# HPV Types in Recurrent Respiratory Papillomatosis


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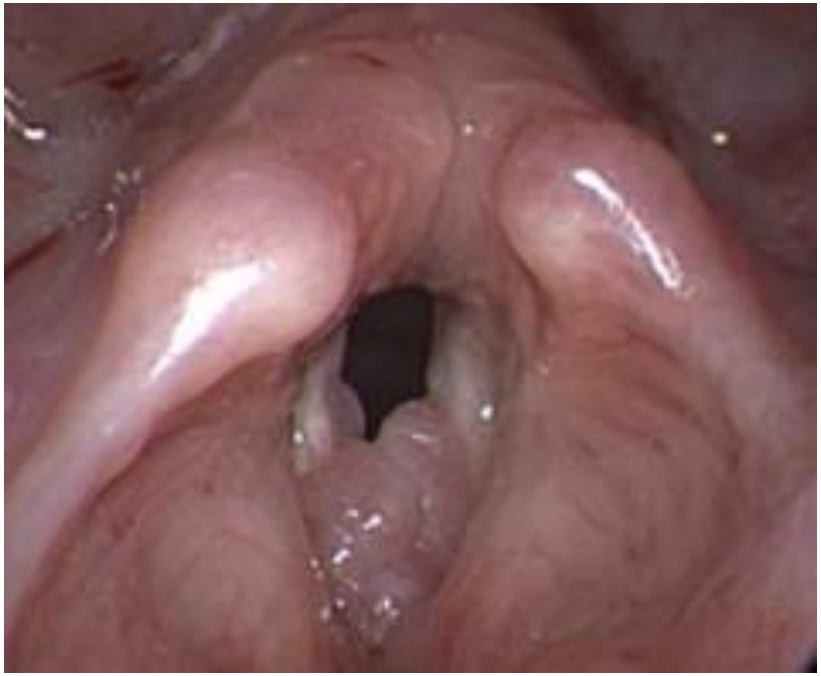
# RECURRENT RESPIRATORY PAPILLOMATOSIS

- Papillomas of the respiratory tract
- Most common benign tumour of the larynx
- Caused by Human Papilloma Virus





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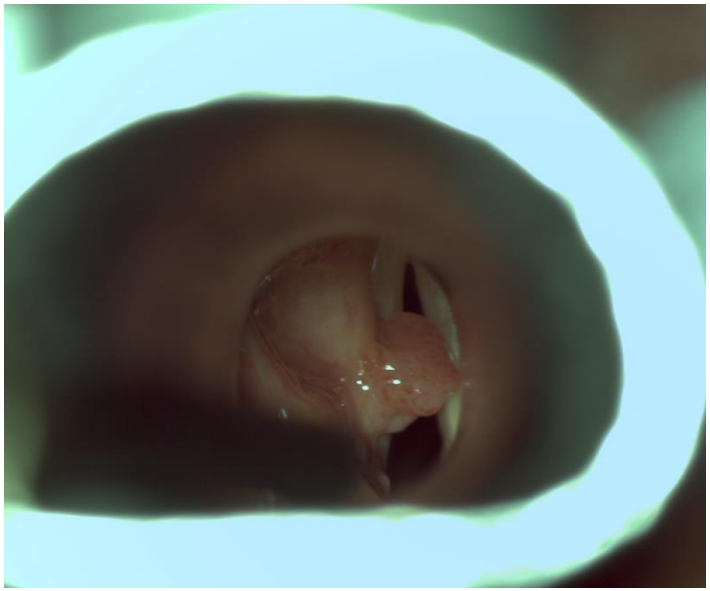
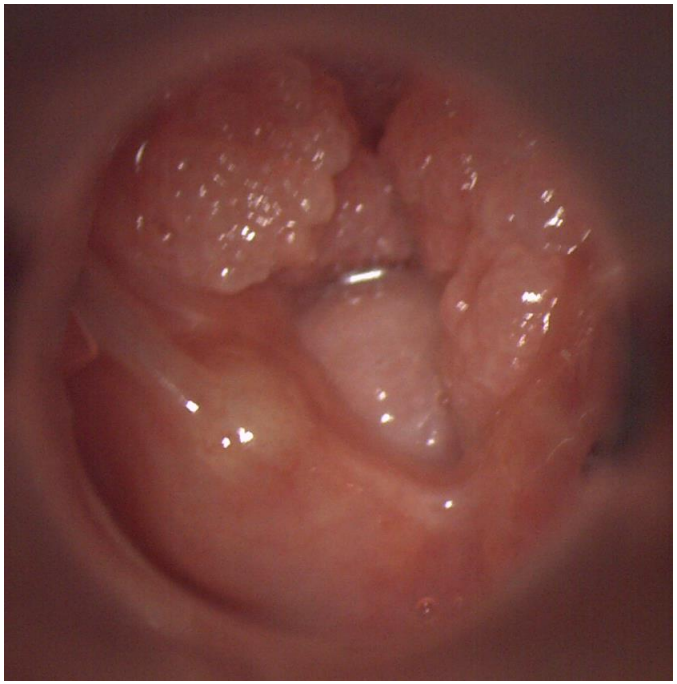
# RECURRENT RESPIRATORY PAPILLOMATOSIS

