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Distribution of Genital Wart Human Papillomavirus Genotypes in China: A Multi-Center Study

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Abstract

Although it is understood that low-risk human papillomavirus (HPV) genotypes are associated with genital warts, there have been very few published studies reporting the genotype-specific prevalence of HPV among Chinese population. The aim of the study was to assess the prevalence of HPV genotypes in genital warts across China, and thus to evaluate the potential benefit of a quadrivalent HPV vaccine in this population. The tissue samples of a total of 1,005 genital warts cases were collected from seven geographical regions of China. HPV genotypes were analyzed using the general primer PCR and sequence-based typing method. Prevalence differences between sexes, geographical regions and age groups were assessed. The overall prevalence of HPV DNA in genital warts patients was 88.7% (891/1,005). Low-risk genotypes predominated, with a prevalence of 78.1% (785/1,005). The most prevalent genotypes were HPV-6 (41.3%), HPV-11 (37.6%) and HPV-16 (10.4%). Among HPV positive patients, single infections were more frequent (866/891, 97.2%) than co-infections (25/891, 2.8%). Both the overall prevalence of HPV DNA and that of HPV-6/-11/-16 (positive for any of the three types) decreased with age (P -trend = 0.010 and P -trend = 0.025, respectively). The prevalence of HPV-6/-11 (positive for either HPV type) and HPV-16 varied by geographic region (P = 0.003 and P = 0.001, respectively). The prevalence of HPV-16 in female patients between urban and rural areas showed a marginally significant difference (P = 0.05). In sum, the results provide strong evidence that, in China, the most prevalent HPV genotypes in genital warts are HPV-6, HPV-11 and HPV-16. This indicates that a quadrivalent HPV vaccine may decrease the incidence of genital warts in the future.

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Keywords

human papillomavirus; HPV genotypes; distribution; genital warts; China

INTRODUCTION

Genital warts are the most common benign lesions of the anogenital region and can lead to psychosocial distress as well as a high financial treatment burden [Strauss et al., 1996; IARC, 2006]. The incidence of genital warts in United States (US) is 205 per 100,000 person-years [Koshiol et al., 2004]. This gives rise to concerns regarding the disease burden in China, where a complete registration system is lacking.

Human papillomavirus (HPV) related cancers are responsible for a heavy disease burden, especially in developing countries [Ferlay et al., 2010]. To date, more than 200 HPV genotypes have been identified [Bernard, 2005] and classified as either high risk (HR) or low risk (LR) [Schiffman and Castle, 2003]. Of these, approximately 40 HPV types can be found in the genital tract [Munoz et al., 2003]. HPV-16 and HPV-18, the two most prevalent HR types, are both known to cause cervical cancer [de Sanjose et al., 2007; Bosch et al., 2008]. Furthermore, HPV-16 also contributes to the tumorigenesis of vulva, vagina, penis, and anus [IARC, 2006]. In addition, the most common LR types, HPV-6 and HPV-11, have been reported in more than 90% of genital warts [Munoz et al., 2004; Dunne and Markowitz, 2006]. Prevention of HPV infection is the most effective approach to reduce HPV disease burden [Olsson et al., 2009].

In recent years, a quadrivalent (HPV genotypes 6, 11, 16, and 18) vaccine has been developed and is now licensed in some countries [Villa et al., 2005]. Recent studies show that the vaccine is highly effective in preventing disease associated with HPV-6, HPV-11, HPV-16, and HPV-18, including genital warts [Ault, 2007; Barr and Tamms, 2007; Garland et al., 2007; Joura et al., 2007]. However, it is unclear if a quadrivalent vaccine would be equally effective in reducing incidence of genital warts in China because data concerning the HPV genotype distribution among patients with genital warts in China are scarce. A 2008 meta-analysis by Wang and Qiao [2008] was the only previous attempt to assess HPV types among patients with genital warts throughout China. However, prior studies were limited by small sample sizes and differed in specimen collection methods. In addition, it was impossible to compare HPV distributions among patients with genital warts by sexes, age groups and geographical regions. Since this data are not yet available for China, a multi-center study among 18 hospitals covering seven regions of China was performed. The objectives of this study were to estimate the HPV genotype-specific prevalence in genital warts and to evaluate the potential benefit of a quadrivalent HPV vaccine in China.

