testing for HR-HPV, and cervical exfoliated cells smear examinations is adjunct for the screening program, as this substantially increases the sensitivity of detecting neoplasia (Lin et al., 2008; Chen et al., 2012; Chen et al., 2012). In this study, we would like to present the distribution of HPV types in genital warts of a cohort of Chinese in Guangdong to contribute essential data for assessing the potential benefit of HPV vaccines containing HPV 6 and 11.

## Materials and Methods

### *Patient recruitment and sample collection*

Chaozhou City located in easternmost Guangdong province of the People's Republic of China. From August 2009 to October 2014, the study was carried out in Chaozhou Central Hospital. The initial diagnosis of genital warts was made by gynaecologist, dermatologist, or urologist. A standardized sampling protocol was provided to each physician. One representative sample was collected from each patient. Biological samples were obtained from the most typical genital lesions (from coronal sulcus, glans penis, shaft, scrotum, vulva, vagina, perineum, and perianal skin) using a cotton swab (HybriBio Biotechnology Limited Corp.). The cotton swab was then placed in phosphate-buffered saline (PBS), and kept at 4°C until further processing in laboratory. For females with cervical warts, specimens were collected from cervical exfoliated cells based on the method described previously (Chen et al., 2012; Lin et al., 2008; Li et al., 2011), samples of exfoliated cervical cells were collected using plastic cervical swabs. The sampler was inserted 1-1.5 cm into the endocervical canal and rotated 4-5 full turns in counter-clockwise direction. The tip containing cellular material was then placed into transport medium tube and stored at 4 °C immediately. All swabs and store bottle with specimen transport medium were from HybriBio Biotechnology Limited Corp., Chaozhou, China. The study was carried out with the approval of the ethical committee of Chaozhou Central Hospital, and patients consent was obtained for the collection of samples.

### *HPV DNA extraction and HPV GenoArray test*

The swabs and supernatant were removed after the cells were centrifugated for 5 mins with relative centrifugal force of 960 g. DNA was extracted from the sediments with alkaline lysis method Kits (HybriBio Biotechnology Limited Corp.) (Lin et al., 2008; Chen et al., 2012b). HPV GenoArray test was used for HPV detection and genotyping. Genotyping was done by DNA amplification, flow-through hybridization and gene chip by HybriMax (HybriBio Biotechnology Limited Corp., Chaozhou, China). Test was performed according to the manufacture's instructions. Detailed protocols for this assay had been described previously (Lin et al., 2008; Chen et al., 2012a; Chen et al., 2012b). The gene chip

contained type-specific oligonucleotides immobilised on a nylon membrane and could identify 15 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and 6 LR-HPV types (6, 11, 42, 43, 44, and 81) (Lin et al., 2008). The final results were detected by colorimetric change on the chip under direct visualization (Lin et al., 2008; Li et al., 2011; Chen et al., 2012a; Chen et al., 2012b).

### *Statistical analysis*

For age-specific HPV prevalence assessment, the actual age of each study subject was counted with the formula: (interview date - birthday) / 365.25 by Microsoft Excel software. Chi-squared tests were used to assess the statistical significance of any differences in prevalence. All data were analyzed using SPSS software version 16. *P* values were two-sided, and statistical significance was accepted if the *P* value was 0.05 or less. Quantitative data are expressed as mean (SD) and range.

## Results

### *Patient characteristics.*

The subjects of our study was carried out in Chaozhou Central Hospital and most of them were natives. The initial diagnosis of genital warts was made by gynaecologist, dermatologist, or urologist. Male genital warts were from coronal sulcus, glans penis, shaft, scrotum, perineum, and perianal skin and female warts were mostly from vulva, vagina and cervix. Of the 696 samples tested by the GenoArray assays, 675 (96.98 %) samples were found to have definable genotypes. 21 cases (3.02%, 13 males and 8 females) which were negative may not have had virus present, or negative for  $\beta$ -globin, or, had sub-detectable levels on the genital warts, or may not have had genotypes that matched the panel covered by the GenoArray assays, were excluded from the following type distribution analysis.

The remaining 675 samples were successfully genotyped and were further analyzed, including 305 males, mean age 33.25±10.16 (16-65 years), 370 females, mean age 31.06±10.06, (17-67 years), the median age of patients was 32.05±10.16 years (range, 16-67 years) for the entire group, the median age varied slightly between men and women (33.25 vs. 31.06 years;).

