The significance of human papillomavirus receptors related genes variants in cervical cancer screening: a longitudinal study of Chinese population

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Abstract

Objective To investigate the relationship between single nucleotide polymorphisms (SNPs) in human papillomavirus (HPV) receptor gene and HPV susceptibility and the outcomes in Chinese women. Study design A cohort study. Setting Lishui, Zhejiang, China. Population 3066 women were recruited. Methods 29 SNP sites of HPV receptor gene on women with available cytology residual samples were detected. Main outcome measures Develop to cervical intraepithelial neoplasia 2 and worse (CIN2+). Results: 2938 women with sufficient cytology samples performed SNP sites detection. Rs16894821 [GG vs. AA: OR =1.71 (1.08-2.69)] and rs724236 [TT vs. AA: OR=1.73 (1.14-2.62)] in SDC2 increased the HPV susceptibility. TT genotype of rs2575712 in SDC2 was associated with an increased HPV 16/18 susceptibility [OR=2.78 (1.22-6.36)]. Four SNPs (rs1047057 and rs10510097, rs2575735, and rs878949) were significantly associated with HPV persistent infection. In addition, the frequencies of genotype of rs16894821 under recessive model [OR=2.40 (1.12-5.15)] in SDC2, and rs11199993 under dominant model [OR=1.64 (1.01-2.68)] in FGFR2 were significantly associated with the disease progression. Importantly, we found that HPV test in combination with SNPs with sensitivity of 0.51 (0.36-0.66) and specificity of 0.96 (0.96-0.97) in predicting CIN2+ on women with non-HPV16/18+, which had similar performance to HPV test combined with cytology test with sensitivity of 0.44 (0.30-0.60) and specificity of 0.98 (0.97-0.99). Conclusion: Gene variants in HPV receptor related gene may influence the HPV susceptibilities and the outcome of HPV infection. HPV combined with SNPs is a promising alternative to HPV test combined with cytology test in patients with non-HPV16/18 infection.

Introduction

Cervical cancer (CC) is the fourth leading cause of worldwide cancer death for women, accounting for more than 300,000 deaths annually, of which more than 85% CC cases occur in developing countries[1]. Early screening and vaccination are the best methods to decrease the incidence and mortality of CC. High risk Human papillomavirus (HPV) infection, especially persistent infection with HPV, has been recognized as the principal cause of CC [2]. CC is a continuous process from HPV infection to cervical intraepithelial neoplasia (CIN) and finally to invasive disease. Some studies have investigated the correlation between HPV types and different degrees of cervical CIN, and suggested that CIN1 was a self-limited HPV infection, while CIN2-3 was the true precursor of CC[3, 4]. Although vaccination is effective in preventing CC, poor awareness of vaccination, anxiety about adverse reaction of vaccine, and practical issues such as short apply limited the popularization of vaccines in China[5, 6]. Currently, most screening and diagnostic efforts are directed towards early identification of cervical lesions through HPV testing and Pap smear[7]. Only a small proportion of patients with HPV infection that develop to CC, and the development of CC is a complex, multi-step processes including HPV infection, genetic and environmental factors[8, 9]. Therefore, identification of better

biomarkers that associated with persistent of HPV infection and progression to CIN 2 and worse (CIN2+) and to prevent CC early is important to reduce the incidence of CC.

Virus attaches to host cell and interacts with its receptors to infect cells, such as signaling lymphocyte activation molecule (SLAM) and CD6 for measles virus [10], and vitamin D receptor (VDR) for Hepatitis C virus[11]. Many receptors were engaged in the process of HPV infecting human cervical epithelial cells including EGFR, HSPG2, FGFR2, TSPAN1 and SDC2[12-14]. Single nucleotide polymorphisms (SNPs) have been widely used in cancer genetic analyses, and genetic variations in receptors may play a role in the processes of infection and perhaps promote further progresses to cancer[15]. We hypothesized that SNPs in HPV related receptors were associated with the susceptibility of CIN2+ and can predict the risk of CIN2+.

In present study, a series of SNPs in the HPV related receptor genes were selected and evaluated for the associations with HPV susceptibility, persistent of HPV infection and disease progression, aiming to identify the high risk women who are susceptible to persistent of HPV infection and prone to cervical cancer and precancer.