MATERIALS AND METHODS**Study Patients**

From September 2007 to April 2008, a cross-sectional study was performed to investigate the prevalence of specific HPV genotypes in Chinese genital warts cases, as described

previously [Wang et al., 2011; Shi et al., 2012]. This study collected the genital warts specimens from seven geographic regions (northeast, north, northwest, central, southwest, south, and east) in China. Selection of candidate hospitals was based on successful previous research collaborations with Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS) as well as consideration of the medical and economic status of the locale. In sum, 18 hospitals across seven regions of China participated in this study.

Patients who were 18 years of age or older with genital warts diagnosed during clinical examination and confirmed by histology were recruited, regardless of their gender or whether they were an incident or recurrent case. Patients were excluded if they had received cryotherapy or surgery, or were on long term medication. All participants provided written informed consent. Patient data (e.g., age at diagnosis, area of residence, date of sample collection, marital status, education level and occupation) were collected by questionnaire. Institutional review board approval was obtained from the IRB of the Cancer Foundation of China.

Study Procedures

All biopsy specimens were preserved in 10% formalin and were transported to the CICAMS in Beijing by private courier under ambient temperatures. Specimens were later shipped from CICAMS to Sun Yat-sen University in Guangzhou for HPV detection.

Approximately 10 mg of each tissue specimen was soaked in phosphate buffered saline (PBS) for 48 hr, prior to being transferred into 70%, 80%, 90%, 95%, and 100% ethanol sequentially for 12 hr. The specimens were then placed in PBS buffer for 12 hr. Processed tissues were ground under liquid nitrogen and digested in EDTA-proteinase K buffer. The whole genome was isolated using standard phenol/chloroform extraction and ethanol precipitation procedures [Sambrook and Russell, 2001]. The precipitate was re-suspended in 30–50 µl Tris-EDTA buffers (pH 8).

HPV detection and genotyping were performed using the GP PCR-SBT (general primer polymerase chain reaction, and sequence-based typing) method [Gravitt et al., 2003; Wu et al., 2006]. MY09/MY11, GP5+/My09, and GP5+/GP6+ primers were used to perform nested PCR [de Roda et al., 1995; Gravitt et al., 2000]. DNA of each sample was amplified with the L1 gene's consensus primers using a mixture containing 1× buffer, 2 mM MgCl₂, 50 mM KCl, 1.25 units of TaKaRaTaq DNA polymerase, 0.2 mM of each type of dNTP (A,G,C,T), and 400 pmol of each MY09/MY11 primer. Amplification was performed with an ABI thermal cycler at 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 2.5 min, annealing at 55°C for 1.5 min, extension at 72°C for 1.5 min, and with final extension at 72°C for 7 min.

The products were amplified with the MY09-GP5+ primers and the GP5+/GP6+ primer set was used to amplify a 140-bp internal fragment [Jacobs et al., 1997]. The last two amplification mixtures contained 1× buffer, 2mM MgCl₂, 50 mM KCl, 1.25 units of TaKaRaTaq DNA polymerase, 0.2 mM of each type of dNTP (A,G,C,T), and 400 pmol of each primer. HPV was amplified in an ABI thermal cycler at 94°C for 5 min, followed by 30

cycles of denaturation for 45 sec at 94°C, annealing for 30 sec at 53°C and extension at 72°C for 45 min, followed by final extension at 72°C for 7 min.