### *HPV prevalence*

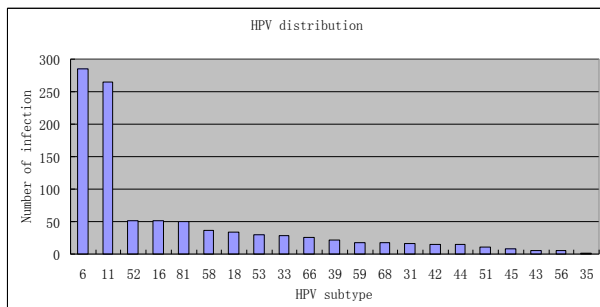
Presence of 1 HPV genotype was only observed in 453 (67.11%) of 675 cases, whereas 222 (32.89%) cases involved infection with  $\geq 2$  genotypes (Table 1). Multiple infections were seen in 29.19% of women and 37.38% of men, and single infection was more common in women than men ( $p < 0.01$ ) (Table 1). On the basis of the low-risk/high-risk genotype classification of previous studies (de Villiers et al., 2004; Chen et al., 2012a; Chen et al., 2012b), presence of  $\geq 1$  low-risk HPV genotype was found in 652 (96.59%) of 675 cases, and presence of  $\geq 1$  high-risk genotype was found in 243 cases (243/675, 36%) (Table 1). These proportions varied significantly with sex; the presence of  $\geq 1$  low-risk genotype was similar between men and women (282/305, 92.46% vs. 327/370, 88.38%;

$P>0.05$ ) (Table 1). In contrast, presence of  $\geq 1$  high-risk genotype was also similar between men and women (120/305, 39.34% vs. 124/370, 33.51%;  $p>0.05$ ) (Table 1). 432 (64%) of 675 genital wart samples demonstrated infection with  $\geq$  low-risk genotype (without high-risk

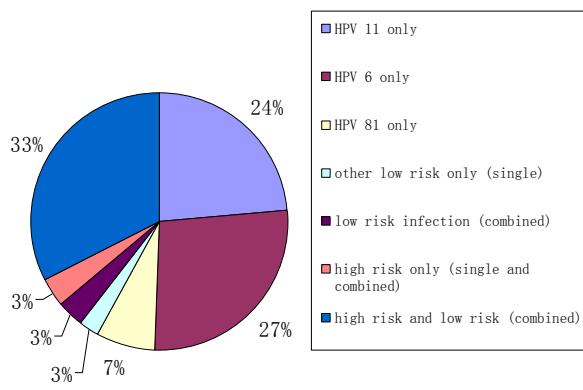
genotypes); 23 (3.4%) demonstrated infection  $\geq 1$  high-risk genotype (without low-risk genotypes), whereas 220 (32.6%) demonstrated co-infection with low-risk and high-risk genotypes (Table 1).

**Table 1. Distribution of HPV Types in 675 Genital Wart Specimens, According to the Low-risk And High-risk Classification**

HPV types	Male (n=305)	Female (n=370)	Total (n=675)
Single-type infection	191	262	453
6	95	86	181
11	70	90	160
81	4	46	50
16	1	2	3
18	4	3	7
31/33/39/45/51/52/	11	22	33
53/58/59/66/42/43/44	5	13	18
Multiple-type infection	114	108	222
Two HPV types	67	69	136
6, 11	3	2	5
6, 16	4	2	6
6, 18	2	2	4
11,16	3	5	8
11, 18	4	2	6
16, 18	0	1	1
Other combination	51	55	106
Three HPV types	26	25	51
Four or more HPV types	16	14	30



**Figure 1. Distribution of HPV types among 675 successfully genotyped genital warts. HPV types were counted regardless of the status of single- or multiple-type infections. 222 specimens with more than one HPV type were counted more than once**



**Figure 2. Distribution of HPV types in 675 successfully genotyped genital warts according to HPV6/11/81 status**

#### Prevalence of specific HPV genotypes

The most frequently encountered HPV genotypes of 675 samples were as follows, by decreasing order of frequency: HPV-6 (285/675, 42.22%), HPV-11 (265/675, 39.26%), HPV-52 (52/675, 7.7%), HPV-16 (51/675, 7.56%), HPV-81 (50/675, 7.4%) and HPV-58 (37/675, 5.48%) (Table 1). The distribution of HPV types, regardless of single- or multiple-type infection, among the 675 HPV-positive samples was shown in Figure 1. In women, the 6 commonest types were HPV-11, HPV-6, HPV-81, HPV-52, HPV-16 and HPV-58, whereas in men, the 6 commonest types were HPV-6, HPV-11, HPV-16, HPV-52, HPV-18, and HPV-58 (Table 1).

Low-risk HPV genotypes 6 and/or 11 were found more frequently in men than in women (267/305, 87.54% vs. 263/370, 71.08%;  $P<0.01$ ).

Conversely, high-risk HPV genotypes 16 and/or 18 distributed equally in men and women (42/305, 13.77% vs. 39/370, 10.54%;  $p>0.05$ ).

Interestingly, low-risk HPV genotypes 81 was more common among women than among men (65/370, 17.57% vs. 17/305, 5.57%;  $p<0.001$ ).

Furthermore, single-type 81 infection was more common among women than among men (46/370, 12.43% vs. 4/305, 1.31%;  $p<0.01$ ).

The distribution of HPV types was analyzed with respect to HPV6 and 11, to assess the potential coverage for genital warts with vaccines containing these types (Figure 2). HPV-6 and/or HPV-11 (alone or in association with each other) were found in 51.26% (346/675) of cases. These 2 genotypes were detected in combination with other HPV types in 27.26% (184/675) of genital wart cases, yielding an overall prevalence of HPV-6 and/or HPV-11 infection of 78.52% (530/675) (Figure 2).