Methods

Study population

Between Oct 2016 and Mar 2020, 3066 women were recruited in one National Medical Products Administration clinical trial (Approval number: 20160149, Shanghai) and received 3-year prospective population-based cervical cancer screening program in Lishui, Zhejiang, China. Eligibility criteria for clinical trial included: aged 21–65 years, no surgical history of cervix uteri, informed consent was obtained. Exclude criteria included the following: pregnancy or within 2 months of the post-partum period; previous total hysterectomy; a history of CIN or worse, vulvar intraepithelial neoplasia or worse, or vaginal intraepithelial neoplasia (VAIN) or worse, invalid HPV results/invalid or unsatisfactory cytology. This study was approved by the Ethics Committee of Women's Hospital, School of Medicine,Zhejiang University (approval number: IRB-20220035-R).

Screening protocol

The flow chart is shown in Figure 1. At baseline, samples of cervical exfoliate cells were collected using a cytology brush (Hologic, Bedford, MA) and stored in the tubes with preservation solution for the cytology test (Hologic, Bedford, MA) and HPV test respectively. HPV was detected by Cobas® 4800Amplification/Detection Kit (Roche Molecular Systems, Pleasanton, CA) according to the manufacture's instruction. Women with positive HPV 16/18 or abnormal cytology results (atypical squamous cells with unknown significance (ASC-US) or worse) were referred to colposcopy with or without biopsy. Due to clinical experience and ethical consideration, women with both negative HPV and cytology results were not referred to colposcopy and were regarded as histological low-grade squamous intraepithelial lesion or less by default. The pathological diagnostic standard was based on the World Health Organization Classification of Tumors of the Female Genital Tract[16]. All women with histological abnormalities were managed according to Guideline for Comprehensive Prevention and Control of Cervical Cancer in China. The HPV, cytology, pathological examinations and SNP detection were independently performed in a blinded manner.

Subsequently, women with positive HPV or abnormal cytology at baseline visit would be recalled for cytology test every 1 year for 2 years and all enrolled women would be recalled for cytology and HPV test at 3rd year. In current study, women who had HPV infection from baseline to the 3rd year of follow-up were defined as persistent of HPV infection. The endpoints were CIN2+. VAIN 2/3 was regarded as CIN2/3.

DNA extraction and SNP detection

The residual of cytology samples at baseline were used for SNP detection. Genomic DNA was extracted using AxyPrep Multisource Genomic DNAMinprep kit (Axygen, Hangzhou, China). The genotypes of 29 SNP sites of HPV receptor gene, including EGFR, FGFR2, HSPG2, INTEGRIN, and SDC2, were determined using the MassArraysystem (Sequenom Inc., San Diego, CA, USA) at Sangon Biotech Company. (Shanghai, China).

This system is based on MALDI-TOF MS and following procedures were applied: PCR amplification of genomic DNA containing the SNP sites, dNTP degradation using by shrimp alkaline phosphatase (SAP), single base extension, desalinization with Spectro CLEAN resin, sample dispensation on Nano dispenser Spectro CHIP, data acquisition and analysis with MassArray TYPER software. Primers used for PCR amplification, single base extension, and 29 SNP sites are listed in Table S1. The detection of 29 SNP sites for each sample was carried out simultaneously in one well.

Statistical analysis

For demographic, categorical variables are expressed as absolute numbers and percentages, which were compared with theχ^2 test. The χ^2 and Fisher exact tests were utilized to analyses the differences in genotypic and allelic distributions between groups. The correlation of genotypic and allelic with the risk of CIN2+ was evaluated via odds ratio (OR) and 95% confident interval (CI) by unconditional logistic regression. The Hardy-Weinberg equilibrium (HWE) was estimated by the goodness-of-χ^2 test. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. We used SAS 9.4 for all statistical analyses.

Results

Basic characteristics

Totally 23.11% (679/2938) women were lost after 3 years of follow-up. Among 313 women with HPV infection at baseline, 57 were HPV 16/18 positive and 256 were non-16/18 HPV positive. Baseline characteristics of participants were listed in Table S2. At 3$^{\text{rd}}$ year, among 255 women with HPV positive, 160 women were newly infected with HPV among HPV negative population at baseline, while 95 were persistent HPV infection and 107 was spontaneously cleared among HPV positive population at baseline. During the study period, a total of 66 patients developed to CIN2+ (34 had CIN2+ at baseline visit) and all of them were HPV positive at diagnosis.