The amplicons were separated by electrophoresis on a 2% agarose gel and purified with an Axgen PCR purification kit. The DNA was then directly sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA) on an ABI 310 DNA analyzer. The nucleotide sequences were aligned and compared with those of known HPV types available through Genbank by using the BLAST 2.0 software server (<http://www.ncbi.nih.gov/blast>). The sample was identified as a particular HPV genotype if the sequence was 95% homologous with the reference standard [Gravitt et al., 2003; Wu et al., 2006].

When a sequence was not unique, another third-round PCR product (50 µl) was purified and inserted into a T-vector for cloning. At least 20 clones were selected randomly for sequencing analysis. Results were compared with the NCBI database to ascertain HPV types.

The quality of DNA extraction was evaluated via the amplification of a 268-bp beta-globin fragment with PC04 and GH20 [Lefevre et al., 2003]. In addition, a 233-bp cytB gene (15,207–15,433 bp of the mitochondrial genome) was amplified by PCR (upstream primer: CCATACATTGGGACAGAC; downstream primer: AGAGAAGTAAGCCGAGGG), and separated by electrophoresis on 2% agarose gels as an internal control [Xia et al., 2007].

The method allows detecting 11 HPV genotypes as follows: low-risk HPV types, HPV-6, HPV-11, HPV-7, HPV-61, and HPV-81; and high-risk HPV types: HPV-16, HPV-58, HPV-59, HPV-45, HPV-66, and HPV-67.

Review of the Literature

Published data on the distribution of specific HPV genotypes in genital warts were searched in PubMed using the terms “HPV” AND “genotype” AND “genital warts.” Additional relevant references cited in retrieved articles were also evaluated. The search was updated on 15 September 2012. Studies including specific-genotype or overall HPV positive numbers were included. If multiple specimens were taken from one individual in some studies, numbers based on specimen volume were extracted if the number on individual was not available. For each included study, the following key information were extracted: country of sample, overall sample size, HPV-6, HPV-11, HPV-16, and/or HPV-18 sample size, displastic areas (external genitalia or mucosal district), HPV DNA source(fresh/fixed biopsies or exfoliated cell by swab).

Statistical Analysis

The sample size was selected to be large enough to accurately estimate the prevalence of specific HPV genotypes. The overall prevalence of HPV genotypes was expressed as the proportion of HPV DNA positive specimens among all cases of genital warts. Qualitative variables were assessed using a 2-sided chi-square test or Fisher's exact test, as appropriate. The median and range were calculated for quantitative variables. The prevalence of HPV genotypes among the seven regions and between males and females were investigated. The

Cochran-Armitage trend test was used to assess the statistical significance of changes in HPV prevalence by 10-year age group. A *P*-value of less than or equal to 0.05 was considered statistically significant. R 2.11.1 software (www.r-project.org) was used for all analyses.

RESULTS

From 2007 to 2008, 1,295 specimens of genital warts were collected from 18 Chinese hospitals. Three subjects were excluded for having incomplete demographic information, and 287 for missing products of cytB amplification, PCR inhibition, or insufficient DNA. There was no significant difference ($P = 0.743$) in the proportion of non-amplifiable specimens in females (22.7%, 132/581) and males (21.8%, 155/711). However, there was a significant difference in the proportion of non-amplifiable specimens among the seven geographical regions ($P < 0.001$), with the highest rate in the Central (94.8%) and lowest rate in the Northwest (65.6%). For the average storage time between amplifiable and non-amplifiable specimens, a significant difference was observed (259 days vs. 455 days, $P < 0.001$). After excluding unqualified specimens, the remaining 1,005 samples were analyzed for the presence of genotype specific HPV DNA (Fig. 1).

Study Patients Characteristics

Overall, the percentage of female patients (55.3%; 556/1,005) was higher than the percentage of male patients (44.7%, 449/1,005; Table I). The mean age was 32 years old (range 17–86) for the entire group, with men being significantly older (male: 34.0 ± 11.4 , range 17–86; female: 30.3 ± 9.6 , range 17–74; $P < 0.001$). There was no significant difference in the age of genital warts patients by geographical regions ($P = 0.501$). The distribution of male cases of genital warts across geographical regions was different from female cases ($P = 0.001$). The Central region had more female cases and the Southwest had more male cases. Marital status among genital warts cases also differed by geographical region ($P < 0.001$). The rate of marriage/co-habitation was highest in the Northeast and the divorced/widowed rate was lowest in the North. In addition, education levels ($P = 0.023$) and occupation ($P = 0.003$) also differed by region.

