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Variants in immune-related genes and genital HPV 16 persistence in men

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ABSTRACT

Objectives: While most human papillomavirus (HPV) infection clears on its own, persistent HPV infection can cause genital warts and anal, penile and oropharyngeal cancers in men. We conducted genetic analysis in a sub-cohort of the HPV infection in men (HIM) study to test the hypothesis that differences in host genes influence HPV persistence in men.

Methods: Baseline and longitudinal genital HPV status at the genitals was measured every 6-months using the Linear Array assay amplified HPV L1 gene fragment using the PGMY09/11 L1 consensus primer system. DNA was extracted from peripheral blood and single nucleotide polymorphisms (SNPs) in the customized genome-wide genotyping array, the "TxArray," were examined using logistic regression in a case-control study design to assess the association with HPV16 persistence/clearance.

Results: Of the total of 737,742 autosomal SNPs in the array, 605,885 passed basic quality control and were examined between 40 men (cases) with $\geq$ 18 months persistent genital HPV 16 infection vs. 151 controls who were HPV 16-positive, but whose infections cleared in < 18 months. The logistic regression analysis from this case-control study showed variants in several gene regions associated with genital HPV 16 persistence, with the strongest association detected with SNPs on chromosomes 20 ($p < 5.72 \times 10^{-6}$) and 15 ($p < 5.89 \times 10^{-6}$), after adjusting for age, smoking status, number of sex partners and four principal components (ancestral background).

Conclusions: Our results provide a preliminary basis for understanding the biological mechanism of oncogenic HPV 16 pathogenesis at the genitals in men. Some of the genes flanking the top hit SNPs are consistent with previous findings in both HPV related and non-related cancers but further genetic studies in larger cohorts are warranted to confirm these and identify novel major susceptibility genes involved in the pathogenesis of genital HPV persistence in men.

1. Background

Human Papilloma Virus (HPV) is the most common sexually transmitted infection (STIs) in both men and women worldwide, including the United States (US) [1]. In men, the HPV genotypes that are most frequently detected in genital warts are HPV 6 and 11 (96–100%), and HPV 16 is the predominant type detected in penile cancer [2]. While penile cancer is considered a rare cancer with 22,000 estimated cases per year worldwide, it is still associated with high morbidity and mortality [2].

Some cohort studies reveal that anogenital HPV incidence is at least as high among men as it is in women [3]. Duration of HPV infections is also comparable between men and women with HPV 16 infections having a longer duration than most other high-risk HPV types in both men and women [4]. However, unlike women where HPV incidence decreases with older age, men remain susceptible to incident HPV infections across their lifespan [5]. Men are also less likely to naturally acquire HPV antibodies compared to women, regardless of the population studied [4]. It is unknown if the systemic immune response to HPV in men is similar to that in women. The majority of HPV infection clears spontaneously, whereas in a subset it persist [1,6], defined as intermediate phenotype, leading to cervical cancer [6]. However, the natural history of HPV-associated penile cancer, including differential HPV persistence is not fully known in men, but similar process as in

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women could potentially be required to induce the cellular changes that lead to pre-cancerous lesions and penile cancer. Findings from a genetic study in men could provide evidence of differences or similarities of the natural history of HPV between men and women [7], which could have tremendous translational and public health importance.

Some studies have assessed social, demographic and behavioral determinants of HPV persistence in men [8]; however, the role of genetically mediated determinants of biological mechanisms for the persistence of HPV in men is not known. Using the *HPV in Men* (HIM) Study cohort population, we conducted a nested case-control study to assess the association between single nucleotide polymorphism (SNP) and the persistence of HPV 16 infection in men.

2. Methods

2.1. Study population

This genetic study is nested within the *HIM study*, the only multinational prospective study of the natural history of HPV infections in men [9,10]. The parent study consists of 4123 participants, 18–70 years (mean age of 33 ± 11 years) residing in Tampa, Florida, USA, Sao Paulo, Brazil and Cuernavaca, Mexico who were followed every 6 months for up to 7 years. A full description of the study procedures has been described previously [5,10]. However, only individuals from Florida and Brazil who consented to the genetic study and with at least one HPV 16-positive visit during the HIM study were included in this genetic study.

This pilot study included only a subset of individuals who were positive for HPV 16 at baseline or during follow-up. Based on the duration of their HPV 16 infections cases and controls were strictly defined: men with an HPV 16 infection that cleared within 18-months and were negative for HPV 16 at the end of the study were classified as "controls" and only men with an HPV 16 infection that persisted for 18-months or longer and were still positive at the end of the study were classified as "cases". This extreme phenotype sampling design, comparing the most severe phenotype with the mildest based on the continuous clearance time (within the spectrum of HPV clearance/persistence, only 10–15% of those infected have persistence infection and others clear relatively quickly over time), will reduce the number of individuals necessary to achieve high power relative to random sampling from the distribution for genetic discovery [11].