Associations between SNPs with HPV susceptibility

We analyzed 29 SNPs genotype and allele distribution among women with and without HPV infection. The genotype distribution of 29 SNPs was inHWE ($p >0.05$). We found a significant association of rs16894821 and rs724236 in *SDC2* with HPV infection (Table 1). For rs16894821, the genotype homozygotes GG is more frequent in the HPV infection group (HPV+ group: 7.99% vs normal: 5.14%), and the homozygotes AA is more frequent in the normal group (HPV+ group: 55.27% vs normal: 60.80%). A statistically significant increase in the HPV susceptibility was found for carriers of the GG genotype compared to the AA genotype (OR =1.71, 95% CI =1.08 to 2.69,p =0.0473). The frequencies of G allele in HPV positive patients was 26.36% (22.17% in the normal controls), which was significantly increased the HPV susceptibility (G vs. A: OR =1.26, 95%CI =1.04 to 1.52, p =0.0181). In addition, we calculated theOR for the dominant model (AG/GG vs. AA: OR =1.26, 95%CI =0.99 to 1.59, p =0.0589), and recessive model (GG vs. AA/AG: OR =1.60, 95% CI =1.03 to 2.50, p =0.0377), which demonstrated recessive model has significant association with HPV infection instead of dominant model. For rs724236, the genotype homozygotes TT is more frequent in the HPV infection group (HPV+ group: 9.90% vs. normal: 6.44%), and the homozygotes AA is more frequent in the normal group (HPV+ group: 51.44% vs. normal: 57.74%). A statistically significant increase in the HPV susceptibility was found for carriers of the TT genotype compared to the AA genotype (OR =1.73, 95% CI =1.14 to 2.62, p =0.0285). For allele, T versus A, the frequencies of T allele in HPV positive patients was 29.23% (24.35% in the normal controls), which was significantly increased the HPV susceptibility (T vs. A: OR =1.28, 95%CI =1.07 to 1.54, p =0.0077). We also found a significant increase in the HPV susceptibility both under the dominant model (AT/TT vs. AA: OR =1.29, 95% CI =1.02 to 1.63, p =0.0333) and recessive model (AA/AT vs. TT: OR =1.60, 95% CI =1.07 to 2.391, p =0.0226). No other genotypes of selected SNPs were found to be associated with the HPV susceptibility.

HPV16 and HPV18 were the two most carcinogenic subtypes. Thus, we further analyzed the susceptibility genes SNPs for HPV 16/18 (Table 2). We found that the TT genotype of rs2575712 in *SDC2* was associated with an increased HPV 16/18 susceptibility compared with GG genotype (TT vs. GG: OR =2.78, 95%

CI =1.22 to 6.36, p =0.0278). This observation is consistent with the increased frequency of the T allele compared with G (T vs. G: *OR* =1.62, 95% *CI* =1.11 to 2.37, p =0.0119) and dominant model (GT/TT vs. GG: *OR* =2.29, 95% *CI* =1.08 to 4.85, p =0.0313). We did not find a significant increase in the risk of TT under the recessive model.

Associations between SNPs and HPV persistent infection

Due to immune mechanisms, most HPV infection spontaneously cleared within one to two years[17, 18]. In present study, the follow up information of HPV were available in 203 patients with baseline HPV infection. 107 (52.97%) were classified as persistent of HPV infection, while 95 (47.03%) HPV were spontaneously cleared (Table S3). The frequencies of genotype and allele of four SNPs (rs1047057 and rs10510097 in *FGFR2* , rs2575735 in *SDC2* , and rs878949 in*HSPG2*) were significantly associated with HPV persistent infection (Table S3). For rs1047057, HPV positive women carried AG genotype (AG vs. GG: *OR* =0.40, 95% *CI* =0.21 to 0.74,p =0.0159) and dominant model (AG/AA vs. GG: *OR* =0.45, 95%*CI* =0.25 to 0.81, p =0.0071) were prone to HPV cleared compared to the GG genotype. For rs10510097, T allele compared to C increased the risk of persistent HPV infection (T vs. C: *OR* =1.74, 95% *CI* =1.08 to 2.80, p =0.0230), while rs2575735 (T vs. C:*OR* =0.59, 95% *CI* =0.36 to 0.98, p =0.0404) and rs878949 (T vs. C: *OR* =0.52, 95% *CI* =0.30 to 0.92,p =0.0241) had the opposite tendency. Dominant model analysis of rs10510097 revealed that CT/TT more likely turn to HPV persistent infection compared to the CC genotype (*OR* =1.83, 95%*CI* =1.03 to 3.22, p =0.0373), while rs878949 (*OR* =0.44, 95% *CI* =0.23 to 0.83, p =0.0099) had the opposite tendency.