Overall HPV Prevalence

HPV DNA was detected in 891 of 1,005 cases of genital warts, yielding an overall prevalence of 88.7% (Table II). The prevalence of any HPV genotype in males (89.5%) and females (87.9%) was not statistically different ($P = 0.492$). With regard to low-risk/high-risk genotype classifications, the presence of 1 LR HPV genotypes was found in 78.1% (785/1,005) cases of genital warts whereas the presence of 1 HR HPV genotypes was found in 11.6% (117/1,005). These proportions varied significantly by sex. LR HPV genotypes were more common among males (81.5% vs. 75.4%; $P = 0.020$) and HR HPV genotypes were more common among females (14.2% vs. 8.5%; $P = 0.006$). A single HPV genotype was observed in 866 (86.2%) cases, whereas 25 cases (2.5%) involved infection with 2 genotypes. These proportions did not vary significantly by sex.

Type-Specific HPV Prevalence

HPV-6 (41.3%, 415/1,005), HPV-11 (37.6%, 378/1,005), and HPV-16 (10.4%, 105/1,005) were the three most common genotypes among the samples; more information on prevalence of other HPV types is presented in Table III. HPV-6/-11/-16 infections (positive for any of the three types) accounted for 86.8% (872/1,005) of HPV positive specimens. All other HPV types combined accounted for only 1.9% (19/1,005) in this study.

Among the 2.5% (25/1,005) cases with multiple infections, HPV-6 and HPV-11 co-infections predominated, accounting for 56.0% (14/25) of the total multiple infections. This was followed by HPV-11 and HPV-16 co-infections (24.0%; 6/25) and HPV-6 and HPV-16 co-infections (16.0%; 4/25). There was only one instance of triple co-infection (HPV-6, HPV-11, and HPV-16). Other HPV types were not involved with HPV co-infections.

The overall and type-specific HPV prevalence by region is shown in Figure 2. The prevalence of any HPV genotype in genital warts cases was highest in Southwest China (95.3%). The prevalence in the other six regions was: 91.9% (North), 92.4% (Northeast), 80.8% (Northwest), 85.9% (Central), 90.1% (South), and 84.9% (East). HPV-6, HPV-11, and HPV-16 were the most prevalent HPV types across all seven regions. There was statistically significant variation in the overall prevalence of HPV positive specimens of genital warts among the seven regions ($P = 0.005$).

Though the overall prevalence of HPV-16 was 10.4%, its prevalence in the seven regions ranged from 5.1% (Northwest) to 22.8% (Central) ($P < 0.001$, seven regions). The prevalence of LR HPV-6/-11 (positive for either of the two) genotypes ranged from 65.2% (Central) to 83.2% (Northeast; $P = 0.008$, seven regions). However, the prevalence of a single infection with either HPV-6 (41.3%) or HPV-11 (37.6%) did not differ significantly by region ($P = 0.089$ and $P = 0.919$, respectively). With the exception of HPV-16 and HPV-6/-11, no differences in the prevalence of any HPV type were observed among the seven regions.

The proportion of genital warts specimens with any HPV type and with HPV-6/-11/-16 decreased with age (P -trend = 0.010 and P -trend = 0.025 respectively; Fig. 3). No other specific genotype showed association with age.

The three most frequent HPV types in both male and female cases were HPV-6, HPV-11, and HPV-16 (data not shown). No significant difference in the prevalence of HPV-6/-11/-16 or any other single HPV type was observed by sex. Only HPV-6/-11 co-infection differed significantly by gender (80.6% in men and 74.8% in women, $P = 0.035$).