- Clinical presentation



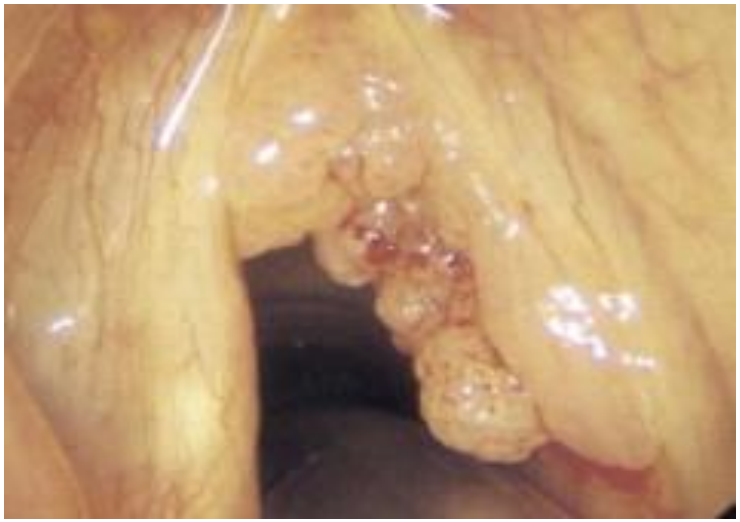


# RECURRENT RESPIRATORY PAPILLOMATOSIS

- Clinical presentation

- Age at presentation
  - Juvenile-onset RRP
  - Adult-onset RRP

- Rare disease





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# The incidence and prevalence of juvenile-onset recurrent respiratory papillomatosis in the Free State province of South Africa and Lesotho

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ABSTRACT

*Background and objective:* : Although the estimated incidence and prevalence of juvenile-onset recurrent respiratory papillomatosis (JORRP) has been determined in countries in North America and Europe and in Australia, no studies have attempted to determine the incidence or prevalence of JORRP in African countries. The aim of this study is to determine the incidence and prevalence of JORRP in the Free State province of South Africa and Lesotho.

*Methods:* This was a retrospective study in which the records of all patients with JORRP from the Free State province of South Africa or Lesotho treated at Universitas Academic Hospital or by otorhinolaryngologists in private practice between 1 January 2011 and 31 December 2013 were reviewed.

*Results:* The estimated incidence and prevalence of JORRP in the Free State were 1.34 and 3.88 per 100,000 population respectively while the estimated incidence and prevalence in Lesotho were 0.49 and 1.04 per 100,000 population respectively. However, these figures are probably an underestimation.

*Conclusion:* The incidence and prevalence calculated for the Free State were generally higher than those found in other studies, while those calculated for Lesotho was similar to those obtained in other studies.



## 1. Introduction

Juvenile-onset recurrent respiratory papillomatosis (JORRP) is a rare condition characterized by recurrent papillomas of the respiratory tract, mainly the larynx, caused by human papillomavirus (HPV), most commonly HPV-6 and HPV-11 [1]. The infection is believed to be acquired from the mother's genital tract during the birthing process [1]. Although the estimated incidence and prevalence of JORRP has been determined in countries in North America and Europe and in Australia [2–10], there have not been any studies that have attempted to determine the incidence or prevalence of JORRP in African countries.

The aim of this study was to determine the incidence and prevalence of JORRP in the Free State province of South Africa and Lesotho.

## 2. Materials and methods

The Free State province of South Africa has a total population of approximately 2.75 million people [11]. Universitas Academic Hospital is the referral centre for the Free State. As it is the only state hospital in the province with an ENT department, all patients seen in the public sector with JORRP in the province are managed at this hospital.

Lesotho is a land-locked country surrounded by South Africa with a total population of approximately 1.9 million people [12]. All patients with JORRP from Lesotho are referred from the Queen Mamohato Memorial Hospital in Maseru to Universitas Academic Hospital for management as the expertise required to manage these patients is not available in Lesotho.

This was a retrospective descriptive study. The records of all patients under the age of fifteen years with histologically-confirmed JORRP who underwent surgery at Universitas Academic Hospital between 1 January 2011 and 31 December 2013 were reviewed and the patients' demographic details, date of diagnosis, place of residence and dates of surgery were recorded. All specialist otorhinolaryngologists in private practice in the Free State were contacted to determine if they had managed any patients with JORRP in this time period and the patients' date of birth, sex, date of diagnosis, place of residence and dates of surgery were obtained from them. Only patients in whom histopathologic confirmation of the diagnosis was available were included. The dates of birth and sex were used to ensure that patients were not included more than once in the study. There are no otorhinolaryngologists in private

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# JORRP INCIDENCE

| Region | Year(s) | Age Range | Incidence (per 100000 population) |
|---|---|---|---|
| Denmark[1] | 1974-1993 | 0-14 years | 0.35 |
| Copenhagen[2] | 1980-1983 | 0-14 years | 0.6 |
| Funen and Jutland, Denmark[3] | 1969-1984 | 0-20 years | 0.38 |
| Norway[4] | 1987-2009 | 0-17 years | 0.17 |
| Atlanta and Seattle[5] | 1996 | 0-18 years | 0.12-2.13 |
| USA[6] | 1993 | 0-14 years | 4.3 |
| USA[7] | 2006 | 0-17 years | 0.51 (Private)<br>1.03 (Public) |
| Canada[8] | 1994-2007 | 0-14 years | 0.24 |
| Free State[9] | 2011-2013 | 0-14 years | 0.52-2.23 |
| Lesotho[9] | 2011-2013 | 0-14 years | 0.30-0.59 |

1.Silverberg 2004 2.Bomholt 1987 3.Lindeberg 1990 4.Omland 2012 5.Armstrong 2000 6.Derkay 1995 7.Marsico 2014 8.Campisi 2010 9.Seedat 2014



