Abstract number: THAB0203

Thuy NGUYEN, Constance DELAUGERRE, Marc-Antoine VALANTIN, Corinne AMIEL, Emmanuelle NETZER, Thomas L’YAVANC, Michele OHAYON, Pierre-Marie GIRARD, Nesrine DAY, Georges KREPLAK, Gilles PIALOUX, Vincent CALVEZ, Jean-Michel MOLINA, Anne-Geneviève MARCELIN, and Eve TODESCO

Shared HCV Transmission Networks among HIV-1 Positive and Negative Men Having Sex with Men by Ultra-Deep Sequencing in Paris
Introduction

- **HCV incidence in men having sex with men (MSM)**
  - Outbreaks of Acute hepatitis C in developed countries [1-3]
  - In HIV+ MSM
    - ↑ HCV incidence from 2012 to 2016 in France (4.3/1000 PY to 11.1/1000 PY) [4]
    - High HCV reinfection rate in Paris (218/1000 PY) [5]
    - Pooled HCV incidence in HIV+ MSM was 7.8/1000 PY [6]
  - In HIV- MSM
    - Pooled HCV incidence was 0.4/1000 PY [6] but can reach 14/1000 PY in MSM included in the ANRS IPERGAY Pre-Exposure Prophylaxis (PrEP) trial [7]

- **HCV transmission networks**
  - Large European HCV transmission networks in HIV+ MSM (78% of sequences formed 11 clusters of 4-37 individuals) [2]
  - Spread of HCV from HIV+ to HIV- MSM [8,9]

Introduction

- **Transmission chains**
  - Viral sequences more genetically similar to each other than expected by chance, demonstrated by a tight cluster on phylogenetic trees
  - Satisfying branch support and genetic distance threshold requirements\(^1\)
  - Sequencing of polymerase (NS5B)\(^2\) or hypervariable region (HVR)\(^3\)

- **Available techniques**
  - **Sanger sequencing**: gold standard for clinical monitoring of patients but unable for a deep characterization of intra-host genetic diversity
  - **Ultra-deep sequencing (UDS)**: allows an extensive analysis of viral population (minority variants)

---

Objectives

- To identify and deeply characterize HCV transmission chains in HIV+ and HIV- MSM in Paris

- To detect closely related HCV transmission events among them (directness inference)
Methods

- Sanger sequencing and UDS on polymerase fragment (NS5B, 388 bp)

- Approximative maximum likelihood with GTR model (FastTree v 2.1) -> Phylogenetic tree construction

- Transmission chain determination at different thresholds of maximum genetic distance (MGD) with bootstrap value > 80% (ClusterPicker v 1.2.3)
  - Sanger: 3% of MGD
  - UDS: 3% and 4.5% of MGD
Patients

- 68 patients with acute hepatitis C including

  - 55 (50 HIV+ and 5 HIV-) patients followed at the Pitié-Salpêtrière, Saint-Antoine, and Tenon hospitals, Paris, France or by their referring physicians

  - 13 HIV- patients from ANRS IPERGAY trial (MSM at high risk for HIV acquisition and under PrEP) [1,2]

Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=68)</th>
<th>HIV+ patients (n=50)</th>
<th>HIV- patients (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>38.5 (30.5-46.0)</td>
<td>42.5 (34.5-46.0)</td>
<td>32.0 (27.5-35.8)</td>
</tr>
<tr>
<td>Men having sex with men, n (%)</td>
<td>58 (85.3)</td>
<td>43 (86.0)</td>
<td>15 (83.3)</td>
</tr>
<tr>
<td>Unknown sexual orientation, n (%)</td>
<td>10 (14.7)</td>
<td>7 (14.0)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>HCV viral load, log copies/ml, median (IQR)</td>
<td>5.9 (5.3-6.7)</td>
<td>5.9 (5.3-6.9)</td>
<td>5.5 (5.3-5.6)</td>
</tr>
<tr>
<td>HCV genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype 1a, n (%)</td>
<td>32 (47.1)</td>
<td>24 (48.0)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Genotype 4d, n (%)</td>
<td>28 (41.2)</td>
<td>20 (40.0)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>Genotype 3a, n (%)</td>
<td>6 (8.8)</td>
<td>5 (10.0)</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Genotype 2k, n (%)</td>
<td>2 (2.9)</td>
<td>1 (2.0)</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>ALAT (IU/L), median (IQR)</td>
<td>320.0 (146.5-535.5)</td>
<td>315.0 (144.8-480.8)</td>
<td>467.0 (234.0-647.0)</td>
</tr>
<tr>
<td>HIV co-infection (%)</td>
<td>50 (73.5)</td>
<td>50 (100.0)</td>
<td>/</td>
</tr>
<tr>
<td>HCV reinfection (%)</td>
<td>15 (22.1)</td>
<td>14 (28.0)</td>
<td>1 (5.6)</td>
</tr>
</tbody>
</table>
More HCV transmission chains detected by UDS but fewer subjects identified in each chain