DISCUSSION

To our knowledge, the current study is the first study of the prevalence of HPV in genital warts with a large sample size in China with participation of more than 1,000 genital warts patients. The primary strengths of the study are the large sample size and geographic distribution of study sites across China. Also, hospitals in both rural and urban areas were

enrolled in this study. Therefore this research provides an accurate assessment of the HPV type-specific distribution among genital warts patients throughout China comparatively.

In this study, the data of the multi-hospital study were compared with a meta-analysis by Wang and Qiao [2008] and a literature review performed by the study group. The systematic review of the literature in this study allowed the identification of 84 papers in PubMed and our retrieved references of which 12 fulfilled the inclusion criteria including a total of 1,844 individual's data [Yun and Joblin, 1993; Vandepapeliere et al., 2005; Potocnik et al., 2007; Aubin et al., 2008; Garland et al., 2009; Arima et al., 2010; Anic et al., 2011; Ball et al., 2011; Jaberipour et al., 2011; Cremin et al., 2012; Jamshidi et al., 2012; Komlos et al., 2012] (see Appendix).

The overall prevalence of HPV was 88.7%, suggesting that genital warts often occurs in the presence of HPV infection. This was marginally higher than the prevalence reported in a meta-analysis (84.2%, $P < 0.001$) of type-specific HPV among Chinese genital warts patients [Wang and Qiao, 2008] and similar to the rates obtained from literature review (87.6%, $P = 0.45$; Table IV).

The analysis of specific HPV genotypes provides evidence that, in China, the most prevalent HPV genotypes in genital warts patients are HPV-6, HPV-11, and HPV-16. In the meta-analysis, HPV-6 and HPV-11 predominated in genital warts specimens, followed by HPV-40. [Wang and Qiao, 2008]. Conversely, in the literature review, the three major prevalent types are HPV-6, HPV-11, and HPV-16, consistent with our result.

HPV-6 and HPV-11, either alone or in combination with other HPV genotypes, were present in 77.4% of cases (Fig. 1, "HPV types in all regions"). The rate previously reported in the literature ranged from 53.6% to 96.4% [Potocnik et al., 2007; Aubin et al., 2008; Wang and Qiao, 2008; Garland et al., 2009; Anic et al., 2011; Komlos et al., 2012]. The rate 77.4% is significantly lower than the results of the meta-analysis by Wang et al. and the literature review (both P -values < 0.001). On one hand, considering the fact that the population or tissue-processing method of the meta-analysis and the literature review, both of which were with very high rates of HPV-6 and/or HPV-11, were not consistent, the prevalence of HPV-6/-11 with 77.4% in the multi-center study varied from either the meta-analysis by Wang et al. or by our literature review (83.8%; Table IV). On the other hand, because the overall HPV positive rate was similar to other studies, the proportion of HPV-16, present in 10.4% cases of genital warts and was the third most common HPV genotype in our study, was higher than that reported in some previous studies (ranged from 1.3% to 9.6% in our literature review). It may cause the smaller proportion of HPV-6/-11. This result suggests that there might be some variation in the prevalence of HPV-16 (and HPV-6/-11) in genital warts patients. Confirmatory investigations may be needed to firmly establish the prevalence of HPV-16 in genital warts.

The proportion of HPV-6 (46.6%) and HPV-11 (42.4%; Fig. 1, "HPV types in all regions") in genital warts reported above is not consistent with studies from western countries which found the proportion of genital warts with HPV-6 to be much higher than that with HPV-11 [Potocnik et al., 2007; Garland et al., 2009]. However, the findings in the multi-hospital

study were similar to that reported in the meta-analysis of Chinese genital warts (HPV-6: 54.9%; HPV-11: 41.1%) [Wang and Qiao, 2008]. Therefore, the HPV-6/HPV-11 ratio may differ between Chinese and Western populations.

