Human Papillomavirus Genotypes in Invasive Cervical Carcinoma in HIV-Seropositive and HIV-Seronegative Women in Zimbabwe.

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HPV genera and species

- According to the most recent ICTV classification, the *Papillomaviridae* family includes two subfamilies *Firstpapilomavirinae* with more than 52 genera and *Secondpapillomavirinae* with one genus and one species”(Van Doorslaer et al., 2018) and a total of 133 species.

- Over 200 human papillomavirus (HPV) genotypes have been described with most falling into the Alpha, Beta and Gamma genera.

- HPVs associated with malignant anogenital cancers are found in the Alpha-PV genus.

- Very little has been published on Beta and Gamma-PVs in genital infections and almost nothing is known about these viruses in Africa. There is no published information on genital Beta- and Gamma-PVs in South Africans.
Background HPV Biology

- Papillomaviridae family
- Non-enveloped virus
  - Double stranded circular DNA virus.
  - Genome is approximately 8kb
  - Typically it has 8 ORFs the early genes are for replication and transcription and the late are structural proteins.
- Establish productive infections in epithelial cells of the skin (cutaneous types) or mucous membranes (mucosotropic)

BACKGROUND:

• Invasive cervical carcinoma (ICC) accounts for 23% of all cancer-related deaths in Zimbabwean women. Trials for a national program of genotype-specific human papillomavirus (HPV) vaccines are underway to prevent cervical carcinoma, but the distribution of HPV types among women with ICC according to HIV status is unknown.
METHODS:

To determine prevalence and distribution of high-risk HPV genotypes by HIV status in women with ICC.

We performed a cross-sectional study on women referred for ICC testing at 4 urban referral hospitals in Zimbabwe from June 2014 to December 2015.

Cervical biopsies were obtained for histology and HPV genotyping. HIV serology testing was performed. HPV testing was performed using MY09/MY11 polymerase chain reaction followed by typing using dot-blot hybridization.
RESULTS:

• Of 107 participants with histologically proven ICC, HIV prevalence was 49.5% (53/107). HIV-positive women tended to be younger (median age 44 years) than HIV-negative women (median age 59 years).

• HPV prevalence was 94% (101/107), ranging from 1 to 5 genotypes per participant.

• HPV 16 (81.5%), 18 (24%), 33 (13%), 35 (11%), 56 (9%), and 45 (7.4%) were the most prevalent genotypes among HIV-negative participants; HPV 16 (67.9%), 18 (43.4%), 56 (18.9%), 45 (15.1%), 33 (11.3%), and 58 (9.4%) were the most prevalent among HIV-positive participants. Eighty-three percent of women were infected with either HPV-16 or HPV-18.
CONCLUSIONS:

- Effective vaccination programs against HPV 16 and HPV 18 could prevent up to 83% of cases of cervical cancer in Zimbabwe. HIV may influence distribution of some HPV genotypes given the significant increase in prevalence of HPV 18 among HIV-positive participants.
Human Papillomavirus Genotypes in Invasive Cervical Carcinoma in HIV-Seropositive and HIV-Seronegative Women in Zimbabwe

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Altini Mario, BDS, MDent, DSc (Medicine), FCPATH (SA) Oral*

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Conclusions: Effective vaccination programs against HPV 16 and HPV 18 could prevent up to 83% of cases of cervical cancer in Zimbabwe. HIV may influence distribution of some HPV genotypes given the significant increase in prevalence of HPV 18 among HIV-positive participants.

Key Words: cervical cancer, HPV, HIV, Zimbabwe, dot-blot hybridization

(J Acquir Immune Defic Syndr 2018;79:e1–e6)
CHARACTERISATION AND EVOLUTIONARY DYNAMICS OF TEN NOVEL GAMMAPAPILLOMAVIRUS TYPES FROM SOUTH AFRICAN PENILE SWABS

Alltalents Tutsirayi Murahwa
PhD Medical Virology (UCT), MPhil (MED) Immunology (UZ), Hons BMLS (UZ)
Laboratory workflow

1. **HPV Circular genome**
   - Linearised 8kb HPV genome
   - Kapa long range PCR
   - Touch down approach

2. **Gel purification**
   - 1kb

3. **pGEM T easy vector/Topo XL vector**

4. **Sanger Sequencing**

5. **Illumina sequencing**
   - MiSeq
     - Up to 2 x 300 bp reads
     - 15 million single reads
     - 2.7 days run time