Identification of HCV transmission chains by Sanger and UDS

<table>
<thead>
<tr>
<th></th>
<th>Sanger 3.0% of MGD</th>
<th>UDS 3.0% of MGD</th>
<th>UDS 4.5% of MGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects included in a transmission chain, n (%)</td>
<td>38 (55.9)</td>
<td>38 (55.9)</td>
<td>43 (63.2)</td>
</tr>
<tr>
<td>Number of transmission chains</td>
<td>10</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Median of subjects (min-max)</td>
<td>3 (2-6)</td>
<td>2 (2-5)</td>
<td>2 (2-6)</td>
</tr>
</tbody>
</table>

MGD: Maximum Genetic Distance

- Subjects in some transmission chains found by UDS were grouped into larger chains by Sanger.
- 3 and 4 hidden transmission chains were detected only by UDS at 3% and at 4.5% of MGD, respectively.
Shared HCV transmission networks among HIV positive and negative subjects

Transmission chains including HIV+ and HIV- subjects

<table>
<thead>
<tr>
<th></th>
<th>Sanger 3.0 % of MGD</th>
<th>UDS 3.0 % of MGD</th>
<th>UDS 4.5% of MGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV transmission chains including HIV+ and HIV- subjects, n/number of chains</td>
<td>8/10</td>
<td>9/17</td>
<td>10/18</td>
</tr>
<tr>
<td>Number of HIV- clustering with HIV+ subjects, n/total number of HIV- subjects</td>
<td>9/18</td>
<td>9/18</td>
<td>10/18</td>
</tr>
</tbody>
</table>

MGD: Maximum Genetic Distance

- High HCV clustering rate among HIV+ and HIV- MSM communities detected by both techniques
Closely related HCV transmission events (MGD < 0.5 %)

5 events detected

Example of 1 event:

**Figure**: Phylogenetic tree constructed from UDS viral sequences of 2 individuals (pink circle and brown triangle)

Red clades: sequences with MGD < 0.5% between 2 individuals.

Blue clades: identical sequences (MGD = 0%) between 2 individuals
Closely related HCV transmission events (MGD < 0.5 %)

**Figure**: Phylogenetic tree constructed from UDS viral sequences of 2 individuals (pink circle and brown triangle)

- **Red clades**: sequences with MGD < 0.5% between 2 individuals.
- **Blue clades**: identical sequences (MGD = 0%) between 2 individuals
Discussion

Both techniques

- High clustering rate of HCV among HIV+ and HIV- MSM communities
  - At least 50% of HIV- subjects enrolled in this study clustering with HIV+ ones

UDS

- Improved discrimination of HCV transmission chains (through transmission of multiple viral strains/minority variants)
  - Detection of closely related HCV transmission events and of hidden transmission chains

- Identified fewer subjects in each chain vs Sanger because of deeper viral population characterization by UDS (↑ viral diversity)
  - Difficult to determine a cut-off of MGD
  - For large-scale prevention and rapid intervention purposes, UDS is not more useful than Sanger
Discussion

- **Closely related transmission events**
  - Suggesting direct transmissions without totally excluding intermediary links

- **Shared HCV transmission networks among HIV+ and HIV- MSM in Paris**
  - Need of better screening and surveillance of HCV infection in the subgroup of MSM with high-risk behaviors whatever the HIV status

- **Further investigation of the high-throughput sequencing data**
  - With European MSM sequences of HCV
Acknowledgement

- This study was funded by Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS).

- We thank the Virology research team, Pitié-Salpêtrière and Saint-Antoine hospitals, Institut Pierre Louis d’Épidémiologie et de Santé publique, INSERM U1136 (Prs. CALVEZ, MARCELIN, MORAND JOUBERT, Drs WIRDEN, SOULIE, LAMBERT-NICLOT, FOFANA, JARY, ABDI and other colleagues MALET, SAYON, DESIRE).

- We thank
  - the INSERM SC10 and the Trial Scientific Committee for IPERGAY trial.

- We thank
  - all the patients who agreed to participate in the study.
  - all the participant doctors who followed the patients, in particular Drs ROUDIERE, LIOTIER, GOSSET, CARDON, GRIVOIS, ISRAEL, KIRSTETTER, LAYLAVOIX, BOTTERO, WORMSER and Pr. KATLAMA.
  - all the participant virologists, Drs AMIEL, ELAERTS and SCHNEIDER.