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Variations in Genes Encoding Human Papillomavirus Binding Receptors and Susceptibility to Cervical Precancer

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ABSTRACT

Background: Cervical cancer oncogenesis starts with human papillomavirus (HPV) cell entry after binding to host cell surface receptors; however, the mechanism is not fully known. We examined polymorphisms in receptor genes hypothesized to be necessary for HPV cell entry and assessed their associations with clinical progression to precancer.

Methods: African American women (N = 1,728) from the MACS/WIHS Combined Cohort Study were included. Two case–control study designs were used—cases with histology-based precancer (CIN3+) and controls without; and cases with cytology-based precancer [high-grade squamous intraepithelial lesions (HSIL)] and controls without. SNPs in candidate genes (*SDC1*, *SDC2*, *SDC3*, *SDC4*, *GPC1*, *GPC2*, *GPC3*, *GPC4*, *GPC5*, *GPC6*, and *ITGA6*) were genotyped using an Illumina Omni2.5-quad beadchip. Logistic regression was used to assess the associations in all participants and by HPV genotypes, after adjusting for age, human immunodeficiency virus serostatus, CD4 T cells, and three principal components for ancestry.

Results: Minor alleles in SNPs rs77122854 (*SDC3*), rs73971695, rs79336862 (*ITGA6*), rs57528020, rs201337456, rs11987725 (*SDC2*), rs115880588, rs115738853, and rs9301825 (*GPC5*) were associated with increased odds of both CIN3+ and HSIL, whereas, rs35927186 (*GPC5*) was found to decrease the odds for both outcomes (P value ≤ 0.01). Among those infected with Alpha-9 HPV types, rs722377 (*SDC3*), rs16860468, rs2356798 (*ITGA6*), rs11987725 (*SDC2*), and rs3848051 (*GPC5*) were associated with increased odds of both precancer outcomes.

Conclusions: Polymorphisms in genes that encode binding receptors for HPV cell entry may play a role in cervical precancer progression.

Impact: Our findings are hypothesis generating and support further exploration of mechanisms of HPV entry genes that may help prevent progression to cervical precancer.

Introduction

Human papillomaviruses (HPV) are non-enveloped, encapsulated double stranded DNA viruses that account for almost all cervical cancer cases worldwide (1–3). Approximately 80% of women are exposed to HPV sometime in their lifetimes, but infection usually is transient, with 70% to 90% of infected individuals "clearing" the virus naturally within 12 to 18 months (4–6). Some women fail to clear HPV infections, resulting in long-term persistent infection (7–13). Epidemiologic and virologic data demonstrate that oncogenic HPVs are the primary causal agents of cervical cancer; oncogenic HPV genotypes are associated with 100 times higher odds of developing cervical cancer (3, 14, 15). Specifically, HPV genotypes 16 and 18 are associated with nearly 70% of all cervical cancer cases and 50% of cervical intraepithelial neoplasia grade-3 (CIN3)—a precancerous lesion (16). However, in most women, multiple factors contribute to persistent infection of HPV and resulting progression to cervical precancer and cancer (16, 17). Epidemiologic studies have shown that younger age at first sexual intercourse, multiple sex partners, oral contraceptive use, multi-parity, human immunodeficiency virus (HIV) infection, smoking, HPV viral load in cervical lesions, and HPV integration in host genome are also associated with HPV persistence, and progression to CIN3 and cervical cancer (17). Although the mechanism for HPV persistence is incompletely understood, several studies implicate immune evasion, involving genetically mediated determinants of the host immune response (18, 19).

Twin and family studies have indicated that the heritability of cervical cancer is 22% to 64% (20–22); however, the contribution of host genetic variation to the development of precancerous lesions is less well understood. A few studies have observed human leucocyte antigen alleles and combination of killer immunoglobulin-like receptors associations with HPV-related cervical precancer (23–25). Other studies have focused on identifying roles of genes associated with DNA damage and DNA repair in cervical precancer and cancer (19). Wang

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and colleagues identified that variation in DNA repair genes (*GTH2F4*, *DUT*, and *DMC1*) were associated with HPV persistence and progression to cervical cancer in Costa Rican women (18). Several other genes involved in DNA repair, ribosomal dysfunction, mitochondrial processes, and/or oxidative stress (e.g., *TMC6*, *TMC8*, *FLJ35220*, *RSP19*, and *PRDX3* have also been shown to be associated with HPV persistence and progression to CIN3/cancer (18, 26).