The proportion of multiple infections (≥ 2 HPV types), 2.5%, was much lower than that reported in other studies. For example, in a study of HPV genotypes in genital warts by Brown et al. [1994], the proportion of multiple infections in non-immunosuppressed people was 68.3% (28/41). Also, in the analysis of the natural history of genital warts from phase III trials of the clinical efficacy of an HPV vaccine, the percentage of multiple infections in genital warts was 28.3% [Garland et al., 2009]. However, the rate of co-infection with only HPV-6 and HPV-11 in the genital warts specimens in our study (1.4%, 14/1,005) did not differ significantly from the rate in a HPV vaccine clinical trial [Garland et al., 2009] (1.5%, 8/520; $P = 0.99$).

The overall prevalence of any HPV genotype and the prevalence of HPV-6/-11/-16 decrease with old age and increased slightly with a second peak at 60–70 years. It was probably related to the natural distribution of HPV on age [Hamlin-Douglas et al., 2008].

The results of the multi-hospital study indicate that 11.6% of all genital warts patients harbor HR HPV DNA. In this study, women had a higher proportion of HR HPV as compared to men (14.2% vs. 8.5%). This was probably related to the fact that women are vulnerable to cervical HPV infection [Dalstein et al., 2004; Pretet et al., 2008]. In addition, the distribution of HR HPV in genital warts patients between urban areas and rural areas also showed a statistically significant difference (12.8% vs. 6.9%, $P = 0.019$). Though both for male and female patients the HR HPV distribution of patients in urban areas were higher than that of in rural areas, by stratifying, we found that the difference in female patients was statistically significant (urban vs. rural: 15.6% vs. 7.4%; $P = 0.05$) whereas in male patients was not (urban vs. rural: 9.1% vs. 6.4%; $P = 0.435$). From the natural distribution aspect, the prevalence of HR HPV are higher in women compared with that in men [Shin et al., 2004]. Besides, because women in urban areas may have more opportunities to have social activity, it is easy to understand that the HR HPV distribution in urban women is higher than that in rural women.

The overall positive rate of HPV genotypes was different significantly between the seven regions. The meta-analysis also reported variation of HPV prevalence in Chinese genital warts patients by region [Wang and Qiao, 2008]. Because we looked at regions differently from the meta-analysis so a perfect comparison cannot be made. The variation may be caused by different lifestyle and economic status of regions.

Prevention of genital warts is essential due to the psychological and financial burden associated with these lesions [Wang et al., 2011]. The three predominant genital warts associated HPV types (HPV-6, HPV-11, and HPV-16) in our study are all preventable by the existing quadrivalent HPV vaccine. Although the proportions of the three types varied from the results of the literature review, which may be resulted by the fact that most of the publications in the literature review are based on Western population, the overall HPV positive rate keeps consistent. This suggests that implementation of a quadrivalent HPV

vaccination program in China may reduce the burden of disease associated with genital warts and achieve performance as good as in Western countries. For example, it has been reported that the quadrivalent HPV vaccine could prevent 62.0–87.0% cases of genital warts in France [Aubin et al., 2008]. Furthermore, a cost-effectiveness analysis conducted in China reported that adding a quadrivalent HPV vaccine to a combined screening strategy was a cost-effective method for reducing the disease burden of cervical cancer, precancerous lesions and genital warts caused by HPV genotypes 6, 11, 16, and 18 [Canfell et al., 2011]. However, based on the results of our study and others, there is variation of HPV prevalence by geographic regions, sexes, and age groups. Therefore, the effectiveness of a vaccine in reduction of disease burden may differ by population.

The current study also has limitations. First, the observed overall prevalence of HPV infection was lower than the values in other studies which used fresh tissue specimens [Aubin et al., 2008]. Executing a multi-center study covering vast distances necessitated preserving collected tissues in formalin prior to being transported to a centralized lab for HPV genotyping tests. Though this facilitated sample shipping, formaldehyde induced protein-DNA cross-linkages [Tian et al., 2004] may have reduced the efficiency of DNA extraction and polymerase chain reaction. As evidence of this, the length of time that the specimens were kept in formalin directly corresponded to rates of non-amplifiable tests.