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# JORRP PREVALENCE

| Region | Year(s) | Age Range | Prevalence (per 100000 population/year) |
|---|---|---|---|
| Copenhagen[1] | 1980-1983 | 0-14 years | 0.8 |
| Atlanta and Seattle[2] | 1996 | 0-18 years | 1.00-3.97 |
| USA [3] | 2006 | 0-17 years | 1.45 (Private)<br>2.93 (Public) |
| Canada[4] | 1994-2007 | 0-14 years | 1.11 |
| Australia[5] | 1998-2008 | 0-20 years | 0.6-1.1 |
| Free State, South Africa[6] | 2011-2013 | 0-14 years | 3.10-4.60 |
| Lesotho[6] | 2011-2013 | 0-14 years | 1.04 |

1.Bomholt 1987 2.Armstrong 2000 3.Marsico 2014 4.Campisi 2010 5.Novakovic 2010 6.Seedat 2014



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# CLINICAL PRESENTATION - JORRP

- Mean – 5.1 year (0.4-15.5 years) (n=115)
- Mean age at onset of symptoms – 3.5 years (n=59)
- 69 (60%) male : 46 (40%) female

- 65.1% stridor
- 44.6% respiratory distress



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# CLINICAL COURSE - JORRP

- Active disease – 61.5%
- Remission – 35.4%
- Died of airway obstruction – 3.1%

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# HPV types causing juvenile recurrent laryngeal papillomatosis in South Africa

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ABSTRACT

*Objectives:* To identify human papillomavirus (HPV) types associated with juvenile onset recurrent laryngeal papillomatosis (RLP) in southern Africa, to determine if there is a correlation between HPV type and disease aggressiveness and to determine the diagnostic and prognostic value of rapid molecular techniques for detection and typing of HPV using laryngeal biopsies.

*Methods:* Laryngeal biopsies from patients undergoing surgery for RLP were screened for HPV using conventional and real-time PCR techniques. Amplicons were sequenced to determine the HPV type involved. Clinical features were correlated with HPV type.

*Results:* HPV was identified in papillomata from 18 out of 19 patients. Only HPV-6 and HPV-11 were identified, with no co-infections. There was 100% concordance between conventional and real-time PCR techniques. Patients with HPV-11 disease required more procedures and tended to have higher Derkay scores than those with HPV-6 disease. The HPV types identified in our patients were genetically similar to HPV types from geographically distinct regions.

*Conclusions:* RLP in our patient population appears to be exclusively due to HPV-6 or HPV-11. HPV-11 disease appears to be more aggressive than HPV-6 disease. Identification of the HPV types provides motivation for inclusion of vaccines against these types in vaccination programs to protect women against infection and subsequently reduce the incidence of RLP.



## 1. Introduction

Recurrent laryngeal papillomatosis (RLP) is caused by human papillomavirus (HPV) infection of the larynx [1]. Over 100 HPV genotypes have been identified [1], of which HPV-6 and HPV-11 are the most frequent cause of RLP, although other types have also been implicated [2–12]. Most studies have found HPV-11 disease to be more aggressive than HPV-6 disease [2–5,13–16], although some have found no difference [6,7,17]. The only previous study on patients from South Africa suggested that HPV-6 disease was more aggressive [18].

Many patients with RLP presenting at our institution reside in rural areas with limited health care facilities. They travel long distances for treatment and frequently return to hospital only when surgical intervention is an emergency. A prognostic indicator for predicting disease aggressiveness would be a useful tool in our health care environment. If molecular typing of HPV could be used as an indicator of disease aggressiveness it would have an important diagnostic and prognostic role.

Polyvalent vaccines against HPV types associated with cervical cancer and genital warts are available [19]. Acquiring sufficient data on HPV genotypes associated with RLP could provide valuable motivation for vaccination programs to reduce these infections in mothers and ultimately possibly reduce the incidence of RLP in children [19].

## 2. Objectives

The aims of this study were to determine the HPV types in a cohort of patients with RLP in central South Africa, to investigate any potential association between HPV type and disease aggressiveness and to investigate the diagnostic and prognostic role of rapid molecular detection and identification of HPV type.

## 3. Methods

All patients with juvenile onset laryngeal papillomatosis (onset before the age of 16 years) undergoing surgery between January 2008 and September 2009 at Universitas Academic Hospital, a government training hospital in Bloemfontein, South Africa, were enrolled in the study. These patients reside in the Free State and

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# HPV TYPES

- Most studies only identified HPV6 and HPV11
  - Draganov et al. (2006)
  - Duggan et al. (1990)
  - Gabbott et al. (1997)
  - Hartley et al. (1994)
  - Hawkes et al. (2008)
  - Levi et al. (1989)
  - Seedat et al. (2010)
  - Padayachee and Prescott (1993)
  - Rabah et al. (2001)
  - Rimmel et al. (1997)
  - Smith et al. (1993)
  - Terry et al. (1987)
  - Bello de Alford (2001)
- Some studies identified other HPV types
  - Peñaloza-Plascencia et al. (2000) – Types 6, 11, 16, 31, 33, 35, 39
  - Nicollas et al. (2007) – HPV 6 and 16 co-infection
  - Pou et al. (1995) – HPV 16
  - Sanchez (2012) – HPV 16
  - Sakakura et al. (1996) – HPV 18
- HPV types and genomic variants remain stable over time[1]

1. Kocjan 2012



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# HPV TYPES

- HPV11 more aggressive than HPV6
  - Buchinsky et al. (2008)
  - Draganov et al. (2006)
  - Hartley et al. (1994)
  - Maloney et al. (2006)
  - Seedat et al. (2010)
  - Pou et al. (1995)
  - Rabah et al. (2001)
  - Shehata et al. (2008)
  - Wiatrak et al. (2004)
- HPV6 more aggressive than HPV11
  - Padayachee and Prescott (1993)
- No difference between HPV6 and HPV11
  - Abramson et al. (1987)
  - Peñaloza-Plascencia et al. (2000)
  - Gabbott et al. (1997)