Cell surface components used by several viruses for entry mechanism has been well documented (27). Similarly, to establish an infection, HPV must enter the host cell and deliver its DNA into the nucleus; however there is no consensus model for viral entry and trafficking (28). Heparan sulfate proteoglycans (HSPG) serve as the initial entry point for HPV. Removal of HSPGs reduces HPV viral-like proteins (VLP) binding by 80% to 90% (29–33). Previous studies performed both *in vitro* and *in vivo*, have reported on the role of syndecans and glypicans as binding receptors on host cell surfaces for HPV infection. *In vitro*, HPV entry is initiated by binding to a cell surface receptor, whereas, *in vivo*, the basement membrane acts as the primary site of virus binding (34). Heparan sulfate found on syndecans and glypicans has been reported to be associated with pseudo-infection of HPV and binding of HPV VLPs (34–36). It is postulated that HPV L1 capsid protein binds to glycoseaminoglycan (GAG) chains of HSPGs, followed by interactions with cell surface cyclophilin B (CyPB; ref. 37) and cleavage of the L2 capsid protein by furin, as shown in **Fig. 1** (38). This results in reduced affinity for HSPG but to mediate endocytosis a separate secondary receptor is involved. One of the main secondary receptors speculated to be involved in HPV binding and entry is *α6 integrins (ITGA6)*, which initiates further intracellular signaling events for internalization of HPV. Efficient entry of HPV into the cell, facilitated by efficient receptors, can potentially lead to an abundance of viral particles in the cells, which can overwhelm the host immune system and allow the infection to persist longer. Persistent infection can increase the initiation of various mechanisms (e.g., integration into the human genome, disruption of cell-cycle checkpoint, higher inflammation activities; ref. 39) that lead to precancer. Whereas, defective receptors will limit the entry of viruses and allow the immune system to effectively control the infection and block initiation of mechanisms that lead to precancer.

In the United States, cervical cancer incidence and mortality is higher among African American women, compared with whites (26, 40, 41). While race-based social disparities in cervical cancer screening and care have been well documented, there could also be heterogeneity in the genetic background in different racial populations. Thus, it is important to conduct genetic studies in different populations separately to help understand the molecular makeup and pathogenesis of HPV and cervical precancer/cancer in different populations. Further, the role of genetic variants associated with HPV cell entry as a risk factor for development of cervical precancer and cancer has not been explored. The objective of this study was to examine if polymorphisms in receptor genes hypothesized for HPV cell entry were associated with persistent HPV infection and disease progression to precancer in a longitudinal cohort of African American women.

Materials and Methods

Study population

The study was nested within six sites of the MACS/WIHS Combined Cohort Study (MWCCS), a geographically and ethnically diverse prospective cohort study of people living with HIV and without HIV. These study sites included Bronx, NY, Brooklyn, NY, Chicago, IL, Los Angeles, CA, San Francisco, CA and Washington, DC and consisted of women participants enrolled in what was called the Women's Interagency HIV Study (WIHS) during 1994 to 1995 [$n = 2059$ HIV(+), $n = 569$ HIV(-) women], and 2001 to 2002 [$n = 737$ HIV(+), $n = 406$ HIV(-); refs. (42, 43)]. For the parent WIHS study, participants were followed every 6 months. Self-reported data on demographics, lifestyle, socio-economic status, and clinical factors were obtained using structured questionnaires. Participants' physical examinations, laboratory measures, gynecologic examination including cervicovaginal cytology, HIV testing, HPV testing were conducted every 6 months following standardized protocols (42, 43).

Studies have indicated molecular heterogeneity in cervical cancer among different racial and ethnic populations (44); thus, considering allelic and locus heterogeneity between different ancestral groups in general, only African Americans, who comprise the largest group of participants in WIHS were considered for analyses. This analysis included 1,728 self-reported African American WIHS participants with banked cervical lavage and blood specimens between 1994 and 2016 and who had consented for genetic analysis. This included women living with HIV (WLWH; $n = 1337$) and women without HIV ($n = 391$). To account for possible heterogeneity arising from differential ancestry admixture in the self-reported African American population, we adjusted for population stratification as a covariate using principal components analysis (PCA; ref. 45). To maximize the principal component, we used 398,066 SNPs with $r^2 < 0.2$ that are available, as previously described (46).

Study design

This study was an unmatched case–control study using data from all African American women in the WIHS between 1994 and 2016. Participants who developed cervical precancer ("cases" as defined below) and who did not develop cervical precancer ("controls" as defined below) until the last follow-up period were included. Women ($n = 159$) were excluded from the analysis if they did not have a cervix.

Case definitions

Two definitions were used to characterize the cases, each as its own end point as previously defined: (i) histology-based 'CIN3+' cases (47) and (ii) more inclusive cytology-based high-grade squamous intraepithelial lesions (HSIL) cases (which also included both CIN2+ and CIN3+; ref. 48).