In conclusion, the study have reported on the first standardized multi-center study of HPV genotype distributions in genital warts patients across mainland China. The data suggest that prophylactic vaccination against HPV-6, HPV-11, and HPV-16 could play an important role in genital warts prevention. This study have provided reliable data which may inform future vaccine policy in China.

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APPENDIX: Summary of the Studies of the Distribution of HPV Genotypes in GW Patients

Study	Sample size	Population	Time covered	Specimen	HPV-6	HPV-11	HPV-16	HPV-6/-11	Any positive
Jaberipour et al. [2011]	79	Iranian females	NA		–	–	1.3	–	16.5

Study	Sample size	Population	Time covered	Specimen	HPV-6	HPV-11	HPV-16	HPV-6/-11	Any positive
Arima et al. [2010]	22	US males	2003–2009	External genitalia	81.8	–	–	–	90.9
Ball et al. [2011]	31	UK	NA	Frozen biopsy; pos: NA	90.3	32.3	–	–	96.8
Komlos et al. [2012]	39	Slovenian	2005–2011	Anogenital warts; fresh-frozen/paraffin-embedded tissue	79	12.4	–	92.4	–
Jamshidi et al. [2012]	100	Iran females	2009–2010	Frozen stored; pos: NA	49	67	–	–	73
Vandepapeliere et al. [2005]	457	Eight countries	2005	Clinical aspect; anogenital warts	67.2	19.7	–	–	–
Garland et al. [2009]	351 ^a	FUTURE-I & II	2001–2003	NA; NA	74.4	14.2	7.8	84.9	90.8
Anic et al. [2011]	112	HIM (US, Brazil, and Mexico)	2005–2009	Clinical aspect; external genitalia	43.8	10.7	9.8	53.6	80.4
Cremin et al. [2012]	61	NA	NA	Cervical scrapings; external genital warts	–	–	–	–	75.4
Potocnik et al. [2007]	55	Slovenia	NA	Clinical aspect; exophytic GWs on the glans of the penis, the coronal sulcus, or the foreskin	78.2	18.2	3.6	96.4	100
Aubin et al. [2008]	516	French	2007	Clinical aspect; external	69	16.1	8.9	82.9	99
Yun and Joblin [1993]	21	NA	NA	Paraffin-embedded tissue; NA	81	23.8	4.8	–	–
Total	1,844	Mainly western population	2003–2011		69.5	18.7	7.8	83.8	87.6
Wang et al. [2011]	5,244	Mainland China			54.9	41.1	0	83.0	84.2
Current study	1,005	Mainland China	2007–2008		41.3	37.6	10.4	77.4	88.7

^aThe overall and HPV-16 positive rates are based on 520 lesion tissues.

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Fig. 1.
Flow chart of the samples included and excluded.

Fig. 2.
Prevalence of specific HPV genotypes in genital wart cases, by geographic region, China, 2007–2008.

Fig. 3.

The proportion of genital wart cases with specific HPV genotypes, by age group, China, 2007–2008.

TABLE I
 Characteristics of Genital Wart Cases, by Geographic Region, China, 2007–2008

