Variants in human papillomavirus receptor and associated genes are associated with type-specific HPV infection and lesion progression of the cervix

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ABSTRACT

Human papillomavirus (HPV) infects cervical epithelial cells through cellular membrane receptors, and then induces the initiation and progression of cervical cancer. Single nucleotide polymorphisms (SNPs) may impact the susceptibility and outcome of diseases, but it’s still unknown whether variant in HPV receptor and associated genes is associated with type-specific HPV infection and cervical lesion progression. We examined 96 SNPs in 8 genes which may participate in the HPV infection process in 875 samples with HPV negative or single HPV16, 18, 52, 58 positive from 3299 cervical exfoliated cell samples, by Illumina BeadXpress VeraCode platform, and analyzed the correlation between the SNPs and type-specific HPV infection and cervical lesions progression. We found rs28384376 in \textit{EGFR} and rs12034979 in \textit{HSPG2} significantly correlated to HPV16 infection; rs2575738, rs2575712, rs2575735 in \textit{SDC2} and rs6697265 in \textit{HSPG2} significantly correlated to HPV18 infection; rs10510097 in \textit{FGFR2}, rs12718946 in \textit{EGRF} significantly correlated to HPV52 infection; rs4947972 in \textit{EGRF}, rs2981451 in \textit{FGFR2}, rs2575735 in \textit{SDC2} significantly correlated to HPV58 infection. And rs3135772, rs1047057 and rs2556537 in \textit{FGFR2}, rs12034979 in \textit{HSPG2}, rs16894821 in \textit{SDC2} significantly correlated to cervical lesion progression induced by HPV16 infection; rs6697265 and rs6680566 in \textit{HSPG2}, rs16860426 in \textit{ITGA6} by HPV18 infection; rs878949 in \textit{HSPG2}, rs12718946 and rs12668175 in \textit{EGRF} by HPV52 infection; no SNP by HPV58 infection. Our findings suggest that HPV receptor and associated gene variants may influence the susceptibilities to HPV type-specific infection and cervical lesion progression, which might have a potential application value in cervical cancer screening and therapy.

INTRODUCTION

Cervical cancer is the second most common cancer among women, with more than 527,624 new cases and 265,672 deaths \cite{1}, in the world. Among these, 85% of the cervical cancer cases occur in developing countries \cite{2}. The etiology of cervical cancer has already been identified, that is, persistent infection of high-risk HPV is a causal factor for cervical lesions and cervical cancer \cite{3}. So far, there are 160 genotypes HPV have been found, among those, 13 high-risk types are related to the development of cervical cancer \cite{4}. However, infectious frequency of each high-risk type is not same, and HPV16 and 18 are most common. In the worldwide, the prevalence of HPV16 and 18 in women with normal cytology are 2.8% and 1.1%, while the prevalence of HPV52 and 58 are 1.5% and 1.0%,
respectively. Additionally, the prevalence of HPV 52 and 58 in normal cytology in China and East Asia appears some higher, accounting for 2.8% and 1.7%, than that in western areas [1].

The differences of HPV genotype distribution are also found in different grades of cervical lesions. A meta-analysis showed that HPV16 positivity was gradually increased from normal/ASCUS/LSIL/CIN1 (20-28%), through CIN2/HSIL (40/47%) to CIN3/ICC (58/63%) [5]. Another study revealed that HPV16 and HPV18 were 2-fold and 1.5-fold, respectively, more common in SCC than those in HPV-positive LSIL, while HPV52 and 58 were less common in SCC compared with HPV-positive LSIL, with SCC/LSIL ratios of 0.28 to 0.85 [6]. Those different distributions of HPV genotypes represent the different infectious and carcinogenic potentials of HPVs, but it is still unknown whether they are also related to the genetic susceptibility of individual to HPV type-specific infection.

Previous studies have revealed that viruses infect host cells through the relevant receptors, such as herpes virus entry mediator (HVEM) for Herpes virus, CD4, as well as CXCR4 and CCR5, for HIV [7], and sodium taurocholate cotransporting polypeptide (NTCP) for HBV [8]. Further, researches also show that single nucleotide polymorphisms (SNPs) in virus receptors can affect the infection process and outcome of pathogens. For example, a mutation in the chemokine receptor CCR5-Δ32, a co-receptor for macrophage-tropic (M-tropic) HIV-1 strains, can increase the host tolerance and promote the progression of the disease [9], and SNPs in syndecan 2 gene were associated with HIV DNA levels [10]. It has been known that multiple receptor engagements are involved in the process of HPV infecting human cervical epithelial cells [11–16]. According to studies on HPV16, virion binds to heparan sulfate proteoglycans (HSPGs) on either the epithelial cell surface or basement membrane through interactions with the L1 major capsid protein [17–18]. Growth factor receptors become activated through HSPG/growth factor/virion complexes that initiate signaling cascades during early virion-host cell interactions. After binding to HSPGs, virion undergoes conformational changes, leading to isomerization by cyclophilin B and proprotein convertase (FURIN) mediated L2 minor capsid protein cleavage that increases L2 N terminus exposure. Along with binding to HSPGs, virion binds to alpha 6 integrins, which initiate further intracellular signaling events. Following these primary binding events, HPV16 binds to a newly identified L2-specific receptor, the annexin A2 heterotetramer. Therefore, as opposed to a sequential handoff of the virion from one receptor to another, we hypothesize that a receptor complex coalesces and includes HSPGs (HSPG2 and SDC2), CyPB (PPIB), alpha 6 integrin (ITGA6), tetranspanins (TSPAN1), GFR (EGFR and FGFR2) and A2t. Thus, we selected eight genes, which have been identified as mainly molecules involved in HPV infection process, into our study. The eight genes included EGFR, PPIB, HSPG2, FGFR2, FURIN, ITGA6, TSPAN1 and SDC2. Considering the association of individual SNP with pathogen infection and disease outcome, we assume that SNPs in HPV receptor and associated genes may influence the susceptibility to type-specific HPV infection and cervical lesions progression.