CIN along with grades 1, 2, or 3 were used to describe the thickness of the cervical lining that contained abnormal cells (49). Consistent with prior WIHS and other studies, precancer from abnormal Pap smears and colposcopy were defined as either (i) CIN-3+ or (ii) CIN-2 if concurrent with a cytologic diagnosis of HSIL (17, 50). CIN-2 without cytologic HSIL was not considered to be precancer given the high degree of uncertainty that exists with a histologic diagnosis of CIN-2 (51), even with the use of an expert pathology panel review. As the first outcome, all CIN3+ cases were compared with everyone else who did not have CIN3 or cervical cancer during follow-up (controls).

As a second outcome, following the 2001 Bethesda system criteria for cytologic diagnosis, squamous lesions were divided into four categories: (i) atypical squamous cells of undetermined significance (ASCUS), and cannot exclude HSIL (ASC-H); (ii) low-grade squamous intraepithelial lesions (LSIL); (iii) HSILs; and (iv) invasive cancer (52). As the second outcome, all HSIL cases were compared with women who only had ASCUS or ASC-H, LSIL or no precancer lesions during the WIHS study period until the last available follow-up visit (50).

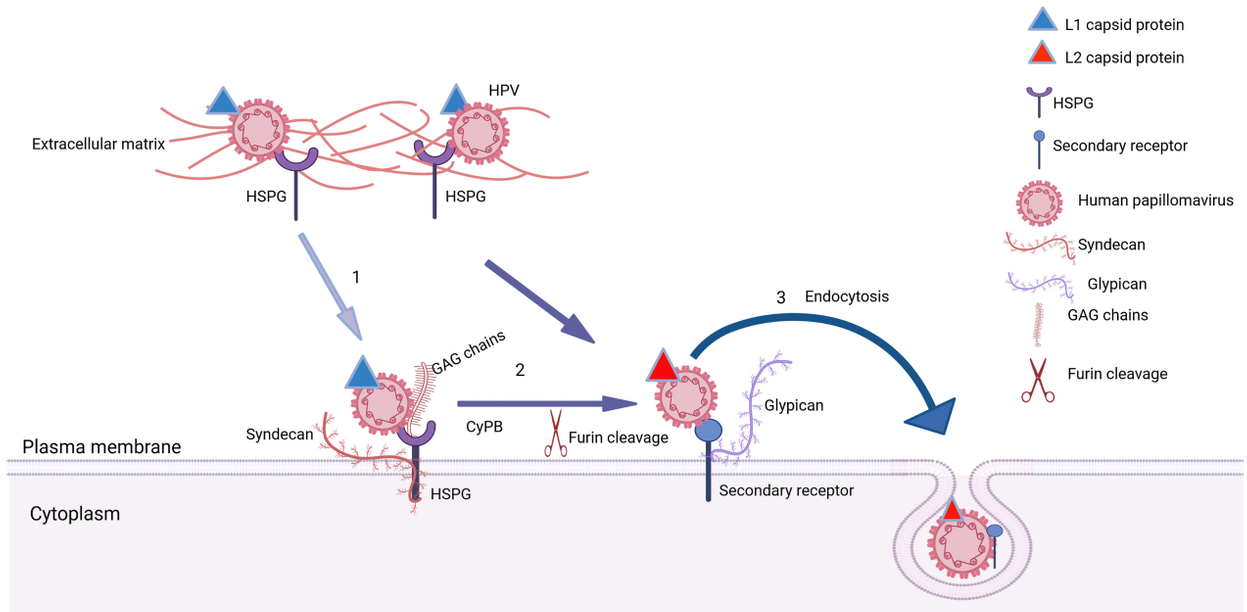

Figure 1.
Schematic illustrations of HSPGs involvement as co-receptors in early stage of HPV cell entry. 1. HSPG is suggested as the primary receptor for HPV cell entry. L1 capsid protein binds to HSPG GAG chains. Heparan sulfate is mostly found on two membrane-bound proteoglycans – syndecans and glypicans; 2. Interaction of capsid with CyPB results in conformational changes in L1 and L2. This is followed by furin cleavage of L2 and increased affinity to secondary receptors; 3. Furin cleavage of L2 triggers actin-dependent receptors. (Created with BioRender.com)