6. **Plasmid purifications (lanes 1-12, A, B) run on 0.8% agarose gel. The NEB 1kb DNA ladder (L) and the non-recombinant vector (3.5kb p CR-XL-TOPO vector) were included (V).**
Discovery, characterisation and genomic variation of six novel *Gammapapillomavirus* types from penile swabs in South Africa

Alltalents T. Murahwa<sup>a,b,1</sup>, Tracy L. Meiring<sup>a,b,1</sup>, Zizipho Z.A. Mbulawa<sup>a,b,c,4</sup>, Anna-Lise Williamson<sup>a,b,d,4</sup>

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<sup>d</sup>SAMRC Gynaecological Cancer Research Centre, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

**Abstract**

Six novel human papillomaviruses from penile swabs were characterised. Multiple full genome clones for each novel type were generated, and complete genome sizes were: HPV211 (7253bp), HPV212 (7208bp), HPV213 (7096bp), HPV214 (7357), HPV215 (7186bp) and HPV216 (7233bp). Phylogenetically the novel papillomaviruses all clustered with *Gammapapillomavirus*: HPV211 is most closely related to HPV168 (72% identity in the L1 nucleotide sequence) of the Gamma-8 species, HPV212 is most closely related to HPV144 (82.9%) of the Gamma-17 species, HPV213 is most closely related to HPV153 (71.8%) of the Gamma-13 species, HPV214 is most closely related to HPV103 (75.3%) of the Gamma-6 species, HPV215 and HPV216 are most closely related to HPV129 (76.8% and 79.2% respectively) of the Gamma-9 species. The novel HPV types demonstrated the classical genomic organisation of *Gammapapillomavirus*, with seven open reading frames (ORFs) encoding five early (E1, E2, E4, E6 and E7) and two late (L1 and L2) proteins. Typical of *Gammapapillomavirus* the novel types all lacked the E5 ORF and HPV214 also lacked the E6 ORF. HPV212 had nine unique variants, HPV213 had five and HPV215 had four variants. Conserved domains observed among the novel types are the Zinc finger Binding Domain and PDZ domains. A retinoblastoma binding domain (pRB) binding domain in E7 protein was additionally identified in HPV214. This study expands the knowledge of the rapidly growing *Gammapapillomavirus* genus.
Complete Genome Sequences of Four Novel Human
Gammapapillomavirus Types, HPV-219, HPV-220, HPV-221,
and HPV-222, Isolated from Penile Skin Swabs from South
African Men

© Alltalents T. Murahwa, Tracy L. Meiring, Zizipho Z. A. Mbulawa, Anna-Lise Williamson

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ABSTRACT Four novel human gammapapillomaviruses were characterized from penile specimens using genome amplification, cloning, and sequencing. The HPV-219 L1 gene showed 87% nucleotide identity to that of HPV-213 of species gamma-13, HPV-220 had 72% identity to L1 of HPV-212 (gamma-17), HPV-221 had 80% identity to L1 of HPV-142 (gamma-10), and HPV-222 had 73% nucleotide identity to L1 of HPV-162 (gamma-19).
## Human Reference clones

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Showing 1 to 10 of 10 entries (filtered from 220 total entries)
Evolutionary dynamics of ten novel Gamma-PVs: insights from phylogenetic incongruence, recombination and phylodynamic analyses

Altaltens T. Murahwa1,2, Fredrick Nindo3, Harris Onyweru1,2, Tracy L. Meiring1,2, Darren P. Martin3,4 and Arna-Lise Williamson1,2

Abstract

Background: Human papillomaviruses (HPVs) are genetically diverse, belonging to five distinct genera: Alpha, Beta, Gamma, Mu and Nu. All papillomaviruses have double stranded DNA genomes that are thought to evolve slowly because they co-opt high-fidelity host cellular DNA polymerases for their replication. Despite extensive efforts to catalogue all the HPV species that infect humans, it is likely that many still remain undiscovered. Here we use the sequences of ten novel Gammapapillomaviruses (Gamma-PVs) characterized in previous studies and related HPVs to analyse the evolutionary dynamics of these viruses at the whole genome and individual gene scales.

Results: We found statistically significant incongruences between the phylogenetic trees of different genes which imply gene-to-gene variation in the evolutionary processes underlying the diversification of Gamma-PVs. We were, however, only able to detect convincing evidence of a single recombination event which, on its own, cannot explain the observed incongruences between gene phylogenies. The divergence times of the last common ancestor (LCA) of the Alpha, Beta, Mu, Nu and Gamma genera was predicted to have existed between 49.7–53.5 million years ago, before splitting into the five main lineages. The LCA of the Gamma-PVs at this time was predicted to have existed between 45.3 and 67.5 million years ago: approximately at the time when the simian and tarsier lineages of the primates diverged.

