

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

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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

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

More HCV transmission chains detected by UDS but fewer subjects identified in each chain

Identification of HCV transmission chains by Sanger and UDS

	Sanger 3.0% of MGD	UDS 3.0% of MGD	UDS 4.5% of MGD
Number of subjects included in a transmission chain, n (%)	38 (55.9)	38 (55.9)	43 (63.2)
Number of transmission chains	10	17	18
Median of subjects (min-max)	3 (2-6)	2 (2-5)	2 (2-6)

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

	Sanger 3.0 % of MGD	UDS 3.0 % of MGD	UDS 4.5% of MGD
HCV transmission chains including HIV+ and HIV- subjects, n/number of chains	8/10	9/17	10/18
Number of HIV- clustering with HIV+ subjects, n/total number of HIV- subjects	9/18	9/18	10/18

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:

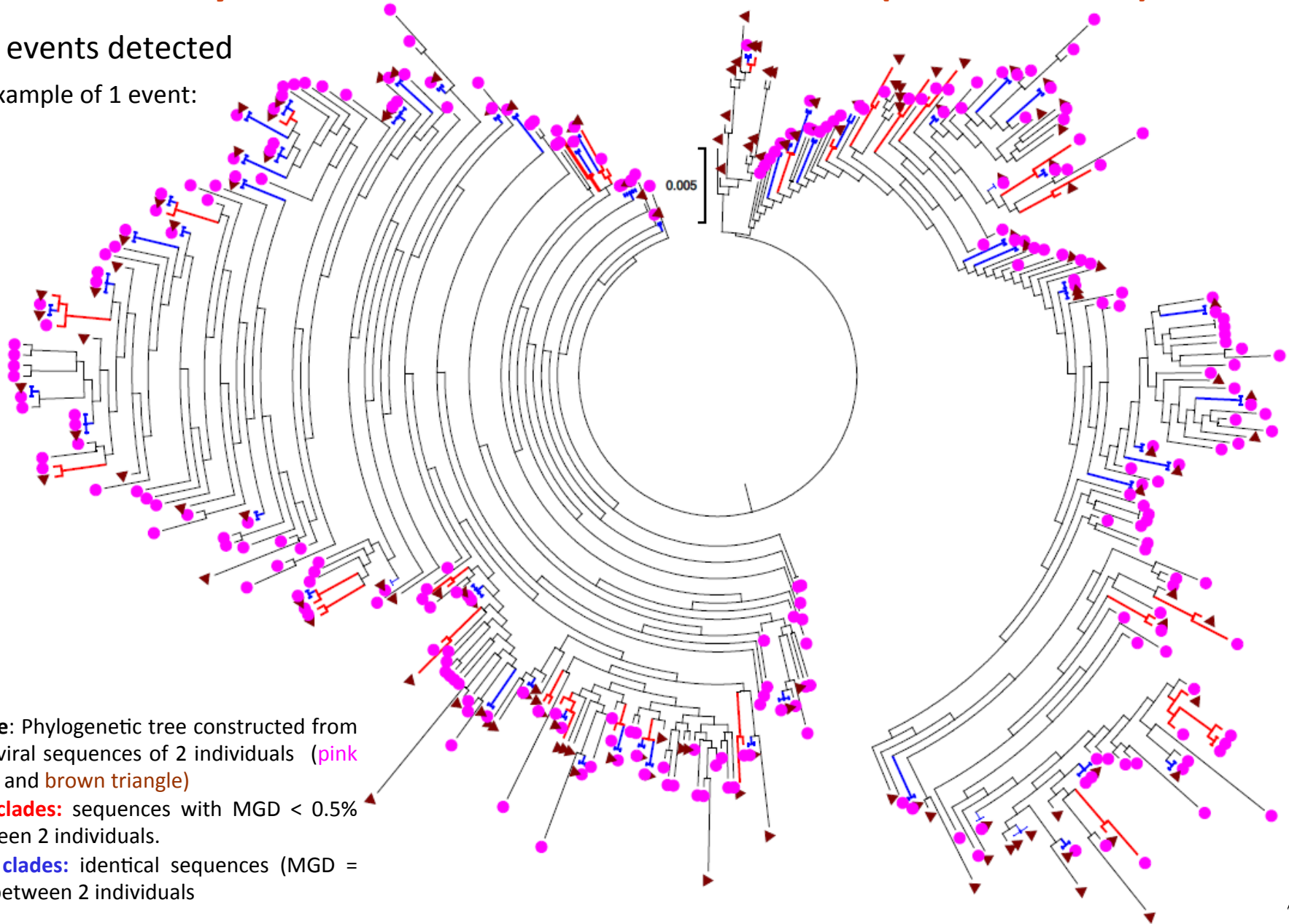


Figure: Phylogenetic tree constructed from UDS viral sequences of 2 individuals (pink circle and brown triangle)

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 %)

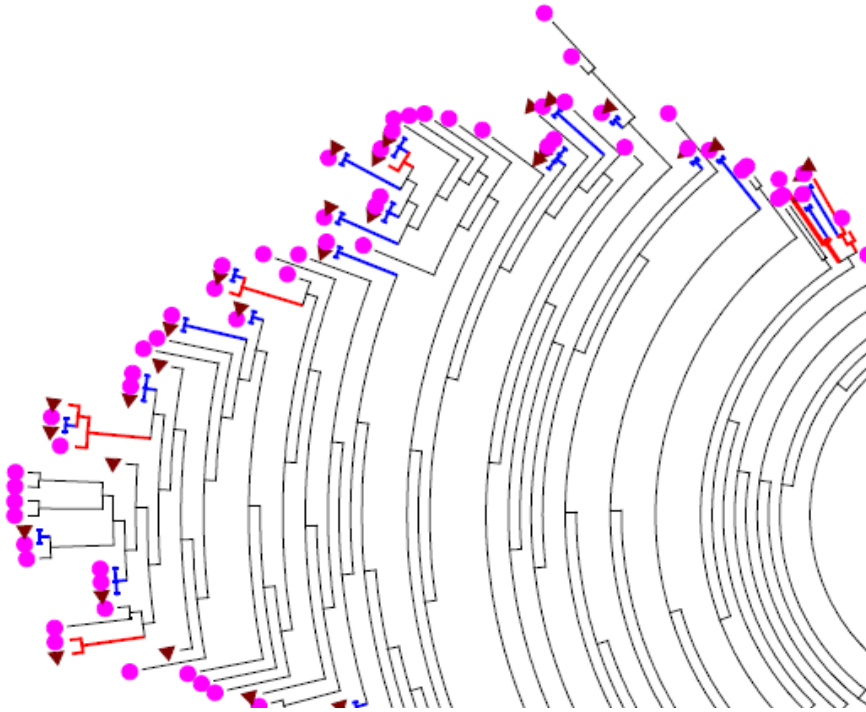


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Red clades: sequences with MGD < 0.5% between 2 individuals.

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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

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