Candidate genes

HSPGs act as HPV binding receptors and are required on host cell surfaces for HPV infection (29, 31). Heparinase treatment is known to reduce pseudo-infection of HPV (31). HPV VLPs' ability to transfer genes into COS-7 cells, is also reported to require heparan sulfate (35). Heparan sulfate is mostly found on two membrane-bound proteoglycans- syndecans and glypicans. Syndecans and glypicans differ in structure of the core protein domain (53). Syndecans have a transmembrane and cytoplasmic domain, whereas glypicans are anchored to the extra-cytoplasmic face of the plasma membrane via glycosylphosphatidylinositol (GPI). Syndecans and glypicans bind proteins of the extracellular environment via their heparan sulfate chains, regulating a wide spectrum of biological activities, including cell proliferation and differentiation, morphogenesis, wound repair, and host defense. Syndecans comprise a family of four distinct genes (designated SDC1–4) encoding integral membrane proteins. Glypicans are a family of at least six different gene products that are linked to the cell membrane via a GPI anchor (34, 36, 54). Others have implicated alpha(6) integrin (*ITGA6*) as another receptor for HPV type 16; correlation between HPV-like particle (HPV-16 L1-VLP) and *ITGA6* has been reported in literature (55).

Given that most heparan sulfates on host cell surfaces are members of membrane bound proteoglycans syndecans and glypicans (53), we selected the following candidate genes based on previous literature and biologic significance (encoded genes): Syndecan 1 (*SDC1*) in chromosome 2, Syndecan 2 (*SDC2*) in chromosome 8, Syndecan 3 (*SDC3*) in chromosome 1, Syndecan 4 (*SDC4*) in chromosome 20, Glypican 1 (*GPC1*) in chromosome 2, Glypican 2 (*GPC2*) in chromosome 7, Glypican 3 (*GPC3*) in chromosome X, Glypican 4 (*GPC4*) in chromosome X, Glypican 5 (*GPC5*) in chromosome 13 and Glypican 6 (*GPC6*) in chromosome 13. Integrin subunit alpha 6 (*ITGA6*) in chromosome 2 was also included in this study (55).

DNA and genotyping

Blood samples were collected at each semiannual visit for laboratory testing. Genomic DNA was isolated from peripheral blood mononuclear cells using the Pure-gene DNA isolation kit (Gentra Systems, Minneapolis; ref. 56). Informed consent was requested from all participants via protocols approved by institutional review boards (IRB) at each affiliated institution of the parent study. All women who consented to participate in studies of host genetics were genotyped using the Illumina Omni2.5-quad beadChip array (Illumina, San Diego). Of 2,120 women with genomic data, 1,728 women who were screened for precancer during the study period as described above were included in the analyses.

Genotype quality control

All SNPs were checked for completeness (by SNP and by subject), rare variants, and deviation from Hardy-Weinberg Equilibrium (HWE). SNPs with a call rate < 90% and HWE $P > 1\times10^{-4}$ and were included. SNPs with minor allele frequency differences > 0.2 compared with the most closely related 1000 Genomes (1000G) reference panel (RRID:SCR_006828, ASW for African American population) were removed.

HPV detection and typing

Cervicovaginal lavage samples were collected using 10 mL of normal saline (0.85% sodium chloride). Protocols for semiannual HPV testing have been described previously (50, 57, 58). Briefly, HPV DNA were detected using well-established MY09/11 primer system PCR assays, followed by hybridization with HPV type–specific probes. HPV was measured from samples on or closest date available to precancer diagnosis visit for cases and last visit for the controls. Secondary analyses were conducted limited to cases and controls who were infected with the same HPV types and phylogenetic groups. We chose

to group HPV types by phylogeny because it is based on the L1 capsid sequence which would likely use a similar mechanism for cell entry. HPV genotyping was grouped as follows:

a. Any oncogenic/ high risk HPV (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)
b. Alpha-9 HPV (16, 31, 33, 35, 52, 58, 67)
c. Alpha-9a - Alpha-9 HPV other than HPV16 (31, 33, 35, 52, 58, 67)
d. HPV16
e. Alpha-7 HPV (18, 45, 39, 59, 68, 70, 85, 97)
f. Alpha7a - Alpha-7 HPV other than HPV18 (45, 39, 59, 68, 70, 85, 97)

PCA for population substructure

For PCA, first linkage disequilibrium (LD) pruning was performed with all the SNPs in the microarray, where all correlated SNPs (n = 1,149,632) were removed from the SNP set. Minor allele frequency of at least 2% and LD r^2 value of ≤ 0.2 were used as inclusion criteria for including SNPs in the PCA. There were 398,066 independent SNPs included in the PCA conducted with the Eigenstrat software (45). There is heterogeneity in the admixed African American population so PCs 1–3 were included in the analyses to correct for population substructure within the subset of WIHS women who self-reported as African American.