Written consent was obtained from all participants and the study protocols for the parent study were approved by institutional review boards (IRB) at all sponsoring organizations and conformed to human-experimentation guidelines set forth by the United States Department of Health and Human Services. The Human Subjects Committees of the University of South Florida (USF), the Ludwig Institute for Cancer Research, and the CRT-AIDS (Centro de Referência e Tratamento de Doenças Sexualmente Transmissíveis e AIDS), São Paulo, Brazil, approved study procedures for the parent study. The study protocol of the parent study and this genetic sub-study were reviewed and approved by the University of Alabama at Birmingham IRB (IRB-080619006).

2.2. Penile/scrotal sampling and HPV 16 detection

Baseline and longitudinal HPV status (every 6 months up to 7 years) are known for the men participating in the ongoing parent HIM study, as previously described [10]. By use of Dacron (Digene, Gaithersburg, MD, USA) swabs pre-wetted with saline, four separate specimens were obtained from the coronal sulcus, glans penis, penile shaft, and scrotum, and were combined and placed in 450 μL of Specimen Transport Medium as one sample. All specimens were stored at −70 °C until PCR analyses and genotyping were undertaken. The validity of these three anatomical sites in the assessment of HPV status and the high sampling reproducibility for the detection of HPV DNA by use of this method has been thoroughly established [12]. Further, DNA from these samples was extracted using the QIAamp DNA Mini Kit (QIAGen, Valencia, CA, USA) and HPV was tested in the DNA using PCR for target amplification of the HPV *L1* gene fragment. The PGMY09/11 L1 consensus primer system was used and genotyped with the Linear Array method, as previously described [5,10] (Roche Molecular Diagnostics, Alameda, CA, USA).

2.3. Genome-wide genotyping platform

The DNA was extracted from banked whole blood aliquots and genotyping of 737,742 autosomal SNPs was performed using the TxArray, with the Axiom Affymetric technology (Affymetrix CA) developed by the iGeneTRAIN consortium. The 'TxArray' provides comprehensive, cost-effective and accurate coverage of HLA/MHC regional markers, SNPs around 600 transplantation-related loci, and known functional variants and pharmacogenomic markers [13]. While our goal was to conduct a hypothesis-generating pilot study, the array also provided opportunities to examine variants across *HLA*, *KIR*, and other non-HLA immune-related genes.

2.4. Data quality control (QC) and analysis

All SNPs were evaluated for standard QC thresholds. Individuals with inconsistent duplicates were removed from the analysis based on the monomorphic, minor allele frequency (MAF). Of the 737,742 SNPs examined, SNPs based on both Hardy Weinberg Equilibrium (< 0.01) and MAF (< 0.01) were excluded, resulting in 605,885 SNPs included in the final analysis.

To test for potential confounding effects of population stratification in our study cohort, principal component (PC) analysis was performed. Self-reported race (US white, US black, and Brazilian white) was confirmed using PC-based clustering analysis. Logistic regression analysis was used to estimate per-allele odds ratios (OR) and 95% confidence intervals (95% CI) for the association of each SNP using an additive model. All models were adjusted for age at first HPV-positive visit, smoking status (never vs former, current), number of male anal sex partners, number of female sex partners, and four principal components (ancestral background based on genetic data). Quantile-quantile (Q-Q) plots of p-values were constructed to evaluate deviations from the expected test statistic distribution. Tx-array Chip-based Manhattan plots were generated to visualize the results.

3. Results

Of the 191 HPV infected men included in the genetic study, 40 had HPV infections that persisted for at least 18 months and were still positive at the end of the study. In contrast, 151 had HPV infections that did not persist for at least 18 months and were negative at the end of the study. The basic demographics and characteristics of the participants are described in Table 1. The Manhattan plot (Fig. 1) summarizes the results from the association of P-values of SNPs in the TxArray (all shown in Supplement Table 1). The Q–Q plot showed no evidence of systematic bias in the results from the statistical routines employed ($\lambda = 0.913$). Table 2 reports the top-hit SNPs based on their highest significant p-values. Of the 605,885 SNPs that passed the QC criteria we described above, the strongest association with genital HPV 16 persistence was observed for rs1293153 ($p < 5.72 \times 10^{-6}$) and rs405103 and 15 ($p < 5.89 \times 10^{-6}$) in chromosomes 20 and 15, respectively, after adjusting for age, smoking status, number of male anal sex partners, number of female sex partners, and four principal components (adjusting for ancestral background). A few other SNPs in *SLC12A6, KCC3, DCC,* and *CSMD1* genes (Table 2) seem interesting, as these genes may have relevant biology reported in HPV-associated cancers.