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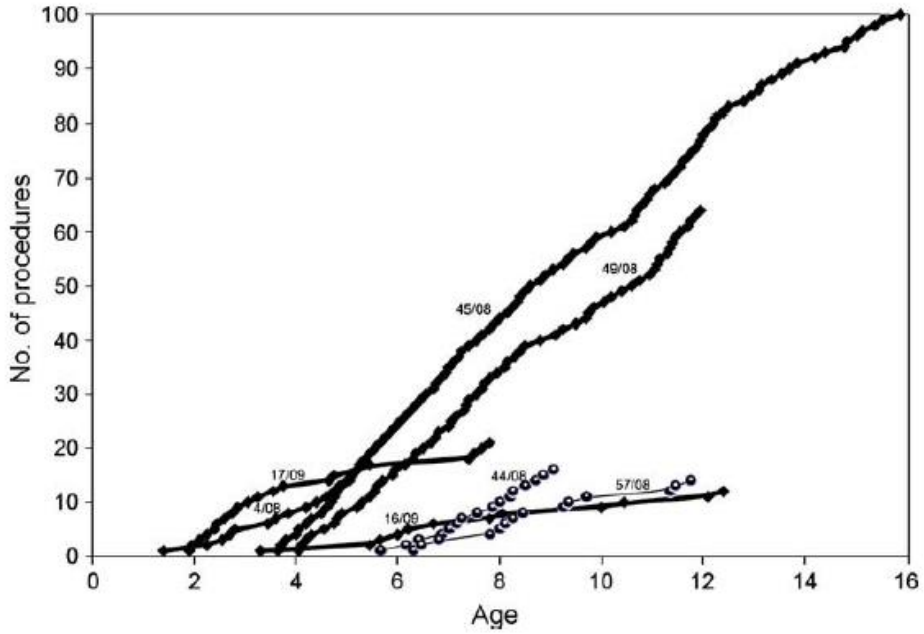
# HPV TYPES

- Padayachee and Prescott (1993)
  - 20 children
  - HPV6 – 5 patient (25%)
  - HPV11 – 15 patients (75%)
  - HPV6 infection more clinically aggressive than HPV11
  - No correlation between age and either aggression or prolonged clinical course

# HPV TYPES

- Seedat et al. (2010)
  - 18 patients
  - Patients with HPV-11 disease required significantly more surgical interventions



# RESULTS - JORRP

| | HPV6 | HPV11 | P-value |
|---|---|---|---|
| Patients | 51 | 42 | |
| Age at diagnosis | 5.7 | 4.2 | 0.010 |
| Total procedures | 8.4 | 19.8 | 0.016 |
| Average procedures/year | 5.3 | 10.8 | 0.006 |
| Average Derkay score | 16.2 | 23.9 | 0.0002 |
| Tracheostomy | 3 (5.9%) | 7 (16.7%) | |
| Pulmonary involvement | 0 | 2 (4.8%) | |

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# FRET-based detection and genotyping of HPV-6 and HPV-11 causing recurrent respiratory papillomatosis

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Real-time PCR
Recurrent respiratory papillomatosis (RRP)

ABSTRACT

Recurrent respiratory papillomatosis (RRP) is a potentially life-threatening disease caused by human papillomavirus (HPV), usually HPV types 6 and 11. The conventional method used for detection and typing the RRP isolates in our laboratory is the polymerase chain reaction (PCR) and DNA sequencing method. A real-time PCR assay based on fluorescence resonance energy transfer (FRET) probe technology was developed for the detection and rapid genotyping of HPV-6 and-11 isolates from biopsy material. The primers and probes were designed using multiple alignments of HPV-6 and HPV-11 partial E6 and E7 sequences that included prototypic and non-prototypic variants. Real-time PCR followed by probe-specific melting-curve analysis allowed differentiation of HPV-6 and HPV-11. HPV-6 and HPV-11 amplicons were used to determine detection limits and inter- and intra-assay variability. The detection limit of the assay was 12.8 DNA copies for HPV-6 and 22.5 DNA copies for HPV-11. A total of 60 isolates were genotyped using the FRET real-time PCR assay and a 100% concordance was obtained when results were compared with genotyping based on conventional DNA sequencing. The real-time PCR assay based on FRET technology was able to detect and rapidly genotype HPV from tissue biopsy obtained from patients with RRP. The assay reduces the time required for genotyping from three working days to less than a day.



## 1. Introduction

Human papillomaviruses (HPV) are a group of viruses which may cause warts on the cutaneous epithelium of the skin or cause cancer and warts in the mucosal epithelium of the anogenital and oral region of both men and women (de Villiers et al., 2004; Maver et al., 2010; Molijn et al., 2005). To date, more than 150 HPV types have been identified and fully characterized (Burk et al., 2011). These HPV types have been divided into low risk and high risk types according to their ability to induce malignancy (Bonagura et al., 2010; Draganov et al., 2006; Molijn et al., 2005; Seaman et al., 2010). Low risk HPV types include HPV-6 and HPV-11, which are primarily associated with genital warts and recurrent respiratory papillomatosis (RRP) (Bonagura et al., 2010; Burk et al., 2011; Garland et al., 2007; Kocjan et al., 2011; Maver et al., 2011).