Statistical analysis

PLINK 1.90 was used to perform an association analysis based on an additive model. As described above, two different case control study designs were used, and secondary analyses limited to specific type/group of HPV genotype were also conducted. Logistic regression analyses were used to assess the association between each SNP and the odds of developing cervical precancer. All models were adjusted for age, HIV serostatus, CD4 T cells counts (on or closest date available to precancer diagnosis visit for cases and last visit for the controls) and three principal components (for population substructure, see above) to estimate per-allele adjusted odds ratios (aOR) and 95% confidence intervals (CI). CD4 T cells count was included as a categorical variable: < 200, 200–499, 500–999, and ≥ 1,000 cells/mm^3. Separate analyses were also performed by each HPV genotyping group. Initially there were 4.880 loci; after removing 3 redundant loci, 4.877 loci were available for analysis. Correction for multiple testing was performed based on the effective number of tests which resulted after pairwise correlations between markers (59). There were 2,692 effective number of tests (adjusted P value 1.86×10^{-5}). Corrections for multiple testing of the 4,880 SNPs were applied using the 2,692 effective number of tests; however, uncorrected results are also presented.

Ethics statement

The parent study and this sub-study was performed in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the University of Alabama at Birmingham IRB. Written informed consent was obtained, and IRB approval was obtained for the protocol at each of the participating sites.

Data availability

The data generated in this study are not publicly available because this study is part of the MWCCS. Data can be made available upon reasonable request from the corresponding author following DSMB approval.

Results

Characteristics of the study population

Demographic and clinical characteristics of the study population by CIN3+ and HSIL status are shown in **Table 1**. The study included 1,337 (77%) WLWH and 391 (23%) women without HIV African American study participants. Overall, 103 patients had CIN3+ lesions and 148 had HSIL. Patients with CIN3+ lesions were significantly younger than controls (P < 0.0001) and a similar trend was observed with HSIL as the outcome.

Table 1. Demographic and clinical characteristics of African American study participants in the WIHS by CIN3+ lesion and HSIL status.

		CIN3+			HSIL		
Variables of interest	**All (n = 1,728)**	**Cases (n = 103)**	**Controls (n = 1625)**	***P* value**	**Cases (n = 148)**	**Controls (n = 1580)**	***P* value**
Age in years [Median (IQR)]	46.4 (39.8–53.4)	38.6 (33.1–45.9)	46.7 (40.2–53.7)	<0.0001	40.6 (33.9–46.7)	46.9 (40.3–53.8)	<0.0001
HIV status							
Sero-positive	1337 (77.4)	90 (87.4)	1247 (76.7)	0.008	131 (88.5)	1206 (76.3)	0.0003
Sero-negative	391 (22.6)	13 (12.6)	378 (23.3)		17 (11.5)	374 (23.7)	
CD4 T cells count (cells/mm^3)[a]							
<200	416 (24.3)	37 (36.3)	379 (23.6)	<0.0001	58 (39.5)	358 (22.9)	<0.0001
200–499	418 (24.4)	40 (39.2)	378 (23.5)		50 (34.0)	368 (23.5)	
500–999	485 (28.4)	12 (11.8)	473 (29.4)		22 (15.0)	463 (29.6)	
≥1000	391 (22.9)	13 (12.8)	378 (23.5)		17 (11.6)	374 (23.9)	
HPV infection[b]							
HPV 16	47 (3.0)	5 (5.2)	42 (2.9)	0.2485	9 (6.4)	38 (2.7)	0.0308
A9	209 (13.5)	22 (22.7)	187 (12.9)	0.0109	37 (26.2)	250 (11.2)	<0.0001
A9a	162 (10.8)	17 (18.5)	145 (10.3)	0.0232	28 (21.2)	134 (9.8)	0.0002
A7	197 (12.7)	24 (24.7)	173 (11.9)	0.0004	31 (22.0)	166 (11.8)	0.0013
A7a	178 (11.9)	21 (22.8)	157 (11.1)	0.0022	26 (19.7)	152 (11.1)	0.0065
Onc	331 (21.4)	37 (38.1)	294 (20.3)	<0.0001	55 (39.0)	276 (19.6)	<0.0001

[a]CD4 T cells count missing = 18, measured on or closest date available to precancer diagnosis visit for cases and last visit for the controls.
[b]Measured from samples on or closest date available to precancer diagnosis visit for cases and last visit for the controls, HPV status missing = 604; HPV Types: Onc, Any oncogenic/ high risk HPV (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68); A9, Alpha-9 HPV (16, 31, 33, 35, 52, 58, 67); A9a, Alpha-9 HPV other than HPV16 (31, 33, 35, 52, 58, 67); A7, Alpha-7 HPV (18, 45, 39, 59, 68, 70, 85, 97); A7a, Alpha-7 HPV other than HPV18 (45, 39, 59, 68, 70, 85, 97).