Table 1
Demographic characteristics of men who cleared or persisted HPV infection.

	Cases (n = 40) (persisted for at least 18 months)	Controls (n = 151) (Cleared within 18 months)	p-value
Age in years, mean (std)	30.875 ± 3.1	31.4 ± 1.8	0.69
Female sex partners, mean (std)	3.0 ± 0.85	2.6 ± 0.97	0.02
Male anal sex partner, mean (std)	0.20 ± 0.52	0.17 ± 0.49	0.65
PCA-based Race, n (%)			0.71
Brazilian White	15 (37.5)	62 (41.1)	
US White	21 (52.5)	69 (45.7)	
US Black	4 (10.0)	20 (13.2)	
Smoking, n (%)			0.17
Never	21 (52.5)	58 (38.4)	
Past	7 (17.5)	46 (30.5)	
Current	12 (30.0)	47 (31.1)	

4. Discussion

To our knowledge, this is the first study to investigate differences in inherited DNA variations of host genes on HPV 16 clearance/persistence in men. Specifically, two SNPs, rs1293153 and rs405103 in chromosomes 20 and 15 respectively (Table 2), had the statistically strongest association with clearance/persistence of HPV 16 infection in men. However, these novel SNPs and their associated genes or gene regions are not well-characterized. Although we are still unsure about the potential differences in host biology and immunology between men and women and their possible mechanism in HPV natural history and pathogenesis, some SNPs in this study, interestingly, are associated with HPV persistence. However, the site-specific nature of HPV infections and the epithelial cell differences between men and women limit an exact analogous comparison of HPV pathogenesis. On the other hand, this discrepancy could potentially help explain the lack of genetic similarities between men and women for their respective HPV infection outcomes.

Even though our top hits surpass the multiple correction thresholds of p-values, we acknowledge that samples size is a concern and limitation of the study; however, the identification of "extreme" cases of HPV persistence provides efficiency and power to the study design. We were, therefore, rigorous in our definition of HPV clearance, HPV infection follow-up, and QC thresholds for the SNPs, which accounts for potential confounders and actually provides robustness to our study, increasing power to detect a plausible association, despite small sample size. Among the other top hit variants (Table 2), one was a missense in *DBX2* gene, though no clear relation of HPV related cancer is known, and most were in intron or intergenic regions with unknown function. Although the findings consistent with the HPV or cancer literature require cautious interpretations, they are noteworthy for replication in future studies. For instance, a SNP rs7176426, an intronic variant in the *SLC12A6*, also known as *KCl cotransporter 3 (KCC3)* gene, was associated with clearance of HPV 16 in our study. The *KCC3* gene has been