Both HPV-6 and HPV-11 belong to the genus *Alphapapillomavirus*, species 10, of the *Papillomaviridae* family (Bernard et al., 2010; Burk et al., 2011; de Villiers et al., 2004). Based on the genetic relationship and the percentage differences of the nucleotide sequence of aligned complete genomes, Burk et al. classified HPV-6 into two lineages, lineage A and lineage B, and three sublineages, B1, B2 and B3 (Burk et al., 2011). Lineage A contains the prototype HPV-6b, while the non-prototypic variants HPV-6a and HPV-6vc belong to sublineages B3 and B1, respectively. HPV-11 has been classified into two sublineages, A1 and A2, with sublineage A1 containing the HPV-11 prototype (Burk et al., 2011).

RRP, predominantly caused by HPV-6 and HPV-11 (Derkay and Wiatrak, 2008; Goon et al., 2008; Larson and Derkay, 2010; Seedat et al., 2010) is a chronic disease characterized by benign exophytic, verrucous proliferation within the respiratory tract (Derkay and Wiatrak, 2008; Larson and Derkay, 2010; Wiatrak et al., 2004). RRP usually involves the larynx of the patient, but may also extend to the esophagus, trachea, nasal cavity and even the lungs (Derkay and Wiatrak, 2008; Draganov et al., 2006; Wiatrak et al., 2004). RRP has the potential to cause significant morbidity and episodic mortality due to complete airway obstruction or malignant transformation (Bonagura et al., 2010; Goon et al., 2008; Larson and Derkay, 2010; Wiatrak et al., 2004). The course of the disease is unpredictable. Patients may recover completely after the first presentation of the disease, while others may present with aggressive disease requiring multiple surgical debridement of the papillomas (Bonagura et al., 2010; Larson and Derkay, 2010; Wiatrak et al., 2004).

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# Novel HPV-6 variants of human papillomavirus causing recurrent respiratory papillomatosis in southern Africa

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## SUMMARY

There is currently no information regarding the genetic diversity of HPV-6 variants circulating in South Africa. The aim of this study was to determine the HPV-6 variants affecting patients with recurrent respiratory papillomatosis, to determine whether mutations correlate with disease severity and identify molecular determinants of virulence with prognostic relevance. HPV-6 variants were identified based on genome changes within the 712-991 bp region encompassing the non-coding region (URR) of the genome, with variations in length resulting from insertions and duplications, and the 453-bp gene encoding the E6 protein. Based on manual comparison of sequence data from the URR, the isolates were identified as HPV-6a and HPV-6vc variants. Three novel HPV-6 variants were identified: one based on a mutation in the E6 region; two based on changes in the URR including a unique substitution detected in three isolates and an insertion and 170-bp duplication in the URR genome in one patient, who had clinical features of severe disease.

**Key words:** Human papillomavirus (HPV), recurrent respiratory papillomatosis.

## INTRODUCTION

Papillomaviruses are members of the Papillomaviridae family [1]. To date, over 100 human papillomavirus (HPV) types have been identified [2]. The viral genome is an ~8000-bp molecule of double-stranded circular DNA [1]. The genomic organization comprises three main regions: early, late and the long control region (LCR) [3]. The early region encodes for the regulatory proteins (designated E1–E7) associated with replication, transcription and cell cycle control while the late region encodes for the structural proteins. The LCR, also referred to as the upstream regulatory region (URR), is a non-coding region that includes a variety of transcriptional regulatory motifs, the early promoter and viral coded E2 regulator binding sites. It extends from the termination of the L1 gene to the first methionine of the E6 gene [3].

Recurrent respiratory papillomatosis (RRP) is caused by HPV infection of the respiratory tract, usually the larynx. It is commonly associated with HPV types 6 and 11, with most studies finding HPV-11 disease to be more aggressive than HPV-6 disease [4–6]. Molecular characterization has indicated that HPV-6 isolates can be divided into three

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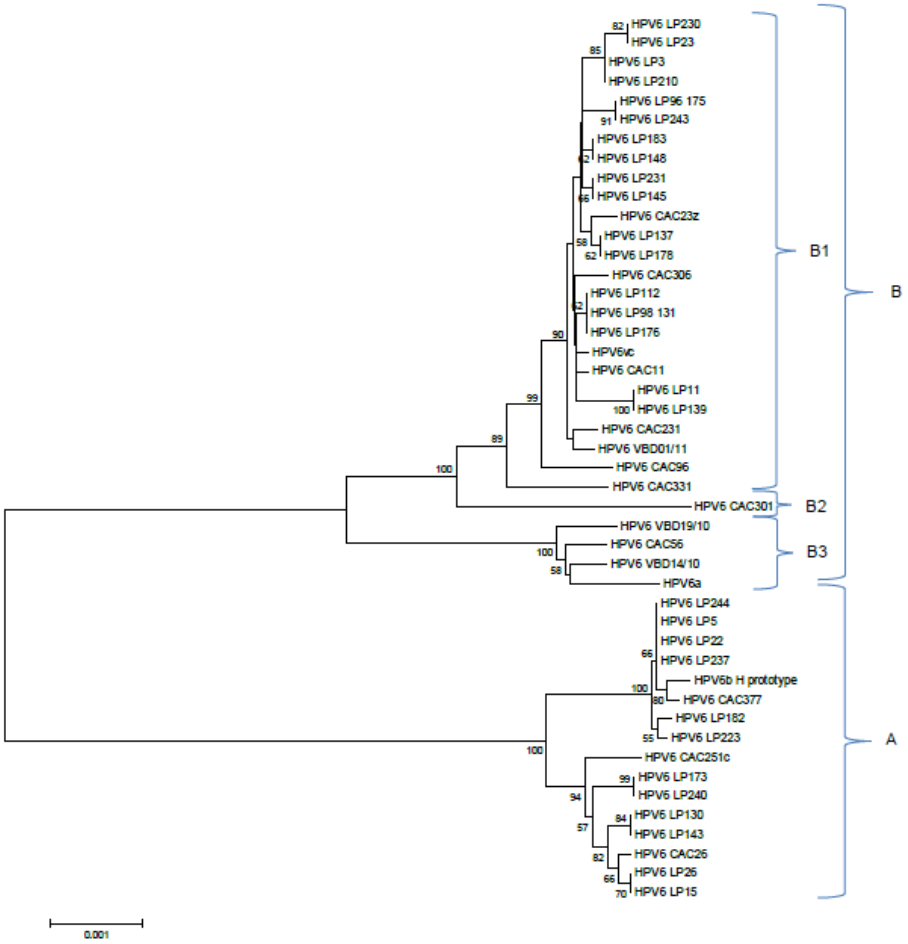
# HPV6 VARIANTS