Overall association of SNPs with cervical precancer lesions

While sixty SNPs individually showed potential association with precancer (at P value ≤ 0.01) in African American study participants in the WIHS cohort, none of these SNPs were statistically significant after correcting for multiple comparisons ($P < 1.86\times10^{-5}$). The odds ratios and 95% CI of individual SNP association before multiple comparisons are shown in Supplementary Table S1. The majority of SNPs were either in the intron or in the intragenic regions. Overall, 10 SNPs (rs77122854 in *SDC3*, rs79336862 and rs73971695 in *ITGA6*, rs57528020, rs201337456 and rs11987725 in *SDC2*, rs115738853, rs115880588, rs35927186 and rs9301825 in *GPC5* were consistently associated with both CIN3+ lesions and HSIL. A forest plot showing the association (aOR and 95% CI, before adjusting for multiple comparisons) between SNPs and both CIN3+ and HSIL as outcome endpoints for cervical precancer is shown in **Fig. 2**. The minor allele in rs35927186 (*GPC5*) was associated with reduced odds of CIN3+ and HSIL, while the minor alleles of the remaining 9 variants were associated with increased odds of CIN3+ and HSIL. While both WLWH and women without HIV were included in the model, both HIV serostatus and CD4+ count were adjusted for in the models to account for any differences due to immune status.

Association of SNPs with cervical precancer lesions by HPV genotype

Table 2 shows SNPs that were associated (aOR, 95% CI) with precancer (CIN3+ and HSIL) prior to adjusting for multiple comparisons. Among individuals with any oncogenic HPV infections, minor allele of rs12866859 in *GPC5* was associated with susceptibility to HSIL and a similar association trend was shown with CIN3+.

Alpha-7/alpha-7a HPV types

Among individuals with A7 infection, minor allele in SNPs rs12866859 in *GPC5* was associated with increased odds of CIN3+ and had a similar association trend with HSIL. Likewise, minor alleles in rs10737383 in *SDC3* and rs13392912 in *ITGA6* were associated with increased odds of CIN3+; similar trends were observed with HSIL. Association in rs12866859 was also observed among those who were infected with all other A7 HPV types but not HPV18.

Alpha-9/alpha-9a HPV types

Alpha-9 was the most common (14%) HPV-genotype reported. Minor alleles in SNPs rs16860468 in *ITGA6*, rs11987725 in *SDC2*, and rs3848051 in *GPC5* were associated with increased odds of CIN3+; minor allele in rs2356798 in *ITGA6* was associated with increased odds of both CIN3+ and HSIL. Among those who were infected with all other A9 HPV types but not HPV16, minor allele in rs722377 in *SDC3* was associated with increased odds of CIN3+ lesions; similar trends were observed with HSIL.

Discussion

The current study reports polymorphisms in several genes that encode receptors (syndecan and glypican family), potentially used for cell entry by HPV and their associations with development of precancer in African American WLWH and women without HIV. Specifically, novel associations with four SNPs in *GPC5*, three in *SDC2*, two in *ITGA6* and one SNP in *SDC3* with CIN3+ and HSIL were identified.

The majority of SNPs reported in this study are intronic variants and have not been reported previously in the literature. While we could not establish the clinical relevance of these observations, the results provide evidence of potential associations with development of precancer. Almost all the associated SNPs had no known functional implications reported in the current literature, specifically with any cancer related pathways. Furthermore, there were differences in frequencies with some of the rare and common variants in African American populations compared with European whites, as reported in dbSNP. For example, two SNPs rs115738853 and rs115880588 in *GPC5* (but not in perfect LD) and rs73971695 in *ITGA6*, all have MAF 2% in African Americans, but are monomorphic (i.e., do not exist) in European whites. Although low in frequency, minor alleles were more prevalent among precancer patients; thus, associations featured large aOR but broader CI. Similarly, rs57528020 in *SDC2* have MAF 4% in African Americans, but is also not polymorphic in European whites. SNPs rs893225 (MAF ∼ 10%) and rs166604 (MAF 20%) have similar frequencies in both populations. While this is one of the first studies conducted in African American women, additional studies will be needed to assess whether some of these associations are functional, in LD with other variants, specific to African Americans.