Similarly, HPV-16 and/or HPV-18 (alone or in association with each other) were observed in 1.63% (11/675) of specimens. HPV-16 and/or HPV-18 were further detected in combination with other HPV genotypes in 9.93% (67/675) of genital wart cases, yielding an overall prevalence of infection with HPV-16 and/or HPV-18 of 11.55% (78/675). HPV-6, HPV-11, HPV-16, and/or HPV-18 (alone or in association with each other) were observed in 56.6% (382/675) of all cases. Finally, at least 1 of these 4 genotypes (alone or in combination with other genotypes) was found in 82.52% (557/675) of cases.

## Discussion

Human papillomavirus (HPV) infection is a common sexually transmitted infection worldwide. HPV may cause several reproductive tract diseases, including genital warts and cervical cancer, the later is the most serious health problem due to persistent HR-HPV infection. Although cervical cancer showed a declining trend over the past 3 decades in China, it remains a major health burden in Chinese females especially women living in rural area. The

disease burden is believed to be underestimated given the relatively high HPV prevalence shown in recent studies (Chan et al., 2009).

Up to date, about 120 kinds of HPV subtypes have been characterized, above 40 kinds of subtypes was associated with genital tract infection, they were classified into high risk and low risk based on their carcinogenic capability. The pathogen of genital warts was low risk HPV (LR-HPV). HPV6, 11, 42, 43, 44 and 81 was generally considered as low-risk, the main subtypes for genital warts, whereas HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 53 and 66 were classified as high-risk (de Villiers et al., 2004). Generally, HR-HPV could incorporated into genome of host, persistent infection or recurrent infection of HR-HPV was the main factor of cervical cancer, the pathogenic ability of HPV was closely associated with HPV subtypes (Walboomers et al., 1999), therefore, HPV subtyping and evaluation were very important for clinical practice.

To obtain the first data concerning HPV and genital warts in eastern Guangdong, 696 genital wart tissue specimens were tested for the presence of HPV DNA using GenoArray test, the samples from 675 cases of genital warts were successfully genotyped for HPV. Single infection ratio was 67.11% (453/675), mainly HPV11 and 6, multiple infection was 32.89%, mainly was mixed infection of HR and LR, double and triple infection was the main multiple infections. HPV-6 or HPV-11 was detected in 78.67% of genital warts studied, which was in agreement with the results of similar recent studies (Aubin et al., 2008; Johnson et al., 2012; Chen et al., 2012b). This study showed that HPV subtypes infection of genital warts of our area were mainly low risk HPV11 and HPV6, consistent to previous study (Chan et al., 2009; Cremin et al., 2012), possibly due to common warts sample of genitals.

According to the recent phylogenetic classification (de Villiers et al., 2004), HPV-81 belongs to the alpha-HPV species 3 together with other HPV genotypes: HPV-72, HPV-83, HPV-84 and HPV-86. Single infection of HPV 81 was more frequently found in the females than in the males in our study. This demonstrated that HPV 81 was more prone to induce epithelial lesion (genital warts) in women other than in males, another explanation was that HPV81 could be eliminated by males quickly, the mechanism underlining it was not clear yet. According to the published data, our study is the first to report the presence of 81 as a single HPV genotype in genital warts tissue specimens, giving this particular HPV genotype a more prominent place in the etiopathogenesis of genital warts than was supposed before.

In our study, which was performed exclusively in individuals without any apparent immunodeficiency, 222 out of 675 genital wart specimens contained more than one HPV genotype. In addition to HPV-6 or HPV-11, which were detected in 28.15% (190/675) samples with mixed HPV infection (Table 1, Figure 1). Our results agreed with the results of similar recent studies, the prevalence of simultaneous infection with different HPV genotypes (mixed HPV infection), ranged from 30% to 54%, mainly depending on the immune status of the individuals

included in the study and the selection of method used for HPV genotyping (Aubin et al., 2008; Chan et al., 2009; Cremin et al., 2012).

119 of 675 samples had one or more of the HR genotypes 31, 33, 45, 52, 58 with or without co-infection with 16 and 18. As indicated by Brown et al (Brown et al., 2009), these HR types have 80% homology with genotypes 16 and 18 and as a clade, the additive efficacy of Gardasil is 25% on a population level. Our study population infected with these additional genotypes should therefore benefit from cross-protection with Gardasil.

The cumulative prevalence of genotypes 6, 11, 16, and 18 was 82.52% (557/675) in our study. Therefore, Gardasil, the quadrivalent vaccine, would have prevented infections in 82.52% of our patient population. HPV16 and/or 18, high-risk types being included in currently available vaccine Cervarix (GSK), were found in genital warts specimens, with a majority of which existed as coinfections with HPV6 and/or 11, would have prevented infections in 11.55% of our patient population.

In conclusion, our study showed that HPV can be found in virtually all tissue specimens of genital warts obtained from patients in eastern Guangdong. This finding suggests that, if the recently approved quadrivalent HPV vaccine proves to be as effective in preventing HPV infections in males as it has proved in females (Brown et al., 2009), this vaccine could prevent the great majority of incidental GWs in males in eastern Guangdong.

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