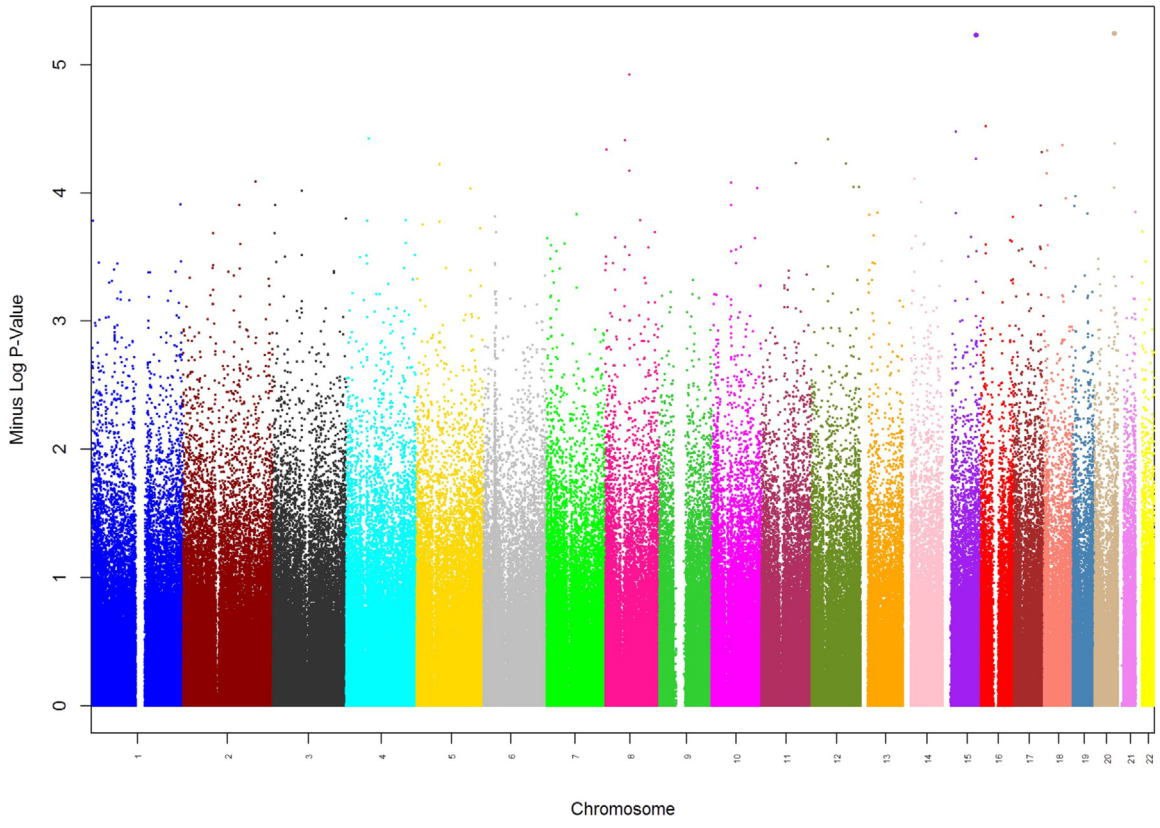

Fig. 1. The Manhattan plot of single nucleotide polymorphisms (SNPs) in the TxArray associated with HPV 16 persistence (> 18 months) in men. The x-axis is the genomic position of each SNP grouped by different chromosome, which is represented by different colors. The y-axis is the p-value for each association test for each SNP, in $-\log 10$ scale, $-\log_{10}$(p-value), using an additive genetic model in logistic regression.

Table 2
Top single nucleotide polymorphisms (SNPs) and gene regions associated with persistence (> 18 months) HPV 16 infection in men after controlling for age, smoking status, number of female sex partners, number of male anal sex partners, and four principal components of ancestry.

SNP	Genomic Position	Gene	beta-value	p-value	Variant type
rs1293153	20:52951047	–	1.617	5.72E − 06	Intergenic
rs405103	15:89617230	*LOC102724566*	2.221	5.89E − 06	Intron
rs7001081	8:64685833	*LINC01289*	− 1.745	1.20E − 05	Intron
rs9924993	16:13639660	*SHISA9*	1.626	3.02E − 05	Intron (lincRNA)
rs7176426	15:34614257	*SLC12A6*	1.695	3.32E − 05	Intron
rs34563630	4:61849734	–	1.784	3.79E − 05	Intergenic
rs2731038	12:45417666	*DBX2*	1.335	3.84E − 05	Missense
rs74961872	8:52968730	–	2.834	3.91E − 05	Intergenic
rs6069487	20:54515066	–	1.38	4.15E − 05	Intergenic
rs11874458	18:50572367	*DCC*	1.313	4.27E − 05	Intron
rs1482207	8:3503182	*CSMD1*	1.581	4.61E − 05	Intron
rs59943563	18:8266569	*PTPRM*	1.488	4.68E − 05	Intron

shown to be an independent prognostic factor in human esophageal squamous cell carcinoma (ESCC) [14]. Another SNP (rs11874458), is located in the intron of the known tumor suppressor *Deleted in Colorectal Cancer* (*DCC)* gene, which has also been shown to be a potential marker for detection of cervical pre-cancer and cancer [15]. Similarly, the variant rs1482207 is in the intron of *CSMD1* gene, which has been suggested to act as a tumor suppressor and frequently deleted in many types of cancers including oropharyngeal squamous cell carcinoma, oral, ovarian, breast, prostate, liver, lung, skin, head and neck cancers, some of which are related to HPV infection [16].

As our study population consists mainly of white men, our results may not be fully applicable to other racial/ethnic population groups. However, because of the genetic basis of this study and principle component adjustments, this might not be a large factor in determining representability. Furthermore, the Txarray may not cover all relevant genes and SNPs but provides direction and hypothesis for future large studies. Of note, there are not many cohorts studying prospective HPV infection in men and thus conducting similar studies may be a challenge. Also, we only assessed the association of persistent HPV 16 infection and whether this association, even if true positive, is specific to HPV 16 or generalizable to other HPV types, especially other high-risk HPV types would be interesting to examine. Nevertheless, HPV in men is a major public health concern, especially given currently there are no HPV screening guidelines in men. In conclusion, our findings suggest that these results provide preliminary evidence for understanding the biological mechanism of oncogenic HPV 16 pathogenesis in men, which require further investigation.

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Conflicts of interest

A.R.G. and L.L.V. are members of the Merck Advisory Board. S.L.S. received a grant (IISP53280) from the Merck Investigator Initiated Studies Program. No conflicts of interest were declared for any of the remaining authors.

This information has not previously been presented or published anywhere.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pvr.2018.08.001.

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