# HPV6 VARIANTS

- 3 novel variants
  - 1 based on a mutation in the E6 region
  - 2 based on changes in the non-coding region
    - Insertion of a 170-bp duplication in the LCR genome in one patient

- In vitro studies using reporter genes suggest that duplication in LCR may influence expression of genes downstream such as E6



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# Global Genomic Diversity of Human Papillomavirus 6 Based on 724 Isolates and 190 Complete Genome Sequences

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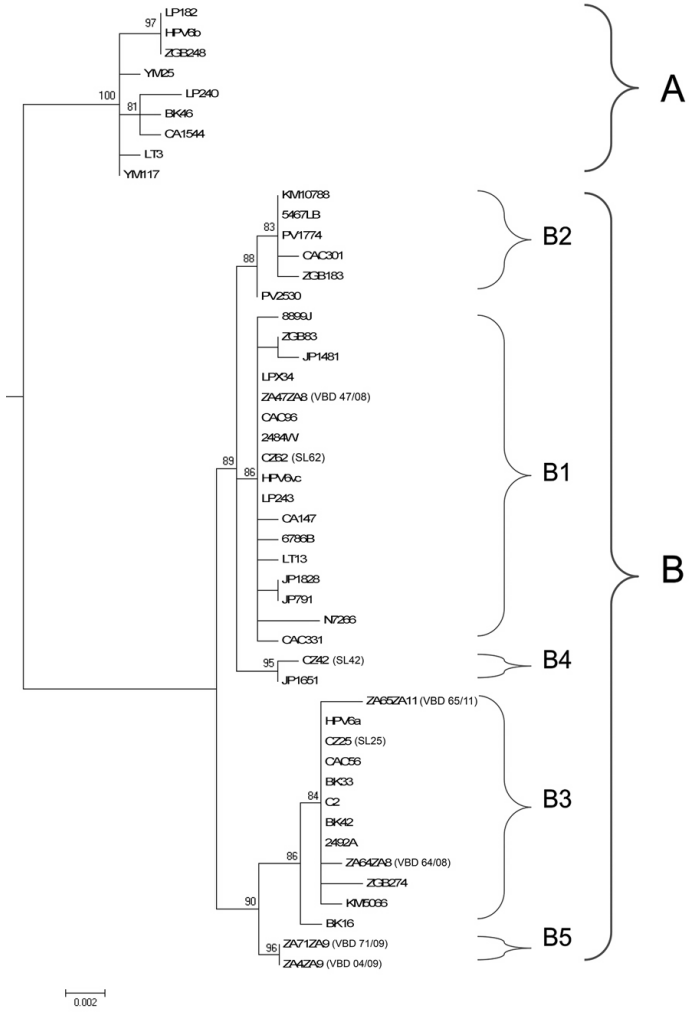
## ABSTRACT

**Human papillomavirus type 6 (HPV6) is the major etiological agent of anogenital warts and laryngeal papillomas and has been included in both the quadrivalent and nonavalent prophylactic HPV vaccines. This study investigated the global genomic diversity of HPV6, using 724 isolates and 190 complete genomes from six continents, and the association of HPV6 genomic variants with geographical location, anatomical site of infection/disease, and gender. Initially, a 2,800-bp E5a-E5b-L1-LCR fragment was sequenced from 492/530 (92.8%) HPV6-positive samples collected for this study. Among them, 130 exhibited at least one single nucleotide polymorphism (SNP), indel, or amino acid change in the E5a-E5b-L1-LCR fragment and were sequenced in full. A global alignment and maximum likelihood tree of 190 complete HPV6 genomes (130 fully sequenced in this study and 60 obtained from sequence repositories) revealed two variant lineages, A and B, and five B sublineages: B1, B2, B3, B4, and B5. HPV6 (sub)lineage-specific SNPs and a 960-bp representative region for whole-genome-based phylogenetic clustering within the L2 open reading frame were identified. Multivariate logistic regression analysis revealed that lineage B predominated globally. Sublineage B3 was more common in Africa and North and South America, and lineage A was more common in Asia. Sublineages B1 and B3 were associated with anogenital infections, indicating a potential lesion-specific predilection of some HPV6 sublineages. Females had higher odds for infection with sublineage B3 than males. In conclusion, a global HPV6 phylogenetic analysis revealed the existence of two variant lineages and five sublineages, showing some degree of ethnogeographic, gender, and/or disease predilection in their distribution.**