Conclusion: Consequently, we report here phylogenetic tree incongruence without strong evidence of recombination.

Keywords: Human papillomavirus, Gamma-PVs, Most recent common ancestor, Phylogenetic incongruence, Recombination, Molecular divergence
Global scenario of PV evolution

Crown-group | Characteristics of the Phenotype
---|---
Alpha-Omikron | -Essentially mucosal PVs -Productive infections -Some of them carcinogenic potential (e.g. HPV16 and HPV18)
Beta-Xi | -Cutaneous PVs -Mainly asymptomatic -Some of them proliferative infections (e.g. HPV4)
Delta-Zeta | -Cutaneous PVs -Productive infections -Some of them carcinogenic potential (e.g. EcPV2)
Lambda-Mu | -Mucosal and cutaneous PVs -Productive and asymptomatic infections

Mya: 300, 250, 200, 150, 100, 50, 0
Paleozoic | Mesozoic | Cenozoic
First amniotes (lay eggs) | Mammals evolve (hair and sweat glands) | Crown radiation of placental mammals
Frequency of *Betapapillomavirus* Infections Among HIV Infected and Uninfected Black Zimbabweans With Cutaneous Lesions

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Human papillomavirus (HPV) types from the *Betapapillomavirus* (β-HPV) genus are plentiful in non-melanoma skin cancers and warts among Caucasians, but there is paucity of information among black Africans. To determine the frequency of β-HPV genotypes in cutaneous infections among Black Zimbabweans, a cross-sectional study was carried out in which blood samples and skin biopsies were collected from patients infected and uninfected with HIV attending a referral hospital. We included 144 participants (72 infected and 72 uninfected with HIV) with clinically apparent cutaneous warts (n = 34), suspected non-melanoma skin cancers (n = 58) and Kaposi sarcoma (KS) (n = 18). The skin biopsies were analyzed for HPV DNA presence and type. β-HPV DNA was identified in

**KEY WORDS:** human papillomavirus; non-melanoma skin cancers; cutaneous warts; Kaposi sarcoma

**INTRODUCTION**

Human papillomavirus (HPV) is the most commonly implicated virus in many human malignancies with 5.2% of all cancers being attributable to HPV infection [Parkin and Bray, 2006]. The World Health Organization estimated that about 9–13% (200 million) of the world population has an HPV infection [Paglions, 2001]. The involvement of HPV in cancer of the penile, oral, genital, and oropharyngeal cancers and cutaneous lesions such as skin warts, squamous cell carcinoma, and basal cell carcinoma has been
Presence of *Betapapillomavirus* in Kaposi Sarcoma Lesions

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Human herpes virus 8 (HHV 8) is recognized as the necessary cause of Kaposi sarcoma (KS) and in the recent past the human papillomavirus (HPV) has been linked to the development of cutaneous basal cell and squamous cell carcinomas. In a cross sectional study investigating Beta HPV infections in skin lesions, an unexpected occurrence of HPV DNA was found in KS lesions of HIV infected individuals. Of the 18 KS cases included in the study 16 (89%) had HPV DNA detected. The most common *Betapapillomavirus* types were HPV14 [15 cases (83.3%)], HPV12 [8 cases (44.4%)], and HPV24 [7 cases (39%)]. Multiple Beta HPV types were detected in 10 (62.5%) of the participants with HPV DNA positive lesions; of these 7 had a CD4+ count below 350 cells/µl and 3 had CD4+ count between 350 cells/µl. The occurrence of occurrence of KS is significantly associated with HIV infection, several studies have shown increased incidence of KS among HIV infected individuals and the synergetic interactions between these two viruses [Cattelan et al., 2001; Newton et al., 2006; Sullivan et al., 2008].

HPV is the most commonly implicated virus in many human malignancies, 5.2% of all cancers are attributable to HPV infection [Parkin and Bray, 2006]. The involvement of HPV in cervical, penile, oral, genital, and laryngeal cancers and cutaneous lesions such as skin warts, squamous cell carcinomas (SCC), and basal cell carcinomas (BCC) has been documented extensively [Alba and Cararach, 2009].

There is an increasing body of evidence linking HPV to non-melanoma skin cancers [Plasmeijer et al., 2011; Viariset al., 2011]. Several molecular epidemiological studies have documented the role of HPV in KS.
Conclusion

• Discovery of more HPV viruses will enable a better understanding of both prophylactic and therapeutic strategies.

• Embracing next generation sequencing technologies is key to this goal.