

# Viral Factor in Development AIDS-related Kaposi's Sarcoma in Zambia

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## Abstract

**Background:** Evidence is now strong that Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiologic agent of all forms of KS. Whether the different KSHV strains vary in infectiousness or virulence is still unclear. We evaluated the relationship between KSHV strains among KS symptomatic and asymptomatic HIV-infected individuals from Zambia.

**Methods:** Children who presented with mucocutaneous or lymphadenopathic KS lesions and asymptomatic HIV-infected individuals were enrolled into the study. DNA was extracted from peripheral blood mononuclear cells (PBMC) and KS tissues. The KSHV K1 gene was amplified by PCR. We have applied a heteroduplex mobility assay (HMA) analysis to determine the diversity of KSHV K1 gene variants within infected individuals, and also determine whether there is compartmentalization of the virus in different tissues, such as PBMC and KS tissues. For each individual, at least 10 independent K1 gene clones were sequenced. DNA sequences of the K1 gene were aligned and analyzed using PAUP version 4.08b software.

**Results:** Surprisingly, HMA showed variation among K1 gene clones obtained from the same individual. We found that different K1 clones obtained from the same subject that exhibited distinct HMA heteroduplexes also had different sequences, and the level of divergence varied in each subject. The analysis of the KSHV K1 genes from KS patients showed that viruses with diverse genotypes were present in the PBMC while restricted K1 gene variants were found in the KS tumor tissue. As KSHV genome shows variation almost exclusively in the K1 gene we compared the K1 gene sequences from 6 children with KS tumors and 10 asymptomatic individuals from Zambia. Analysis of the consensus KSHV K1 gene sequences shows that certain groups of closely related virus isolates were derived from children with KS tumor lesions. The virus isolates from the group had only 0.3 to 1.6% variation for this region on the nucleotide level in contrast with up to 18% variation observed in comparisons to all the other strains.

**Conclusions:** Our study shows that HMA and sequence analysis revealed quasispécies of KSHV in all the infected Zambian individuals. Our study also found that HIV-8 strains may be compartmentalized, with quasispécies existing in PBMC, and more restricted viral genotypes in the KS tumor tissue. These results suggest that KSHV virus isolates have different pathogenic potentials.

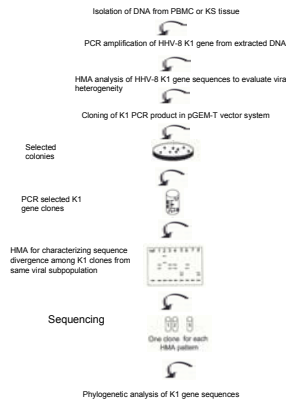
## Introduction

After the onset of the AIDS pandemic, several African countries, including Zambia, reported a significant increase in the number of cases of KS among both adults and children. Some data suggest that AIDS-related KS among children in Zambia presents differently from neighboring African countries in that cases affect mostly a younger age group (< 5 years old). It may correlate with the circulation of exclusive KSHV strains in Zambia which can have the differences in virulence.

To clarify this assumption we have applied a heteroduplex mobility assay (HMA) and phylogenetic analyses to determine the diversity for the hypervariability of KSHV K1 gene sequences among children with AIDS-related KS and asymptomatic individuals. Our results show that only two certain groups of KSHV isolates were delivered from children with KS tumor lesions. Our results demonstrate that in some KS patients, infection with different KSHV strains occurs, and that KSHV sequences may segregate between PBMC and tumor tissues.

## Methods

### Overview of the procedures used for the analysis KSHV K1 gene variability



**Table 1. Characteristics of the patients with KS and healthy individuals involved in the study**

Individual	Kaposi's sarcoma (Yes/No)	Serology		Age/sex
		HIV-1	KSHV	
005i	Yes	+	+	8y/F
005s	No	+	+	5y/F
008i	Yes	+	+	34y/F
008m	No	+	+	34y/F
016i	Yes	+	+	3y/M
072i	Yes	+	+	8y/M
111i	Yes	+	+	5y/M
111m	No	+	+	29y/F
124i	Yes	+	+	7y/M
1609	No	+	+	22y/F
1166	No	+	+	24y/F