	Percentage of cases %						
	North (N = 248)	Northeast (N = 119)	Northwest (N = 99)	Central (N = 92)	Southwest (N = 85)	South (N = 111)	East (N = 251)
Total (N = 1,005)							
Age groups (years) ^a							
<25	27	36.4	24.2	27.8	24.7	27.3	26.6
25 and <30	22.6	21.2	18.2	17.8	18.8	30.9	25
30 and <35	15.7	14.4	17.2	10	17.6	15.5	14.1
35 and <40	11.3	8.47	15.2	16.7	15.3	12.7	10.9
40	23.4	19.5	25.3	27.8	23.5	13.6	23.4
Gender							
Male	48.4	44.5	47.5	21.7	54.1	48.6	43.4
Female	51.6	55.5	52.5	78.3	45.9	51.4	56.6
Education							
Primary school or illiterate	6.5	4.2	7.1	12.0	4.7	3.6	8.4
Junior high school	25.0	25.2	16.2	31.5	18.8	22.5	28.7
Senior high school	35.1	38.7	32.3	28.3	38.8	36.9	39.4
College and above	33.5	31.9	44.4	28.3	37.6	36.9	23.5
Marital status							
Never married	29.8	14.3	27.3	30.4	32.9	44.1	18.3
Married/co-habited	68.5	81.5	68.7	60.9	62.4	54.1	74.9
Divorced/widowed	1.6	4.2	4.0	8.7	4.7	1.8	6.8
Occupation							
Blue collar	28.6	25.2	36.4	27.2	37.6	22.5	28.7
Farmer	15.3	5.9	6.1	12.0	5.9	3.6	11.6
White collar	26.6	26.1	27.3	33.7	28.2	31.5	22.3
Other	29.4	42.9	30.3	27.2	28.2	42.3	37.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

^aThe age of seven cases were missing.

TABLE II

Prevalence of HPV in Male and Female Genital Wart Cases, According to Low-Risk/High-Risk and Single/Multiple Infection Classifications, China, 2007–2008

	No. of cases (%)			<i>P</i> -value ^a
	Male (n = 449)	Female (n = 556)	Total (n = 1,005)	
Any HPV positive	402 (89.5)	489 (87.9)	891 (88.7)	0.492
Low-risk infection ^b	366 (81.5)	419 (75.4)	785 (78.1)	0.023
High-risk infection ^c	38 (8.5)	79 (14.2)	117 (11.6)	0.006
Single infection	395 (88.0)	471 (84.7)	866 (86.2)	0.162
Multiple infection	7 (1.6)	18 (3.2)	25 (2.5)	0.135
HPV6/11	362 (80.6)	416 (74.8)	778 (77.4)	0.035
HPV6/11/16	397 (88.4)	475 (85.4)	953 (94.8)	0.195

^aTwo-sided chi-square test for differences between males and females.

^bLow-risk HPV types: HPV-6, HPV-11, HPV-7, HPV-61, and HPV-81.

^cHigh-risk HPV types: HPV-16, HPV-58, HPV-59, HPV-45, HPV-66, and HPV-67.

TABLE III

Prevalence of Specific HPV Genotypes Among 1,005 Genital Wart Cases, by Decreasing Frequency, China, 2007–2008

HPV genotype	No. of cases (%)		
	Single infection	Multi-infection	Overall
HPV6	396 (39.4)	19 (1.9)	415 (41.3)
HPV11	357 (35.5)	21 (2.1)	378 (37.6)
HPV16	94 (9.4)	11 (1.1)	105 (10.4)
HPV58	7 (0.7)	0 (0)	7 (0.7)
HPV7	3 (0.3)	0 (0)	3 (0.3)
HPV81	3 (0.3)	0 (0)	3 (0.3)
HPV59	2 (0.2)	0 (0)	2 (0.2)
HPV45	1 (0.1)	0 (0)	1 (0.1)
HPV61	1 (0.1)	0 (0)	1 (0.1)
HPV66	1 (0.1)	0 (0)	1 (0.1)
HPV67	1 (0.1)	0 (0)	1 (0.1)

TABLE IV

HPV Distribution Among the Current Study, Meta-Analysis by Wang et al. and the Literature Review

Study population	<u>The current study</u>	<u>Meta-analyses by Wang et al.</u>	<u>Literature review by the current study</u>
	Mainland China		Regions other than China
Findings for prevalence of specific HPV types in GW (%)			
HPV-6	41.3	54.9	69.5
HPV-11	37.6	41.1	18.7
HPV-16	10.4	0	7.8
HPV-6/-11	77.4	83.0	83.4
Any positive	88.7	84.2	87.6