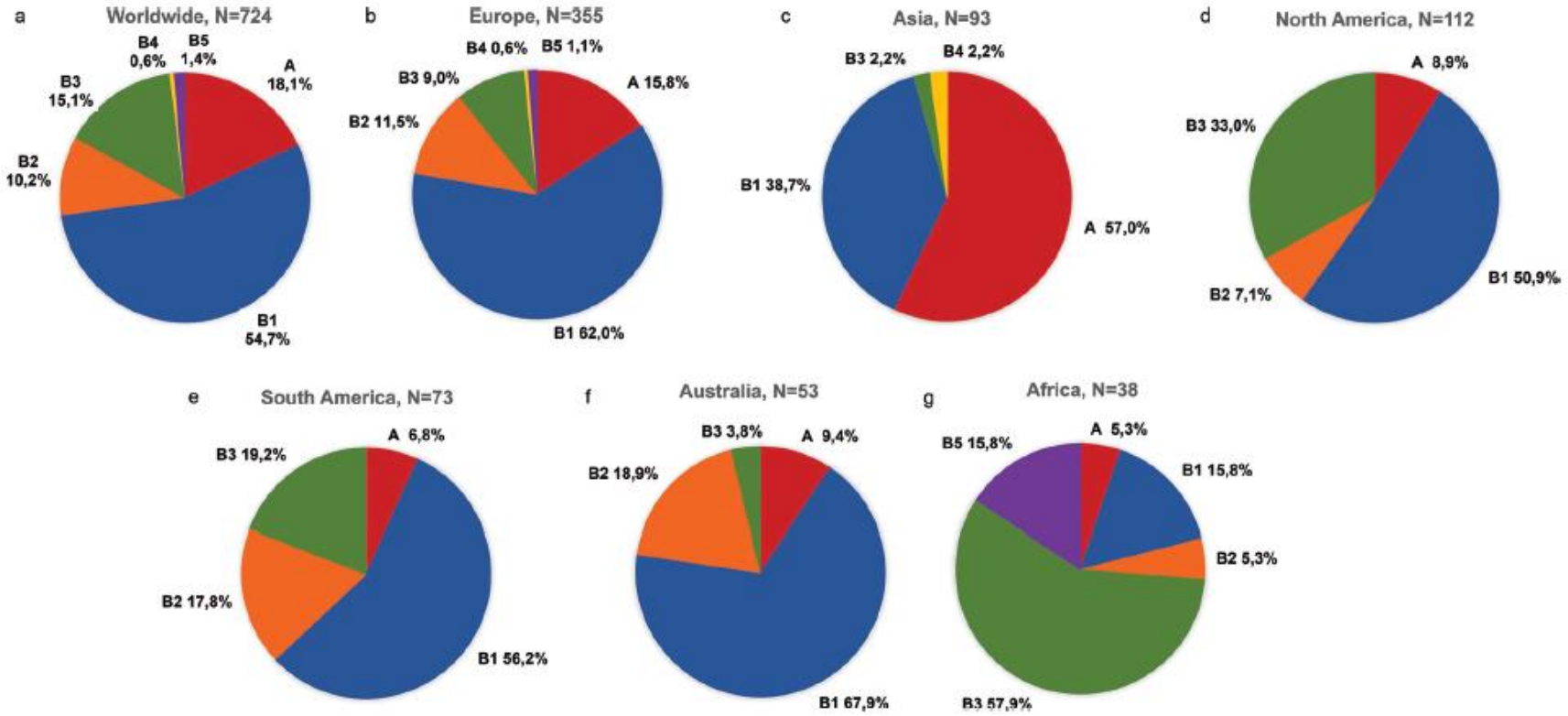
## IMPORTANCE

**This study established the largest database of globally circulating HPV6 genomic variants and contributed a total of 130 new, complete HPV6 genome sequences to available sequence repositories. Two HPV6 variant lineages and five sublineages were identified and showed some degree of association with geographical location, anatomical site of infection/disease, and/or gender. We additionally identified several HPV6 lineage- and sublineage-specific SNPs to facilitate the identification of HPV6 variants and determined a representative region within the L2 gene that is suitable for HPV6 whole-genome-based phylogenetic analysis. This study complements and significantly expands the current knowledge of HPV6 genetic diversity and forms a comprehensive basis for future epidemiological, evolutionary, functional, pathogenicity, vaccination, and molecular assay development studies.**

# HPV6



# HPV6



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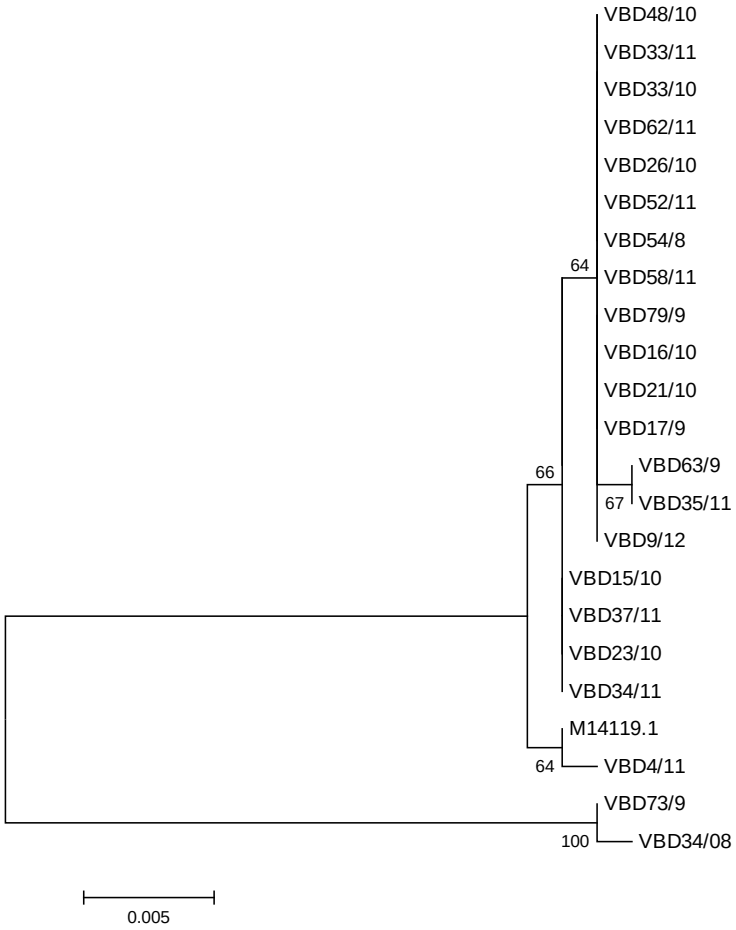
# HPV11