Associations between SNPs and disease progression

66 patients had disease progressing to [?]CIN2 during the study period, and the baseline characteristics of participants of Lishui cohort between <CIN2 and CIN2 + groups were listed in Table S4. There were no differences in age, smoking status, alcohol drinking, education and reproductive age between those groups. The frequencies of genotype and allele of two SNPs (rs16894821 in *SDC2* , and rs11199993 in*FGFR2*) were significantly associated with the disease progression (Table 3). For rs16894821, the genotype homozygotes GG is more frequent in the CIN2+ group, and the homozygotes AA is more frequent in the <CIN2 group. rs16894821 that carried GG genotype versus AA (*OR* =2.57, 95% *CI* =1.16 to 5.68, p =0.0274) would increase the risk of CIN2+. Recessive model analysis revealed that genotype GG also increased the risk of disease progression compared with AA/AG (*OR* =2.40, 95% *CI* =1.12 to 5.15, p =0.0241). For rs11199993, dominant model of rs11199993 carried CG/CC genotype (versus GG, *OR* =1.64, 95% *CI* =1.01 to 2.68,p =0.0468) and minor allele C (versus G, *OR* =1.52, 95%*CI* =1.03 to 2.22, p =0.0330) were also a risk factor for developing to CIN2+.

Evaluation of predictive performance of CIN2+

Next, we explored whether HPV test combined with genetic variations could effectively predict the development to CIN2+ on patients with non-16/18 HPV infection. We evaluated the predictive performance of CIN2+ based on HPV combined with cervical cytology and HPV combined with SNPs (rs16894821 using a recessive model and rs11199993 using dominant model) on women with HPV, respectively. The results showed that combination of HPV test and cytology had a sensitivity of 0.44 (0.30-0.60) and a specificity of 0.98 (0.97-0.99), and a combination of HPV test and SNPs had a sensitivity of 0.51 (0.36-0.66) and a specificity of 0.96 (0.96-0.97) (Table 4), which indicated that SNPs could effectively predict the disease progression in patients with non-HPV16/18 infection and had the potential to replace the work of cytologists on cytology.

Discussion

Main findings

This study was first to evaluate the associations between SNP sites in the HPV related receptor genes with the natural course of HPV infection and assess CIN2+ risk based on a longitudinal study of Chinese population. These results indicated that genetic variation played an important role in predicting HPV susceptibility and

prognosis. In addition, we found that SNPs might be a potential biomarker for predicting CIN2+ in patients with non-16/18 HPV infection and had the potential to replace the work of cytologists on cytology, which could make up for the lack of cytologists in developing countries, and the results of SNP test were more objective than cytology test.

Interpretation

HPV infection is recognized as the principal factor in the development of CC [19]. More than 80% women that in sexually active might be infected by virus[20], but only a very few persist and eventually cause cancer[21]. In practice, once a woman was identified as HPV positive, it can cause anxiety and panic to them. The outcome of HPV infection is a multifactorial related to many genetic and non-genetic factors. The results of our study indicated that SNP sites rs16894821 and rs724236 in *SDC2* gene were associated with the susceptibility of HPV. Allele G of rs16894821 SNP in*SDC2* gene increased the HPV susceptibility and promoted the disease progression as compared to allele A. Significantly elevated GG of rs16894821 was observed as compared to AA carriers in women with HPV+. Allele T of rs724236 SNP in *SDC2* gene increased the HPV susceptibility as compared to allele A, and the same tendency in the dominant and recessive models. High-risk type of HPV are found in over 95% of CC patient[22-24] and the persistence of high-risk HPV infection is associated with an increased risk of precancerous lesion or invasive carcinomas. We found that genotype TT of rs2575712 SNP in *SDC2* gene increased the HPV16/18 susceptibility as compared to GG. For allele of rs2575712, significantly upregulated in allele T as compared to G carriers in patients with HPV 16/18+. Zou et al., had demonstrated that rs2575712 in *SDC2*significantly correlated to HPV18 infection[14]. Due to the limitation of sample size, we did not analyze these two subtypes separately. SNP sites rs1047057 and rs10510097 in *FGFR2* gene, SNP sites rs2575735 in *SDC2* gene, and SNP sites rs878949 in*HSPG2* gene were found to be related with the outcome of HPV. Rs1047057 in *FGFR2* significantly upregulated in AG as compared to GG carriers in patients with infection regression and significantly upregulated rs10510097 in allele T as compared to C carriers in patients with HPV persistent infection. Allele T of rs2575735 in *SDC2* gene was more likely to turn negative compared to G carriers in patients with HPV positive, which was a protective SNP for HPV prognosis. These results indicated that genetic variants of *SDC2* could not only reflected the susceptibility of HPV, but also reflected the outcomes of HPV infection. *HSPG2* is a large multi-domain extracellular matrix proteoglycan, and Zhang et al., reported that *HSPG2* were low expressed in CC patients compared with healthy people[25]. In solid tumor, high expression of *HSPG2* always indicated the disease invasion, metastasis and angiogenesis of solid tumor[26, 27]. Our current study indicated that genetic variants of rs878949 in *HSPG2* gene can also predict the outcomes of patients with HPV infection. Allele T of rs878949 decreased the risk of persistence of HPV infection as compared to allele C. Wang et al., have shown that SNPs for *OAS3* , *SULF1* , *DUT* , and *GTF2H4* were associated with HPV persistence, and *DUT*and *GTF2H4* were genes belonged to DNA repair pathway[28]. Our results revealed the associations between the SNPs in HPV host cell receptor genes and HPV persistence infection.