**Table 2. Level of nucleotide variation among clones**

Name of KSHV isolate and gene	Level of divergence between nucleotide sequences
005m-23 clone of K1 gene	0.0-15%
005m-34 clone of K1 gene	0.0-3%
005m-19 clone of K1 gene	0.0-3%
005i (blood) oforf 26 gene	0.0-3%
111i (blood) of K1 gene	0.15-0.6%
111m (blood) of K1 gene	0.15-0.76%
005i (blood) of K1 gene	0.15-0.45%
005s (blood) of K1 gene	0.15-0.6%
008i (blood) of K1 gene	0.3-12%
008m (blood) of K1 gene	0.16-0.8%
016i (blood) of K1 gene	0.15-1.1%
124i (blood) of K1 gene	0.15-1.64%
124i (KS tissue) of K1 gene	0.15-0.8%
072i (blood) of K1 gene	0.15-0.8%
072i (KS tissue) of K1 gene	0.1-1%

**Table 3. Intra-patient variability of KSHV K1 gene**

Subject	Number of isolates	Number of unique sequences (nucleotide level)	Number of unique sequences (nucleotide level after recombination)	Number of unique sequences (nucleotide level after recombination)	Number of unique sequences (nucleotide level after recombination)	Number of unique sequences (nucleotide level after recombination)	Number of unique sequences (nucleotide level after recombination)
005i	10	10	10	10	10	10	10
005s	10	10	10	10	10	10	10
008i	10	10	10	10	10	10	10
008m	10	10	10	10	10	10	10
016i	10	10	10	10	10	10	10
111i	10	10	10	10	10	10	10
111m	10	10	10	10	10	10	10
124i	10	10	10	10	10	10	10
1609	10	10	10	10	10	10	10
1166	10	10	10	10	10	10	10

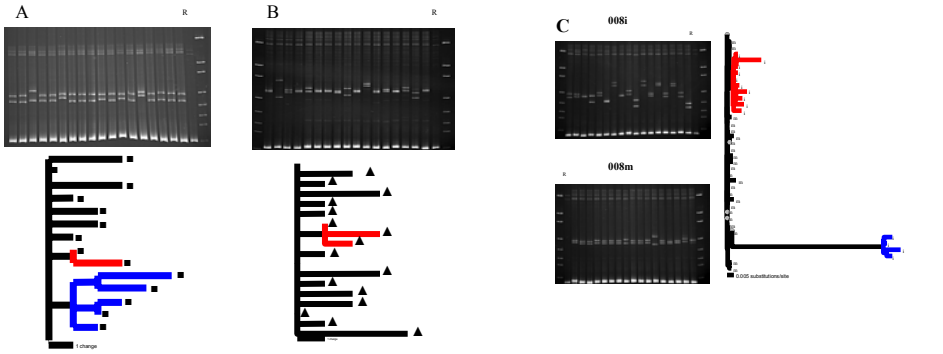


Figure 1. HMA and phylogenetic analyses demonstrating the intra-patient variability of HHV-8 K1 gene sequences and the evolution of KSHV in Zambian individuals. A-111m; B-005i; C-008m and 008i.

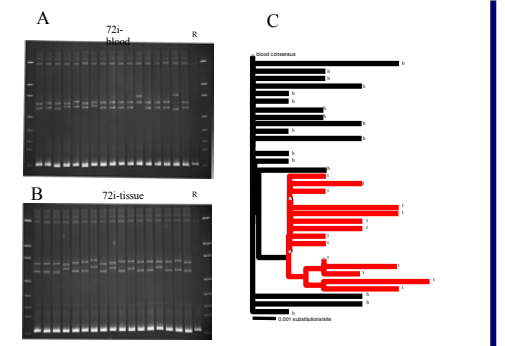


Figure 2. Heteroduplex mobility assay of KSHV K1 gene sequences from PBMC (A) and KS tissue (B) of patient 072i, and their phylogenetic relationships (C).

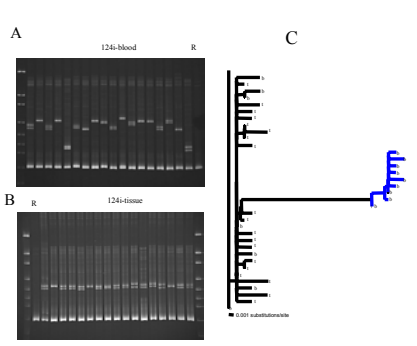


Figure 3. Heteroduplex mobility assay of KSHV K1 gene sequences from PBMC (A) and KS tissue (B) of patient 124i, and their phylogenetic relationships (C).

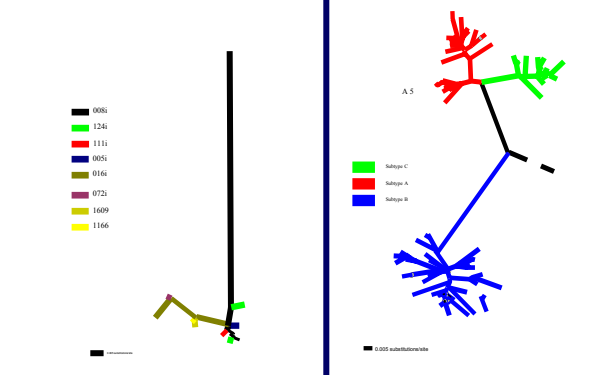


Figure 4. Phylogenetic relationship of the KSHV K1 nucleotide consensus sequences among isolates derived from DNA PBMC of Zambian patients. Figure 5. Unrooted neighbor-joining tree comparing KSHV K1 gene consensus sequences from Zambian patients (dashed lines) to all known isolates.

## Results

Like the situation in HIV, we have demonstrated the convincing examples of KSHV K1 gene heterogeneity or quasispécies within in all analyzed individuals (Table 1-3). We showed that the intra-patient nucleotide variations of K1 gene were significantly higher compare to the rate of *Tag* error mutations detected by carrying out this same procedure an already cloned single plasmid DNA template or when we analyzed ORF 26 gene.

We carried out HMA analysis of PCR products from multiple plasmid clones of the KSHV K1 gene from each individual. In each case, multiple viral variants in PBMC likely reflecting the presence of viral quasispécies in this compartment were detected (Fig. 1-3). We found that ORF-K1 clones from same subject that exhibited distinct HMA heteroduplexes also had different sequences between each other, and the level of divergence varied in each subject. For example, K1 sequences obtained from clones for subject 111i were different from each other, exhibiting 0.15-0.6% nucleotide divergence. Whereas among the KSHV K1 clones for subject 124i, sequence variation between clones differed by 0.15-3%. We also found high level divergence in ORF-K1 among clones from the PBMC of subject 008i, with a 0.3-12% variation at the nucleotide level (Fig. 2-3, Table 2).

We demonstrated that KSHV isolates can change over time as in an individual as in case intra-familial transmission as indicated by phylogenetic analyses (Fig. 1).

Sequence analysis showed that subjects 124i and 008i have the highest level of variation as indicated by both HMA and phylogenetic analyses (Fig. 1; Fig. 3). These two patients appear to have been infected by a minimum of two different KSHV types based on their segregation in the phylogenetic tree for K1.

The KSHV genotyping assay based upon HMA patterns and DNA sequencing of the K1 gene variants from patients 072i and 124i showed that viruses with higher heterogeneity genotypes were present in the PBMC whereas the KS tumor tissue contained virus K1 variants which were clustered more tightly (Fig. 2-3).

An unrooted tree representing all the K1 gene consensus sequences analyzed in this study shows that all K1 sequences from children with AIDS-related KS clustering in two groups (Fig. 4). The diversity of virus isolates within of these groups was only 0.3 to 1.6% variation for K1 region on nucleotide level in contrast with up to 18% variation observed in comparisons to all the other KSHV strains from Zambia.

All K1 sequences except the one in this study segregated with KSHV subtype B which is mainly of sub-Saharan origin (Fig. 5).

## Conclusions

- Our study shows that HMA analysis can effectively be used to detect KSHV K1 gene heterogeneity.
- Both HMA and sequence analysis revealed quasispécies of KSHV in all the infected Zambian individuals.
- Our study also found that KSHV strains may be compartmentalized, with quasispécies existing in PBMC and more restricted viral genotype in the KS tumor tissue.
- The finding of a separate grouping for Zambian patients with AIDS-related KS demonstrate the differences in virulence and associations with the development of KS among KSHV isolates.