Thus, we selected 96 single-nucleotide polymorphism (SNP) sites in the eight genes that were reported to be involved in the process of HPV infection using the haploview software, and evaluated the correlation between the distribution frequency of various SNP sites and type-specific HPV infection and cervical lesion progression in four common HPV genotypes (HPV16/18/52/58) in China and East Asia. Our study aimed to find out the genetic susceptibility to type-specific HPV infection and cervical lesion progression and search for a novel strategy for cervical cancer prevention or therapy.

RESULTS

The age distribution in the samples

The median age of 875 women whose cervical samples were collected was 41 yrs (21-69 yrs), included 40 yrs (22-68 yrs) in 214 controls (HPV negative), 43 yrs (21-69 yrs) in 294 single HPV16 positive, 44 yrs (27-59 yrs) in 55 single HPV 18 positive, 40 yrs (21-69 yrs) in 155 single HPV 52 positive, and 41 yrs (23-68 yrs) in 157 single HPV 58 positive. Further, the median age in 502 women with ≤LSIL and 373 with ≥HSIL was 40 yrs (21-63 yrs) and 43 yrs (21-69 yrs), respectively.

The significant different SNP sites, genotypes and haplotypes between single HPV16/18/52/58 positive and HPV negative in all the samples

All the SNPs in control population of this study were tested by Hardy–Weinberg equilibrium (HWE) as shown in Supplementary Table S1. Further, the differences in frequency distributions of alleles between cases and controls were compared by χ² test and fisher’s test.

SNP sites

Compared with HPV negative, there were two significant SNPs in SDC2 gene (rs2651465, p=0.01449, OR: 0.7154, 95%CI: 0.5487-0.9328 and rs2515127, p=0.03553, OR: 1.409, 95%CI: 1.032-1.924) and one in EGFR (rs4947972, p=0.02629, OR: 1.544, 95%CI: 1.06-2.25) in HPV16 positive group. There were three significant SNPs in SDC2 gene (rs2575712, p=0.001118, OR: 0.4829, 95%CI: 0.3101-0.7519;
rs2575735, p=0.03549, OR: 1.725, 95%CI: 1.069-2.782 and rs2575738, p=0.04269, OR: 1.639, 95%CI: 1.026-2.62) and two in HSPG2 gene (rs3767137, p=0.00345, OR: 3.469, 95%CI: 1.016-2.742 and rs6658920, p=0.04755, OR: 0.435, 95%CI: 0.1927-0.9822) and one in TSPAN1 gene (rs10890384, p=0.03999, OR: 0.4111, 95%CI: 0.1719-0.9831) in HPV18 positive group. There was one significant SNP in SDC2 gene (rs2589205, p=0.02931, OR: 1.402, 95%CI: 1.044-1.884), one in HSPG2 gene (rs6680566, p= 0.0302, OR: 0.7112, 95%CI: 0.5229-0.9674) and one in PPIB gene (rs2253557, p=0.04421, OR: 0.4602, 95%CI: 0.2208-0.9591) in HPV52 positive group. There were two significant SNPs in EGFR gene (rs11770506, p=0.01617, OR: 1.466, 95%CI: 1.082-1.985 and rs4947972, p=0.02831, OR: 1.629, 95%CI: 1.065-2.493) and one in FURIN gene (rs17514846, p=0.02622, OR: 0.5896, 95%CI: 0.3725-0.9332) and one in SDC2 gene (rs2575712, p=0.03558, OR: 0.7214, 95%CI: 0.537-0.9692) in HPV58 positive group. The detailed data were shown in Supplementary Table S1.

Genotypes

Compared with HPV negative, there was one protective SNP genotype “TA” of rs2651465 (OR: 0.624314, 95%CI: 0.419429-0.926093, p=0.011555) and one in HSPG2 (OR: 0.5896, 95%CI: 0.3725-0.9332) and one in SDC2 gene (rs2254357, p=0.04221, OR: 0.393682, 95%CI: 0.2208-0.9591) in HPV52 positive group. There were two susceptible SNP genotypes “AA” of rs2575712 (OR: 0.54901, 95%CI: 0.339422-0.884265, p=0.011555) in HPV52 positive group. The detailed data were shown in Supplementary Table S2.

Haplotypes

Compared with HPV negative, there was no significant different haplotype in HPV16 positive group. There is one protective haplotype “GGG”(rs2254357, rs878949, rs6680566) in HSPG2 (Freq(case)=0.326, Freq (control) =0.403, χ²=4.421, Chi square’s P value=0.0355, Fisher’s P value =0.036331) and one susceptible haplotype “GGA” (rs2254357, rs878949, rs6680566) in HSPG2 (Freq (case) =0.295, Freq (control) = 0.228, χ²=4.305, Chi square’s P value =0.038, Fisher’s P value=0.039358) in HPV52 positive group. There were two protective haplotypes “GG” (rs11770506, rs763317) in EGFR (Freq (case) =0.578, Freq (control) =0.667, χ²=6.133, Chi square’s P value=0.0133, Fisher’s P value =0.01617) and “AA” (rs17514846, rs4702) in FURIN (Freq (case) =0.097, Freq (control) =0.152, χ²=4.724, Chi square’s P=0.0297, Fisher’s P value=0.043411) in HPV58 positive group. The detailed data were shown in Supplementary Table S3.

The significant different SNP sites, genotypes and haplotypes between single HPV16/18/52/58 positive and HPV negative in the normal samples

All of the tested SNPs in the table were in Hardy–Weinberg equilibrium (HWE) in the control population from the normal samples (p>0.05), as shown in Table 1A. The different genotypes of individual SNPs in each analyzed genes in HPV negative samples (214 cases) were shown in Supplementary Table S4.

SNP sites

Compared with HPV negative in the normal samples, there was one significant SNP in EGFR gene (rs28384376, p=0.01865, OR: 2.642, 95%CI: 1.267-5.509) and one in HSPG2 gene (rs12034979, p=0.02327, OR: 3.114, 95%CI: 1.209-8.021) in HPV16 positive group. There were three SNPs in SDC2 gene (rs2575738, p=0.02623, OR: 2.128, 95%CI: 1.108-4.086; rs2575712, p=0.04014, OR: 0.4909, 95%CI: 0.258-0.934 and rs2575735, p=0.04542, OR: 2.056, 95%CI: 1.034-4.088) and one in HSPG2 gene (rs6697265, p=0.03901, OR: 1.978, 95%CI: 1.059-3.695) in HPV18 positive group. There was one SNP in FGFR2 gene (rs10510097, p=0.02673, OR: 1.711, 95%CI: 1.081-2.709) and one in EGFR gene (rs12718946, p=0.02796, OR: 0.6108, 95%CI: 0.3986-0.9357) in HPV52 positive group. There was one SNP in EGRF gene (rs4947972, p=0.02882, OR: 1.977, 95%CI: 1.088-3.59) and one in FGFR2 gene (rs2981451, p=0.02921, OR: 0.5318, 95%CI: 0.3039-0.9306) and one in SDC2 gene (rs2575735, p=0.03599, OR: 1.77, 95%CI: 1.063-2.947) in HPV58 positive group. The detailed data were shown in Table 1A.

Genotypes

Compared with HPV negative in the normal samples, there were two susceptible SNP genotypes “AA” of 28384376 (OR: 21.32744, 95%CI: 1.621665-1158.865,
Table 1A: The significant different SNP sites in target genes between single HPV16/18/52/58 positive and HPV negative in the normal samples

| VS HPV negative | SNP_ID       | Gene | chr | position       | A1 | A2 | A1 | A2 | A1 | A2 | HWE p value in control | OR(95%CI)            | P value |
|-----------------|--------------|------|-----|----------------|----|----|----|----|----|----|------------------------|----------------------|---------|
| HPV16           | rs28384376   | EGFR | 7   | 55233121       | A  | C  | 1  | 2.642(1.267-5.509) | 0.01865             |
|                 | rs12034979   | HSPG2| 1   | 22259146       | A  | G  | 1  | 3.114(1.209-8.021) | 0.02327             |
| HPV18           | rs2575736    | SDC2 | 8   | 97530402       | A  | G  | 1  | 2.128(1.108-4.086) | 0.02623             |
|                 | rs6697265    | HSPG2| 1   | 22256725       | G  | C  | 0.4326 | 1.978(1.059-3.695) | 0.03901             |
|                 | rs2575712    | SDC2 | 8   | 97576436       | A  | C  | 0.8779 | 0.4909(0.258-0.934) | 0.04014             |
|                 | rs2575735    | SDC2 | 8   | 97534651       | A  | G  | 0.6067 | 2.056(1.034-4.088) | 0.04542             |
| HPV52           | rs10510097   | FGFR2| 10  | 123327876      | A  | G  | 1  | 1.711(1.081-2.709) | 0.02673             |
|                 | rs12718946   | EGFR | 7   | 55221447       | G  | C  | 1  | 0.6108(0.3986-0.9357) | 0.02796             |
| HPV58           | rs4947972    | EGFR | 7   | 55161043       | G  | C  | 1  | 1.977(1.088-3.59)   | 0.02882             |
|                 | rs2981451    | FGFR2| 10  | 123278914      | A  | G  | 1  | 0.5318(0.3039-0.9306) | 0.02921             |
|                 | rs2575736    | SDC2 | 8   | 97534651       | A  | G  | 0.6067 | 1.77(1.063-2.947)  | 0.03599             |

A1: Minor allele name
A2: Major allele name
OR: Estimated odds ratio (for A1, A2 is reference)
HWE: Hardy–Weinberg equilibrium

Table 1B: The significant different genotypes in target genes between single HPV16/18/52/58 positive and HPV negative in the normal samples

| VS HPV negative | SNP Number/Gene | genotype | No.(frequency) in case | No.(frequency) in control | OR(95%CI)            | P fisher |
|-----------------|-----------------|----------|------------------------|--------------------------|----------------------|---------|
| HPV16           | rs28384376 EGFR | AA       | 3(10.7%)               | 1(0.6%)                  | 21.32744(1.621665-1158.865) | 0.007919 |
|                 |                 | AC       | 6(21.4%)               | 30(17.5%)                | 1.470486(0.442615-4.260336) | 0.418286 |
|                 |                 | CC       | 19(67.9%)              | 140(81.9%)               | ref                  | ref     |
|                 | rs12034979 HSPG2| AA       | 0(0.0%)                | 0(0.0%)                  | 0(0-1INF)            | 1       |
|                 |                 | AG       | 7(25.0%)               | 15(8.8%)                 | 3.437163(1.059898-10.29703) | 0.019656 |
|                 |                 | GG       | 21(75.0%)              | 156(91.2%)               | ref                  | ref     |

(Continued)
| VS HPV negative | SNP Number/ Gene | genotype | No.(frequency) in case | No.(frequency) in control | OR(95%CI) | P fisher |
|-----------------|-----------------|----------|------------------------|--------------------------|-----------|----------|
| HPV18           | rs2575738 SDC2  | AA       | 4(17.4%)               | 8(4.7%)                  | 5.092509(0.954255-23.63242) | 0.028565 |
|                 |                 | AG       | 9(39.1%)               | 57(33.7%)                | 1.637461(0.553659-4.778623) | ref      |
|                 |                 | GG       | 10(43.5%)              | 104(61.6%)               | ref       | ref      |
|                 | rs6697265 HSPG2 | GC       | 15(65.2%)              | 89(52.0%)                | 4.600737(1.008827-42.96709) | 0.0334   |
|                 |                 | CC       | 2(8.7%)                | 55(32.2%)                | ref       | ref      |
|                 | rs2575712 SDC2  | AC       | 10(43.5%)              | 86(50.9%)                | 0.444594(0.151795-1.299012) | ref      |
|                 |                 | CC       | 10(43.5%)              | 38(22.5%)                | ref       | ref      |
|                 | rs2575735 SDC2  | AG       | 8(34.8%)               | 48(28.1%)                | 1.620441(0.538477-4.632181) | ref      |
|                 |                 | GG       | 12(52.2%)              | 117(68.4%)               | ref       | ref      |
| HPV52           | rs10510097 FGFR2| AG       | 27(39.1%)              | 52(30.4%)                | 1.626255(0.854198-3.082385) | ref      |
|                 |                 | GG       | 36(52.2%)              | 113(66.1%)               | ref       | ref      |
|                 | rs12718946 EGFR | GC       | 36(52.2%)              | 83(48.5%)                | 0.854126(0.457694-1.597197) | ref      |
|                 |                 | CC       | 31(44.9%)              | 61(35.7%)                | ref       | ref      |
| HPV58           | rs4947972 EGFR  | GC       | 16(30.2%)              | 34(19.9%)                | 1.823182(0.840407-3.86191) | ref      |
|                 |                 | CC       | 35(66.0%)              | 136(79.5%)               | ref       | ref      |
|                 | rs2981451 FGFR2 | AA       | 0(0.0%)                | 13(7.6%)                 | 0(0-0.894449) | ref     |
|                 |                 | AC       | 18(34.0%)              | 69(40.4%)                | 0.664629(0.324939-1.325018) | ref     |
|                 | rs2575735 SDC2  | AG       | 21(39.6%)              | 48(28.1%)                | 1.822717(0.890965-3.700628) | ref     |
|                 |                 | GG       | 28(52.8%)              | 117(68.4%)               | ref       | ref      |

(Continued)
p=0.007919) and “AG” of rs12034979 (OR: 3.437163; 95%CI: 1.059898-10.29703, p= 0.019656) in HPV16 positive group. Three susceptible SNP genotypes “AA” of rs2575738 (OR: 5.092509, 95%CI: 0.954255-23.63242, p=0.028565), “GG” of rs6697265 (OR: 5.981678, 95%CI: 0.986387-64.47637, p=0.047442) and “GC” of rs6697265 (OR: 4.600737, 95%CI: 1.008827-42.96709, p=0.0334) in HPV18 positive group. There was one protective genotype “GG” of rs12718946 (OR: 0.147506, 95%CI: 0.015969-0.652735, p=0.00389) in HPV52 positive group. There was one protective genotype “AA” of rs2981451 (OR: 0, 95%CI: 0-0.894449, p=0.038732) in HPV58 positive group (Table 1B).

Haplotypes

Compared with HPV negative in the normal samples, there were two critically susceptible haplotypes “GGGG” (Freq(case)= 0.09, Freq (control)= 0.032, χ²=4.149, Chi square’s P value =0.0417, Fisher’s P value =0.059048) and “GGAA” (Freq(case)= 0.108, Freq (control)= 0.243, χ²=4.209, Freq (control)= 0.0402, Fisher’s P value =0.00386) in HPV16 positive group. There was one protective haplotype “GG” in block3 (rs4654770, rs6697265, rs6658920) (Freq(case)= 0.108, Freq (control)= 0.243, χ²=4.209, Fisher’s P value =0.0402, Fisher’s P value =0.040537) and two protective haplotypes “GGA” in block2 (rs2254357, rs878949, rs6680566) (Freq(case)= 0.108, Freq (control)= 0.243, χ²=4.209, Fisher’s P value =0.0402, Fisher’s P value =0.040537) and “GGAGA” in block1 (rs7518070, rs4654771, rs3767137, rs12117402, rs2305562) (Freq(case)= 0.043, Freq (control)= 0.157, χ²=4.26, Fisher’s P value =0.043573) in HPV18 positive group. There were two susceptible haplotypes “GGGAA” (Freq(case)= 0.225, Freq (control)= 0.148, χ²=4.164, Fisher’s P value =0.0413) and “GGA” (Freq(case)= 0.333, Freq (control)= 0.244, χ²=3.958, Fisher’s P value =0.0466) in HPV52 positive group (Table 1B).
(Freq(case)= 0.311, Freq (control)= 0.184, $\chi^2$=7.765, Chi square’s P value =0.0053, Fisher’s P value =0.006766) and one protective haplotype “GA” (Freq(case)= 0.17, Freq (control)= 0.278, $\chi^2$=5.001, Chi square’s P value =0.0253, Fisher’s P value =0.029213) in block 8 (rs3135761, rs2981451) in FGFR2 gene in HPV58 positive group (Table 1C).

Taken above results together, some variants in HPV receptor and associated genes were found to be correlated to type-specific HPV infection, including EGRF and HSPG2 gene to HPV16 infection, SDC2 and HSPG2 to HPV18 infection, EGRF, FGFR2 and HSPG2 to HPV52 infection, and EGRF, FGFR2 and SDC2 to HPV58 infection.

The significant different SNP sites, genotypes and haplotypes between ≥HSIL and ≤LSIL in single HPV16/18/52/58 positive subgroups

All of the tested SNPs in the table were in Hardy–Weinberg equilibrium (HWE) in the control population of this study (p=0.05 except for rs878949 and rs12668175 in HPV52), as shown in Table 2A.

SNP sites

Compared to single HPV16 positive with ≤LSIL, there were three significant different SNPs in FGFR2 gene (rs3135772, p=0.004776, OR: 0.5175, 95%CI: 0.3305-0.8104; rs2556537, OR: 1.863, p=0.009159, 95%CI: 1.165-2.978 and rs1047057, p=0.031276) of rs12718946 in HPV52 positive with ≥HSIL. There was no significant different SNP genotype “GG” (OR: 0.152201, 95%CI: 0.011374-1.184621, p=0.002472) of rs6697265 and two protective SNP genotypes “GG” (OR: 0.333, Freq (control)= 0.594, $\chi^2$=6.25, Chi square’s P value =0.0124, Fisher’s P value =0.021295) in block 2 (rs4654773, rs6697265) in HPV58 positive with ≥HSIL. There was one susceptible SNP genotype “CC” (OR: 8.913675, 95%CI: 1.11492-123.4335, p=0.036075) of rs6697265 and two protective SNP genotypes “GG” (OR: 2.263455, 95%CI: 1.087514-4.74233, p=0.02074) of rs1047057 in single HPV16 positive with ≥HSIL. There was a susceptible SNP genotype “CC” (OR: 8.913675, 95%CI: 1.11492-123.4335, p=0.036075) of rs6697265 and two protective SNP genotypes “GG” (OR: 0.152201, 95%CI: 0.011374-1.184621, p=0.002472) of rs6697265.

Haplotypes

Compared to single HPV16 positive with ≤LSIL, there was one protective haplotype “GG” (Freq(case)= 0.209, Freq (control)= 0.319, $\chi^2$=5.442, Chi square’s P value =0.01753, OR: 0.3534, 95%CI: 0.1577-0.792) in HSPG2 gene and one (rs16894821, p=0.03392, OR: 0.8524, 95%CI: 0.3598-0.9429) in SDC2 gene in single HPV16 positive with ≥HSIL. There were two significant different SNPs in HSPG2 gene (rs6697265, p=0.0213, OR: 2.923, 95%CI: 1.245-6.865 and rs6680566, p=0.02301, OR: 0.3646, 95%CI: 0.1538-0.8643) and one in ITGA6 gene (rs16860426, p=0.04799, OR: 0.3766, 95%CI: 0.1433-0.9895) in HPV18 positive with ≥HSIL. There were two significant different SNPs in EGFR gene (rs16894821, p=0.03583, OR: 1.858, 95%CI: 1.073-3.215) and one in HSPG2 gene (rs878949, p=0.00897, OR: 2.479, 95%CI: 1.287-4.776) in HPV52 positive with ≥HSIL. There was no significant different SNP site in HPV58 positive with ≥HSIL (Table 2A).

Genotypes

Compared to single HPV16 positive with ≤LSIL, there were four protective SNP genotypes “AA” (OR: 0.26792, 95%CI: 0.087236-0.740245, p=0.006378) and “AG” (OR: 0.404562, 95%CI: 0.139783-1.031401, p=0.047019) of rs3135772; “AG” (OR: 0.2899, 95%CI: 0.113434-0.771219, p=0.006208) of rs12034979 and “GA” (OR:0.488314, 95%CI: 0.240851-0.978606, p=0.031276) of rs16894821; two susceptible SNP genotypes “GG” (OR: 3.781242, 95%CI: 1.167225-16.15924, p=0.021072) of rs2556537 and “AG” (OR:2.263455, 95%CI:1.087514-4.74233, p=0.02074) of rs1047057 in single HPV16 positive with ≥HSIL. There was a susceptible SNP genotype “CC” (OR: 8.913675, 95%CI: 1.11492-123.4335, p=0.036075) of rs6697265 and two protective SNP genotypes “GG” (OR: 0.152201, 95%CI: 0.011374-1.184621, p=0.002472) of rs6697265.

It is well known that the occurrence and development of human cervical cancer are related to high-risk HPV infection. HPV 16 binds to heparin sulfate proteoglycans (HSPGs) on either the epithelial cell surface or basement membrane through interactions with its L1 major capsid protein, and afterwards HSPG/growth factor/HPV16 complexes activate growth factor receptors which initiate signaling cascades during early virion-host cell interactions [13]. After HPV enters the cell nucleus, E2 ruptures during the virus gene duplication, which helps the virus to integrate into the host cell.
Table 2A: The significant different SNP sites between ≥HSIL and ≤LSIL in single HPV16/18/52/58 positive subgroups

| Pathologic degrees | VS ≤LSIL | SNP_ID       | Gene   | chr | position       | A1 | A2 | HWE p value in control | OR (95%CI)           | P value |
|-------------------|----------|--------------|--------|-----|----------------|----|----|------------------------|----------------------|---------|
| HPV16 ≥HSIL       | rs3135772 | FGFR2        | 10     | 123263616 | A   | G  | 1                       | 0.5175 (0.3305-0.8104) | 0.004776 |
|                   | rs2556537 | FGFR2        | 10     | 123241794 | G   | A  | 0.7489                  | 1.863 (1.165-2.978)  | 0.009159 |
|                   | rs12034979| HSPG2        | 1      | 22259146 | A   | G  | 1                       | 0.3534 (0.1577-0.792) | 0.01753  |
|                   | rs1047057 | FGFR2        | 10     | 123239112 | A   | G  | 0.7456                  | 1.694 (1.067-2.689)  | 0.03118  |
|                   | rs16894821| SDC2         | 8      | 97604112 | G   | A  | 0.3204                  | 0.5824 (0.3598-0.9429) | 0.03392  |
| HPV18 ≥HSIL       | rs6697265 | HSPG2        | 1      | 22256725 | C   | G  | 0.4756                  | 2.923 (1.245-6.865)  | 0.0213   |
|                   | rs6680566 | HSPG2        | 1      | 22229090 | G   | A  | 0.4732                  | 0.3646 (0.1538-0.8643) | 0.02301  |
|                   | rs16860426| ITGA6        | 2      | 173319112| A   | T  | 0.714                   | 0.3766 (0.1433-0.9895) | 0.04799  |
| HPV52 ≥HSIL       | rs878949  | HSPG2        | 1      | 22227091 | A   | G  | 0.01918                 | 2.479 (1.287-4.776)  | 0.00897  |
|                   | rs12718946| EGFR         | 7      | 55221447 | G   | C  | 0.4186                  | 1.887 (1.096-3.248)  | 0.02555  |
|                   | rs12668175| EGFR         | 7      | 55178579 | C   | A  | 0.02953                 | 1.858 (1.073-3.215)  | 0.03583  |

A1: Minor allele name
A2: Major allele name
OR: Estimated odds ratio (for A1, A2 is reference)
HWE: Hardy–Weinberg equilibrium

Table 2B: The significant different genotypes between ≥HSIL and ≤LSIL in single HPV16/18/52/58 positive subgroups

| Pathologic degrees | VS ≤LSIL | SNP Number/ Gene | genotypes | No. (frequency) in case | No. (frequency) in control | OR (95%CI) | P fisher |
|-------------------|----------|------------------|-----------|-------------------------|---------------------------|------------|---------|
| HPV16 ≥HSIL       | rs3135772| FGFR2            | AA        | 49(21.3%)               | 17(35.4%)                 | 0.26792 (0.087236-0.740245) | 0.006378 |
|                   |          | FGFR2            | AG        | 105(45.7%)               | 24(50.0%)                 | 0.139783 (1.031401) | 0.047019 |
|                   |          | FGFR2            | GG        | 76(33.0%)                 | 7 (14.6%)                 | ref        | ref     |
|                   | rs2556537| FGFR2            | GA        | 124(54.1%)               | 22 (45.8%)                | 1.993674 (0.970782-4.102563) | 0.054603 |
|                   |          | FGFR2            | AA        | 62(27.1%)                | 22 (45.8%)                | ref        | ref     |
| Pathologic degrees | SNP Number/ Gene | SNP Number/ Gene | genotype | (frequency) in case | (frequency) in control | OR (95%CI) | P_fisher |
|--------------------|-----------------|-----------------|----------|---------------------|------------------------|------------|----------|
| VS ≤LSIL           | rs12034979 HSPG2 | AA              | 1(0.5%)  | 0(0.0%)             | Inf(0.004612- Inf)     | 1          | 0.006208 |
|                    |                 | AG              | 16(7.0%) | 10(20.8%)           | 0.2899(0.113434-0.771219) | ref        |          |
|                    |                 | GG              | 211(92.5%) | 38(79.2%)            | ref                    | ref        |          |
|                    | rs1047057 FGFR2  | AA              | 2(18.3%)  | 6(12.5%)            | 2.508327(0.887159-8.221692) | 0.077024   |          |
|                    |                 | AG              | 126(55.0%) | 20(41.7%)           | 2.263455(1.087514-4.74233) | 0.02074    |          |
|                    |                 | GG              | 61(22.7%) | 22(45.8%)           | ref                    | ref        |          |
|                    | rs16894821 SDC2  | AA              | 138(60.0%) | 20(41.7%)           | ref                    | ref        |          |
| HPV18 ≥HSIL        | rs6697265 HSPG2  | CC              | 8(44.4%)  | 4(12.5%)            | 8.913675(1.11492-123.4335) | 0.036075   |          |
|                    |                 | CG              | 8(44.4%)  | 18(56.3%)           | 2.179284(0.335237-25.00107) | 0.4528     |          |
|                    |                 | GG              | 2(11.1%)  | 10(31.2%)           | ref                    | ref        |          |
|                    | rs6680566 HSPG2  | GG              | 2(11.1%)  | 8(25.0%)            | 0.152201(0.011374-1.184621) | 0.04718    |          |
|                    |                 | GA              | 7(38.9%)  | 19(59.4%)           | 0.21407(0.040022-1.000252) | 0.040811   |          |
|                    |                 | AA              | 9(50.0%)  | 5(15.6%)            | ref                    | ref        |          |
|                    | rs16860426 ITGA6 | AA              | 1(5.6%)   | 4(12.5%)            | 0.240602(0.004308-2.938929) | 0.333333   |          |
| HPV52 ≥HSIL        | rs878949 HSPG2  | AA              | 2(5.7%)   | 5(4.5%)             | 1.919333(0.170181-12.9159) | 0.606343   |          |
|                    |                 | AG              | 15(42.9%) | 19(17.1%)           | 3.771844(1.490165-9.6238) | 0.002348   |          |
|                    |                 | GG              | 18(51.4%) | 87(78.4%)           | ref                    | ref        |          |
|                    | rs12718946 EGFR  | GC              | 21(60.0%) | 56(50.9%)           | 2.544463(0.889958-8.410202) | 0.073346   |          |
|                    |                 | CC              | 6(17.1%)  | 41(37.3%)           | ref                    | ref        |          |
|                    |                  | CC              | 12(35.3%) | 24(21.6%)           | 2.778045(0.90185-9.022859) | 0.068632   |          |
|                    | rs12668175 EGFR  | CA              | 14(41.2%) | 42(37.8%)           | 1.864266(0.651283-5.687745) | 0.237231   |          |
|                    |                 | AA              | 8(23.5%)  | 45(40.5%)           | ref                    | ref        |          |

(Continued)
chromosome, and consequently the over-expression of E6/E7 gene leads to the development of cervical cancer [19–21]. Although the mechanisms by which HPV induces cervical carcinogenesis have been described already, it is still unknown that the relationship between the variants in HPV receptor and associated genes and the susceptibility to type-specific HPV infection and cervical lesions progression. A haplotype-based association analysis is an increasingly accepted approach for genetic association studies [22], thus we firstly performed a haplotype-based study to analyze the relationship between HPV receptor and associated gene variants and the susceptibility to type-specific HPV infection and cervical lesion progression in Chinese women.

In an analysis on the susceptibility of HPV receptor and associated gene variants to type-specific HPV infection, we found two susceptible SNP sites rs28384376 in EGFR gene and rs12034979 in HSPG2 gene, and two susceptible genotypes “AA” of rs28384376 and “AG” of rs12034979, and two risk haplotypes “GGAA” and “GGGG” in block10 (rs2912780, rs2981575, rs2936870) in HSPG2 gene and one protective haplotype “GA” in block7 (rs2464474, rs16894821) in SDC2 gene in HPV16 positive group, suggesting that genetic variants in EGFR, HSPG2, FGFR2 and SDC2 genes may be associated with type-specific HPV16 infection. The differences of haplotype frequency distributions in target genes between cases and controls were significant by χ² test (p<0.05) while not by fisher’s test (p>0.05), suggesting that each kind of haplotype may be susceptible to type-specific HPV16 infection and there is no dominant susceptible or protective haplotype. The OR of rs28384376 and rs12034979 are 2.642 and 3.114 respectively, and there was no protective SNP site except for one critical haplotype. All those phenomena might be employed as an explanation that HPV16 possesses the highest prevalence among all high-risk genotypes in the worldwide. Again we found three susceptible SNP sites rs2575738 and rs2575735 in SDC2 gene and rs6697265 in HSPG2 gene, and one protective SNP site rs2575712 in SDC2 gene, and three susceptible genotypes “AA” of rs2575738 and “GG”, “GC” of rs6697265, and one susceptible haplotype “GGG” in block3 (rs4654773, rs6697265, rs6658920) and two protective haplotypes “GGA” in block2 (rs2254357, rs878949, rs6680566), “GGAGA” in block1 (rs7518070, rs4654771, rs3767137, rs12117402, rs2305562) in HSPG2 gene in HPV18 positive group, suggesting that genetic variants in HSPG2 and SDC2 genes are associated with type-specific HPV18 infection. Similarly, there was one susceptible SNP site rs10510097 in FGFR2 gene and one protective SNP site rs12718946 in EGFR gene, and one protective SNP genotype “GG” of rs12718946; and two susceptible haplotypes “GGA” in block2 (rs2254357, rs878949, rs6680566), “GGGAA” in block1 (rs7518070, rs4654771, rs3767137, rs12117402, rs2305562) in HSPG2 gene in HPV52 positive group, suggesting that genetic variants in HSPG2, FGFR2 and EGFR genes are associated with type-specific HPV52 infection. And again, there was two susceptible SNP sites rs4947972 in EGFR gene and rs2575735 in SDC2 gene and one protective SNP site rs2981451 in FGFR2 gene, and one protective SNP genotype “AA” of rs2981451, and one susceptible haplotype “GC” and one protective haplotype “GA” in block8 (rs3135761, rs2981451) in FGFR2 gene in HPV58 positive group, suggesting that genetic variants in EGFR, SDC2, and FGFR2 are associated with type-specific HPV58 infection. Thus, variants of HPV receptor and associated genes in SNP sites, genotypes and haplotypes may influence significantly the susceptibility of the individual to type-specific HPV18, 52 and 58 in Chinese women, but seems unobvious to type-specific HPV16 infection.

Furthermore, we analyze the correlation between HPV receptor and associated gene variants and cervical lesion progression in each single HPV positive group. In HPV16 positive group, there were two susceptible SNP sites rs2556537 and rs1047057 in FGFR2 gene, and three protective SNP sites rs3135772 in FGFR2 gene, rs12034979 in HSPG2 gene and rs16894821 in SDC2 gene, and two susceptible SNP genotypes “GG” of rs2556537 and “AG” of rs1047057 and four protective SNP genotypes “AA”, “AG” of rs3135772, “AG” of rs12034979, “GA” of rs16894821, and one

| Hpv genotype | VS≤LSIL | Block | GENE | Haplotype | Freq (case) | Freq (control) | χ² | P. Chi Square | OR (95%CI) | P. fisher |
|--------------|--------|-------|------|-----------|-------------|---------------|----|--------------|------------|----------|
| HPV16 ≥HSIL  | 6      | SDC2  | GG   | 0.209     | 0.319       | 5.442         | 0.0197 | 0.553624     | (0.333747-0.931329) | 0.022419 |
| HPV18 ≥HSIL  | 2      | HSPG2 | GG   | 0.333     | 0.594       | 6.25          | 0.0124 | 0.345927     | (0.132108-0.867713) | 0.021295 |
| HPV52 ≥HSIL  | 2      | HSPG2 | CAA  | 0.271     | 0.131       | 7.681         | 0.0056 | 2.470592     | (1.20591-4.988233)   | 0.00897  |

Table 2C: The significant different haplotypes between ≥HSIL and ≤LSIL in single HPV16/18/52/58 positive subgroups
In the study, include 214 HPV negative, 294 single HPV16 positive, and 157 single HPV58 positive. All the samples had the histological diagnosis, and the diagnostic criteria were followed by the American Society for Colposcopy and Cervical Pathology (ASCCP) 2006 guidelines [23]. Sample collection for the study was approved by the Ethics Committee of the Hospital.

HPV test and genotyping

The HPV test was performed by the Hybrid Capture 2 (HC2) test (Qiagen Digene) or Cervista test (Hologic). HC2 test is used for detecting the pool of 13 high-risk HPV genotypes including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Cervista test is used for detecting 14 genotypes (above 13 types plus 66), with a report of A5/A6(51, 56, 66), A7(18, 39, 45, 59, 68), and/or A9 (16, 31, 33, 35, 52, 58) positive or negative. Hybridio Rapid GenoArray test kit (GA) is used for HPV genotyping, including 6 low-risk types (6, 11, 42, 43, 44 and CP8304), 15 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 66 and 53). All the tests were performed according to the manufacturer’s protocol [24–26].

SNP selection and genotyping

The Haploview software 4.2 (Mark Daly’s lab of Broad Institute, Cambridge, MA, Britain) was used to analyze the tagSNPs and haplotype block based on the CHB (Chinese Han Beijing) population data of HapMap (HapMap Data Rel 27 PhaseII +III, Feb09, on NCBI B36 assembly, dbSNP b126 (International HapMap Project), a total of 96 SNPs in 8 HPV receptor and associated genes (EGFR, PPIB, HSPG2, FGFR2, FURIN, ITGA6, TSPAN1 and SDC2) were genotyped. Validated tagSNPs were selected with a MAF > 5% in the HapMap Asia population. SNPs that satisfied the following criteria were considered for detection: 1) tagSNPs were preferentially selected, 2) those SNPs were previously reported to be frequent in Chinese population (http://www.ncbi.nlm.nih.gov/snp). The total genomic DNA was extracted from the cervical exfoliated cells using KoningTM Mutisource Genomic DNA Extration Kit-Mini and PureLink® Genomic DNA Kits (invitrogen). The DNA concentration was detected, agarose gel electrophoresis was run and the final concentration was quantified to 50 ng/μl. All the SNPs were genotyped by Illumina BeadXpress VeraCode platform (USA), according to the manufacturer’s protocol.

Statistical analysis

All statistical analyses were performed using PLINK version 1.07 [27]. All p values in this study were two-sided by CHISQ and fisher test [28]. A p<0.05 was considered as the threshold for statistical significance. Allele frequencies, genotype frequencies and haplotypes frequencies for each SNP of all the subjects were compared using the CHISQ.
and Fisher test. ORs and 95% CIs were calculated by unconditional logistic regression analyses adjusted for age [29]. Genotypic frequencies in control subjects for each SNP were tested for departure from HWE using an exact test. Each HPV16/18/52/58 group was divided into two subgroups according to the pathological grade (≥HSIL and ≤LSIL) for analyzing the correlation between SNPs and lesion progression. Population was not stratified because all participants’ ethnicity was Han Chinese.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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