As we know, women with HPV16/18 infection would be directly referral to colposcopy, while women with non-16/18 HPV infection would receive cervical cytology triage and abnormal results ([?]ASC-US) required further colposcopy. However, the performance of cytology was heavily dependent on the experience and abilities of trained cytologists. In addition, a lack of well-trained cytologists had limited the application of cytology in developing country. In current study, we found that genotype CG/CC of rs11199993 in *FGFR2* gene was upregulated in CIN2+ group compared to GG and the same tendency was observed in genotype GG of rs16894821 in*SDC2* gene compared to AA/AG. Importantly, our results demonstrated that HPV test in association with SNPs (rs16894821 using a recessive model and rs11199993 using dominant model) had a similar effect in predicting disease progression as in association with cytology test in women with non-HPV16/18 infection. To the best of our knowledge, our study is the first to provide evidence that SNPs in HPV host cell receptor gene can be used to predict the disease progression of HPV infection in Chinese populations.

FGFR2 gene, located on human chromosome 10q26, encodes*FGFR2b* and *FGFR2c* isoforms functioning as *FGF*receptors [29], which plays an important role in the tumorigenesis by regulating cell proliferation,

apoptosis, metastasis, and angiogenesis[30]. Increasing evidence reported that *FGFR2* was associated with cancers, such as endometrial uterus cancer, gastric cancer, and ovarian cancer[31-33]. Studies on CC revealed that *FGFR2* is highly expressed in CC tissues and cell lines [34]. Sun et al., revealed that *FGFR2* is a direct and functional downstream target of miR-889 in CC cells, and miR-889 overexpression suppressed CC viability and invasion by targeting FGFR[35], and *FGFR2*has become a new target for female reproductive system cancers therapy[36]. *SDC2* is one of the syndecan family of proteins, which could promote cell adhesion and is associated with cell proliferation. Previous studies have reported that *SDC2* was overexpressed and enhanced invasion in a number of cancers [37, 38]. Decreasing *SDC2* expression led to cell cycle arrest and reduced the occurrence of cancers, such as colon and breast cancer[39, 40]. In addition, Oh et al., identified that methylated *SDC2* can be used as a serum DNA biomarker for early detection of colon cancer[41]. In current study, we found that variants in *SDC2* can be utilized to identify women with HPV infection.

Strengths and limitations

We identified biomarkers from genetic variation on HPV related receptor genes to predict the natural history and progression to CIN2+. Major limitation of this study is no full typing of HPV infection in women at the time of screening, so a stratified subgroup analysis not be performed by typing. Detection on full HPV types is necessary to identify biomarkers that can be utilized to predict the progression under different HPV types.

Conclusion

Understanding the genetic variations could promote the development of early detection biomarkers, predict the outcome of HPV infection and disease progression, and to make aggressive treatment to reduce the incidence of adverse outcomes in high-risk population. The present findings provide additional information for understanding the HPV genotype distribution at different phase of natural course of HPV infection and predict the prognosis of patients of HPV infection, which need to be validated by prospective large-sample studies. Additional studies are needed to address the functional consequence of these genetic alteration in the natural course of HPV infection.

Disclosures of interest

The authors declare that they have no competing interests.

Contribution of authorship

The study was conceived and planned by L.X, X.X. The analysis of the data was performed by X.H.Y and W.M.J. Interpretation of data by Y.L.F and L.Y. The manuscript was written by X.H.Y, W.M.J and L.X.

Details of ethics approval

This study was approved by the Ethics Committee of Women's Hospital, School of Medicine,Zhejiang University (approval number: IRB-20220035-R).

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