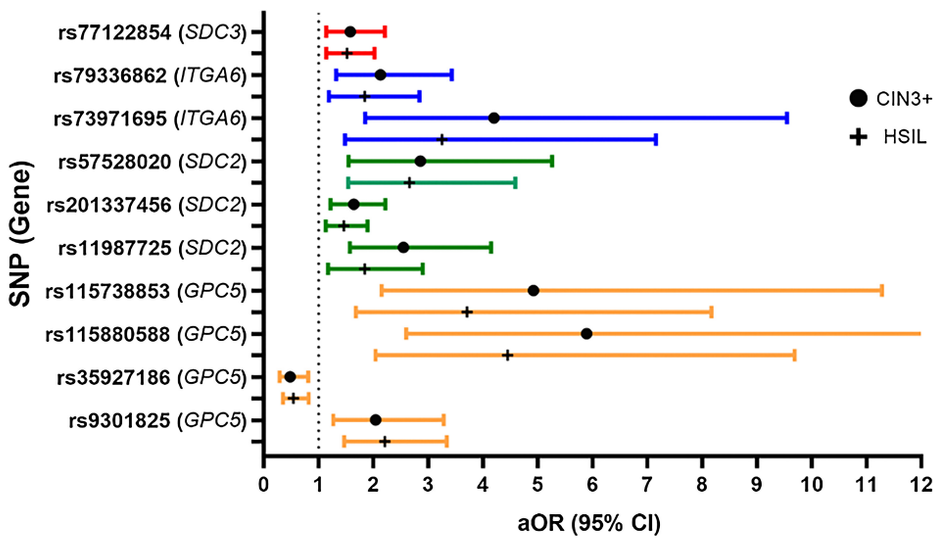

Figure 2.
Forest plot showing the individual association (aOR and 95% CI) between genetic variants and both CIN3+ and HSIL as outcome endpoints for cervical precancer (adjusted for age, HIV sero-status, CD4 T-cell counts, and three principal components for genetic ancestry; SNPs with P value ≤ 0.01 are presented (before adjusting for multiple comparisons).

Table 2. Individual SNPs associated with increased odds of precancer (CIN3+ and or HSIL) in African American study participants in the WIHS study, by HPV genotype, before adjusting for multiple comparisons.

		Phenotype CIN3+		Phenotype HSIL	
Gene (Chr) SNP	**HPV genotype**	***P* value**	**aOR (95% CI)**	***P* value**	**aOR (95% CI)**
SDC3 (1)					
rs722377	A9a	0.0007094	4.43 (1.87–10.48)	0.006287[a]	2.33 (1.27–4.29)
rs10737383	A7	0.0002717	3.93 (1.88–8.22)	0.003029[a]	2.62 (1.39–4.97)
ITGA6 (2)					
rs2356798	A9	0.0007891	3.89 (1.76–8.61)	0.000673	2.90 (1.57–5.36)
rs13392912	A7	0.0004786	15.82 (3.36–74.51)	0.00315[a]	9.16 (2.11–39.88)
rs16860468	A9	0.000755	6.22 (2.15–17.99)	0.004407[a]	3.89 (1.53–9.89)
SDC2 (1)					
rs11987725	A9	0.0005637	9.97 (2.70–36.86)	0.05944[a]	2.76 (0.96–7.96)
rs11987725	Onc	0.00005142	5.30 (2.36–11.88)	0.008344[a]	2.65 (1.28–5.46)
GPC5 (13)					
rs3848051	A9	0.0006686	7.08 (2.29–21.87)	0.02268[a]	3.26 (1.18–8.99)
rs3848051	A9a	0.0007759	8.09 (2.39–27.39)	0.02028[a]	3.60 (1.22–10.63)
rs12866859	A7	0.0002813	13.72 (3.34–56.37)	0.000354	11.01 (2.95–41.06)
rs12866859	A7a	0.0003207	17.06 (3.64–80.00)	0.00261[a]	8.32 (2.09–33.05)
rs12866859	Onc	0.0005586	5.98 (2.16–16.49)	0.000139	6.07 (2.40–15.38)

Note: HPV Types: Onc, Any oncogenic/ high risk HPV (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68); A9, Alpha-9 HPV (16, 31, 33, 35, 52, 58, 67); A9a, Alpha-9 HPV other than HPV16 (31, 33, 35, 52, 58, 67); A7, Alpha-7 HPV (18, 45, 39, 59, 68, 70, 85, 97); A7a, Alpha-7 HPV other than HPV18 (45, 39, 59, 68, 70, 85, 97).
[a]*P* value > 0.001. Models were adjusted for age, HIV serostatus, CD4 T cells counts and three principal components for race; None of the SNPs were statistically significant after multiple comparisons and we report all SNPs significant at *P* value ≤ 0.001.

