# Phylogenetic Analysis of HIV from PWID in Eastern Europe and Asia: HPTN 074

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## BACKGROUND

People who inject drugs (PWID) have a high risk of HIV infection. Nearly 30% of new HIV infections outside of sub-Saharan Africa occur among PWID. High HIV prevalence among PWID has been reported in Eastern Europe and Asia.

HPTN 074 evaluated the feasibility of using an integrated intervention to reduce HIV transmission among PWID in Indonesia, Vietnam, and Ukraine. The trial enrolled HIV-infected PWID (index participants) and their HIV-uninfected injection partners. Index-partner groups were randomized to receive standard-of-care services for substance use and HIV care (control arm) or supported antiretroviral therapy (ART) and substance use treatment (intervention arm). We used phylogenetic methods to evaluate the relationship between HIV strains in the HPTN 074 study.

## METHODS

### LABORATORY TESTING

HIV diagnostic testing and CD4 cell count testing were performed at study sites. Repeat diagnostic testing, HIV viral load testing, HIV sequencing, and HIV phylogenetic analysis were performed at the HPTN Laboratory Center (Johns Hopkins University, Baltimore, USA).

### SAMPLES USED FOR PHYLOGENETIC ANALYSIS

Phylogenetic analysis was performed using samples collected at study enrollment from 473 index participants and samples from the first HIV-positive visit for seven seroconverters. Additional sequences were obtained using samples collected at a second study visit for the seven index-partner pairs.

### ANALYSIS OF HIV POL SEQUENCES

HIV *pol* was sequenced using the ViroSeq HIV-1 Genotyping System v2.0 for samples with viral loads >400 copies/mL. HIV subtypes were determined by analyzing HIV *pol* sequences with REGA, COMET, and RIP subtyping tools, and were confirmed by phylogenetic analysis using PHYLIP v3.695. Ten background *pol* sequences most closely related to each study sequence were selected using BLAST. Separate phylogenetic trees were constructed for each study site using RAxML v8.2.10. Two or more sequences were considered to form a *pol* sequence cluster if the branch support was ≥90% and the maximum genetic distance between sequences was ