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NEW VARIANT OF KAPOSI'S SARCOMA –ASSOCIATED HERPESVIRUS IN MEN WHO HAVE SEX WITH MEN

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Background

- Kaposi's sarcoma-associated herpesvirus (KSHV) is recognized as the etiologic agent of all epidemiological forms of Kaposi's sarcoma (KS), including classic, endemic, iatrogenic and epidemic (associated to HIV-infection) forms. This virus is also involved in the development of lymphoid malignancies, particularly primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD).
- In individuals of Western countries, KSHV-prevalence is low in general population (<5%) whereas a significantly higher prevalence has been described in men who have sex with men (MSM). Among them, KSHV-acquisition was mainly linked to sexual risk behavior.
- A the end left of KSHV-genome, the open reading frame K1 (ORF-K1) encodes a transmembrane glycoprotein whose amino-acid sequences varies from 20% to 40% between KSHV subtype and from about 10% within subtype.
- The distribution of KSHV-subtype was mainly related to geographic region and ethnicity: subtype A and C were found in Europe, North America, Middle East, Mediterranean basin and Asia, subtypes B and A5 predominantly in Sub-Saharan Africa, subtype D in the Pacific Island and Taiwan, subtype E in Brazilian Indians, subtype Z in a small cohort of Zambian children and subtype F in individuals from Uganda.
- However, few studies also suggested that subtypes differed according to clinical presentation or progression, but this remains to be clarified, results from different studies being contradictory.

Objectives

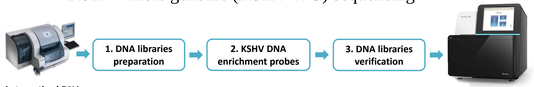
- This study aimed to assess KSHV-diversity in MSM living in France with or without KSHV-associated diseases.
- This study also aimed to find a correlation between KSHV subtype and clinical presentation severity.

Materials and methods

- Patients were included retrospectively in 4 infectious diseases department (Bichat, Pitié-Salpêtrière, Saint-Antoine and Tenon hospitals), Paris, France.
  - 57 MSM HIV-positive with KSHV-associated diseases diagnosed between 2010 and 2017
  - 8 MSM under HIV Pre-exposure prophylaxis (PrEP) with positive KSHV-serology

- ***Samples***
  - 57 whole blood at the time of KHSV-associated diseases diagnosis
  - 8 oral swabs collected from MSM under PrEP

- ***Molecular analysis***
  - Detection and quantification of KSHV-DNA by real time PCR
  - KSHV typing by nested PCR of ORF-K1 (679bp) or VR1 (363bp) and Sanger sequencing
  - KSHV whole genome (KSHV-WG) sequencing



  - KSHV-WG assembly
    - Mapping on GK18 reference sequence (Bowtie2)
    - *De novo assembly* with Spades and Mira
      → KSHV-WG consensus sequence with Mauve

- ***Phylogenic analysis***
  - ORF-K1 amino-acid and KSHV-WG nucleotides sequences
    - Maximum likelihood method (1000 bootstrap) – PhyML 3.0
    - Pairwise genetic distances calculated with Mega 7.0.14

- ***Statistical analysis***
  - GraphPad software
  - Non parametric tests: Mann-Withney U and Fischer t test

Results

1. ORF-K1 phylogenic analysis

- Among the 65 patients included, KSHV-typing was contributive for 19/41 (46%) with KS, 11/12 (92%) with MCD, 4/4 (100%) with PEL and 5/8 (62,5%) under HIV-PrEP.
- Overall, KSHV subtype C was the most prevalent (20/39), of whom 17/39 (44%) fell in variant C3, followed by subtype A (13/39), of whom 7/39 (18%) fell in variant A4. Subtype B was identified in only one patient [Fig 1].
- Genetic distance (GD) calculated between subtypes B and subtypes A or C were respectively 34% and 39% whereas ORF-K1 amino-acid sequences of subtype A and C differed from each other by 19%.

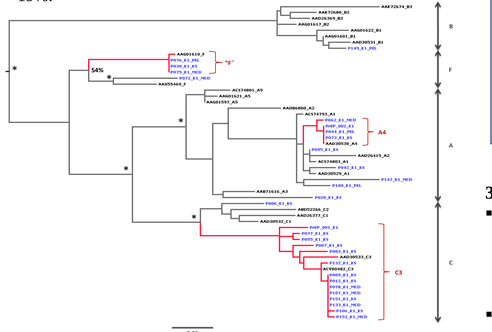


Figure 1: Amino-acid maximum-likelihood tree of ORF-K1 patients and reference sequences available on NCBI database. *Patients' sequences are represented in blue, references sequences in black and the 3 clusters found in red on the tree. Nodes presenting a branch support > 70% (bootstrap analysis using 1000 replicates are indicated by an asterisk.*

- Interestingly, 5 patients were identified as "subtype F": P030, P035, P075, P076 and PrEP004 (See Paragraph 4).

2. KSHV subtype according to patients' origin

- Most of patients came from France (29/39): 15/29 (52%) fell in subtype C (of whom 13/15 in variant C3), 9/29 (31%) fell in subtype A, 1/29 (3%) in subtype B and 4/29 (14%) in "subtype F".
- From Mediterranean basin, 3 belong to subtype A and 2 to subtype C. Patients from Asia fell in subtype C and from South American patients, one fell in subtype A, one in subtype C and one in "subtype F" [Fig 2A].

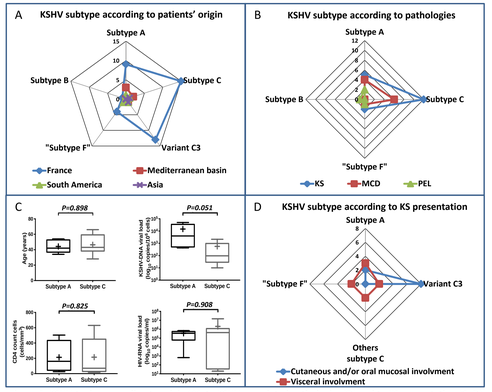


Figure 2: KSHV-typing according to origin (A), to KSHV-associated pathologies (B) and to KS presentation (C, D).

3. Relationship between subtype and clinical presentation

- Proportion of KS and MCD related to each subtype were not significantly different (26% of KS and 36% of MCD to subtype A and 63% of KS and 55% of MCD to subtype C) suggesting that subtype associated with KSHV pathologies may related to the prevalence of each subtype in this population [Fig 2b].
- Among patients with KS
  - KSHV-DNA viral load tended to be higher among patient with subtype A than those with subtype C (*p=0.051*) regardless of the immuno-virological status [Fig 2C].
  - Variant C3 was more associated with only cutaneous and/or oral mucous involvement than others subtypes (Odd ratio = 11.7, IC95% 1.1 – 214.2, *p=0.023*) regardless of the immuno-virological status (CD4 count cells *p=0.776*, HIV-RNA viral load *p=0.749*) [Fig 2D].

4. New variant F characteristics

- "Subtype F" was isolated only from 5 MSM: 2 with visceral KS (P030 and P035), 1 with MCD (P075), 1 with pleural PEL (P076) and also in 1 PrEP user (PrEP004). Patients with lymphoid malignancies were also associated with KS.
- KSHV-associated diseases diagnosis was done at the AIDS stage of HIV-infection.

ORF-K1 amino-acid sequences comparison

- Sequences of our five patients differed from 11% at amino-acid level compared to that of subtype F already described in Uganda (AAX55469_F) [Fig 3] and theirs branches were separated with a bootstrap confidence level of 54% in the phylogenic tree [Fig 1]. Moreover, epidemiological context was different (MSM Caucasian *versus* African patients) .

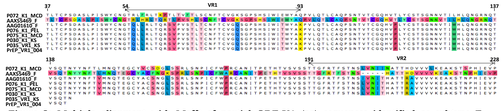


Figure 3: Multiple alignment with Mafft of partial ORF-K1 (aa) sequences identified as subtype F. *Sequence from Uganda (completely colored) is compared with sequence described in 2000 (AAG01610_F), with our five sequences and with KSHV of woman from Congo with MCD (P072_K1_MCD). Mismatches are highlighted with various colors according to the amino-acid concerned and the insertion are represented with dashes.*

- Otherwise, our sequences were closed (GD=10^{-6}) to that KSHV described in a French MSM HIV-positive patient with PEL by Lacoste and al in 2000 (AAG01610_F) [Fig1 and 3].

KSHV-WG phylogenic analysis

- ORF-K1 phylogenic analysis was confirmed by KSHV-WG sequencing of our "subtype F" (P030, P075 and P076) and compared to P072 identified as "African subtype F" (KSHV-WG subtype F reference sequence not available in NCBI database).

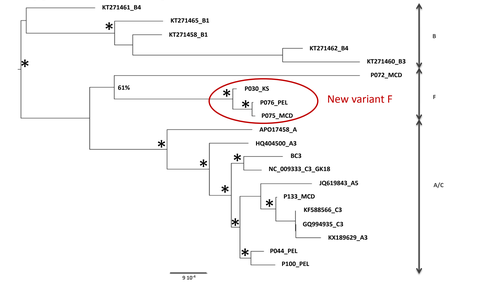


Figure 4 : Nucleotides maximum-likelihood phylogenetic tree of KSHV-WG patients newly sequenced and references sequences available on NCBI database. *By ORF-K1 Sanger sequencing, P044 and P100 were identified as respectively variant A4 and variant A1, P133 as variant C3, P030, P075 and P076 as "Caucasian subtype F" and P072 as "African subtype". Nodes presenting a branch support > 70% are indicated by an asterisk.*

Conclusion

Our study showed that subtype C, and specifically variant C3, was the most prevalent in MSM living in France and tended to be associated with less severe epidemic KS form. We also reported for the first time, 5 new « subtype F » isolated only in MSM and associated with severe diseases. We suggest that, in view of phylogenic (ORF-K1 and KSHV-WG analysis) and epidemiological (« Caucasian » *versus* « African » subtype) finding, subtype F could be subdivided in two genotype variant (F1 and F2).