# LINEAR ARRAY

- Specimens from 63 JORRP patients
- 14 different HPV genotypes detected
  - 7 low-risk types – HPV6, HPV11 HPV55 (subtype of HPV44), HPV67, HPV69, HPV84, HPV89 (CP6108)
  - 6 high-risk types – HPV16, HPV31, HPV35, HPV45, HPV51, HPV82
  - 1 probable high-risk type – HPV66



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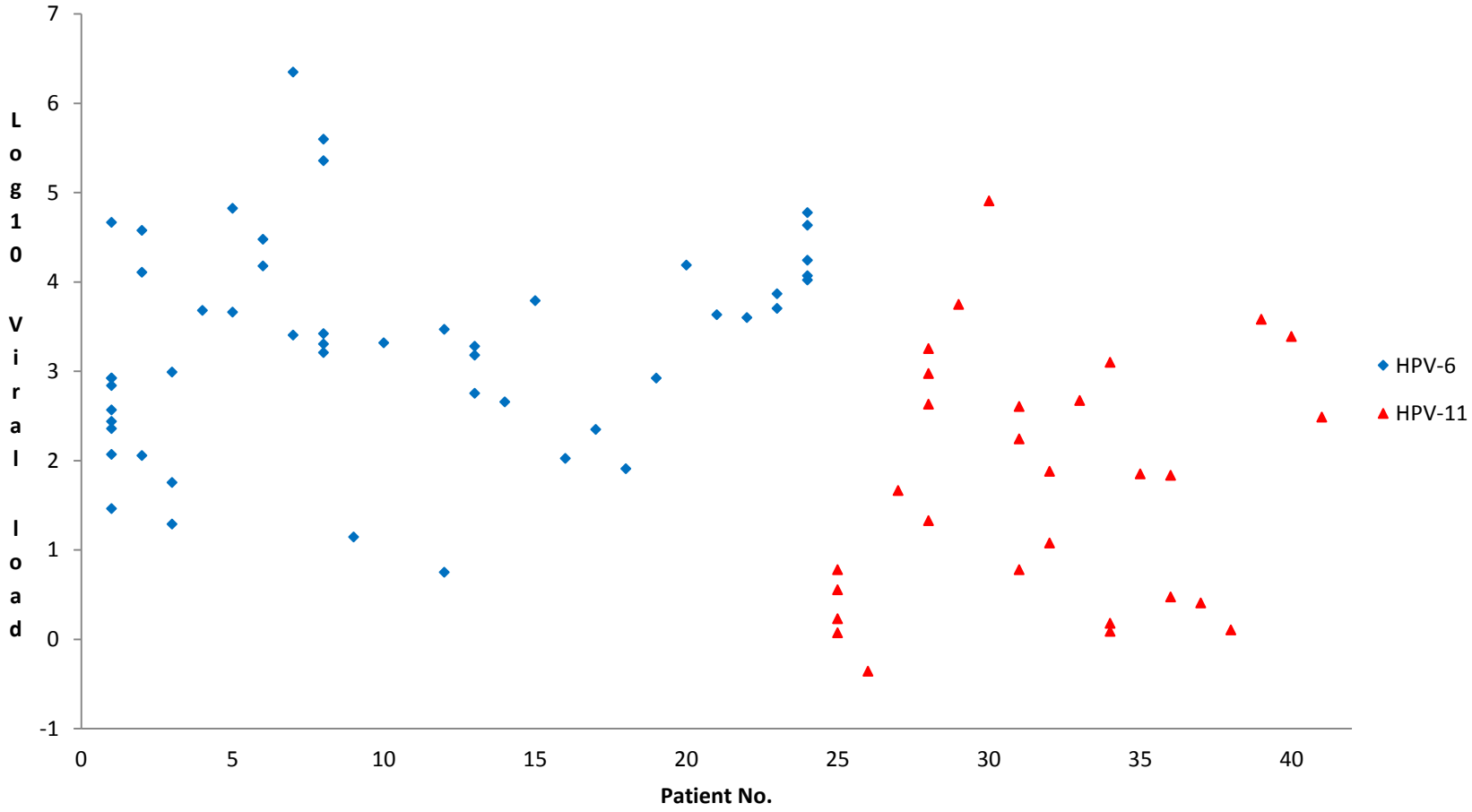
# HPV VIRAL LOAD



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# HIV – JORRP

- HIV status known in 97
  - 86 (88.7%) HIV Negative
  - 11 (11.3%) HIV Positive
    - 7 (7.2%) on ARVs

# HPV-6 JORRP HIV STATUS

| | Negative | Positive |
|---|---|---|
| Patients | 44 | 7 |
| Age at diagnosis | 5.8 | 4.9 |
| Median total procedures | 6 | 8 |
| Average procedures/year | 5.3 | 5.1 |
| Average Derkay score | 16.4 | 20.4 |
| Tracheostomy | 2 | 1 |
| Pulmonary involvement | 0 | 0 |



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# HIV – AORRP

- HIV status known in 16/24
  - 12 (75%) HIV Negative
  - 4 (25%) HIV Positive



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