Even though our sample size is relatively small, this is the first time that genetic variants in these candidate genes were assessed for associations with precancer phenotypes, specifically among African Americans. Given that our study is not based on sequencing but is an array-based analysis done on candidate genes, it is possible that we did not include many of the rare or population-specific variants implicated to be associated with precancer lesions. However, these associations based on candidate gene analysis could be the basis for functional studies in the future. One of the novel approaches of our study is the inclusion of WLWH, as the WIHS cohort is one of the largest HIV cohorts with follow-up data. We were under-powered to conduct the analysis by HIV status and compare differences; however, we adjusted for both HIV serostatus and CD4 T cells counts (also available among women without HIV) in our study. CD4 T-cell counts were a proxy for immune status beyond HIV infection. Overall, more than 24% had CD4 T cells counts less than 200 cells/mm^3 and a higher proportion of CIN3+ cases (36.3% vs. 23.6%) and HSIL cases (39.5% vs. 22.9%) had CD4 T cells counts less than 200 cells/mm^3 compared with their respective controls. In our primary analyses, we included both HPV+ and HPV- individuals. While our defective receptor hypothesis suggest that limited entry of HPV allows the immune system to control the infection, we acknowledge that some individuals with efficient receptors may not have been exposed to HPV. This would potentially bias the effect to the null. However, we also conducted secondary analyses only among HPV+ individuals (by genotyping group). Data on CD4 T cells counts and HPV DNA were collected on or closest date available to precancer diagnosis visit for cases; however, for controls these data were collected on the last visit available. This approach provided a longer window for controls to be exposed to HPV and to include all women in this study with infection, representing an exposed group who had the opportunity to develop precancer but did not.

We carefully examined the differences between cases and controls and adjusted for potential confounders in our analysis. Controls were older, a higher proportion of controls were HIV seronegative, and controls had higher CD4 counts. To account for these differences, we adjusted for all these variables in all genetic models. There was no statistically significant difference in follow-up time between cases and controls (HSIL: 11.7 ± 5.8 years for cases vs. 10.3 ± 6.3 years for controls; CIN2+: 12.2 ± 5.8 years for cases vs. 10.3 ± 6.3 years for controls; CIN3+: 12.8 ± 5.4 years for cases vs. 10.3 ± 6.3 years for controls), thus, survival bias is unlikely to affect our association. Because HPV is the main factor for development of precancer, we agree that future research that matches on the duration of HPV infection will better inform the potential effects of the variants encoding HPV persistence and precancer risk. While HPV exposure time is difficult to assess, we conducted the analysis only among those individuals (both cases and controls) who were infected with-type–specific HPV. Detection bias related to precancerous lesions is unlikely because cervicovaginal cytology was conducted every 6 months following standardized protocols among all study participants.

Overall, 21.4% of participants were infected with oncogenic/high risk HPV genotypes, with alpha-9 phylogenetic group being the most common group; however, it is not clear if all HPV phylogenetic groups use the same receptors for cell entry, or if there are specific receptors for each phylogenetic group. The mechanism of HPV cell entry within each phylogenetic group has not been well characterized. We reported SNPs responsible for HPV cell entry and progression to cervical precancer by HPV genotype. While rs11987725 in *SDC2* was associated with cervical precancer lesions in patients infected with A9 genotypes, variants in *SDC3, ITGA6* and *GPC5* were associated with precancer lesions resulting from both A9/A9a and A7/A7a HPV genotypes. While the exact biologic mechanism of how precancer susceptibility varies by HPV cell entry receptor is still not understood, potential associations from this study suggest that these receptors might be involved with different HPV genotypes. It is possible that increased and efficient cell surface co-receptors would likely support higher HPV replication (viral load) that promote HPV integration or disease progression mechanisms. Therefore, it would be interesting to

estimate if HPV viral load, persistence, and susceptibility to precancer differ by HPV cell entry receptors. The observed associations were not significant after Bonferroni correction for multiple comparisons, perhaps due to limited samples sizes. However, our results are hypothesis generating, supporting additional exploration of variant analyses at aggregate gene level or pathway-based systemic analyses to comprehensively understand which genes are involved in the HPV cell entry pathway and how it affects precancer/cancer progression.

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Authors' Contributions

A. Mukherjee: Data curation, formal analysis, validation, visualization, writing–original draft. **Y. Ye:** Validation, writing–review and editing. **H.W. Wiener:** Data curation, software, formal analysis, visualization, methodology, writing–review and editing. **M.H. Kuniholm:** Validation, writing–review and editing. **H. Minkoff:** Visualization, writing–review and editing. **K. Michel:** Visualization, writing–review and editing. **J. Palefsky:** Validation, visualization, writing–review and editing. **G. D'Souza:** Validation, investigation, visualization, methodology, writing–review and editing. **L. Rahangdale:** Visualization, writing–review and editing. **K.R. Butler:** Visualization, writing–review and editing. **M.C. Kempf:** Resources, investigation, visualization, writing–review and editing. **S.L. Sudenga:** Validation, visualization, methodology, writing–review and editing. **B.E. Aouizerat:** Visualization, writing–review and editing. **A.I. Ojesina:** Validation, visualization, writing–review and editing. **S. Shrestha:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft.

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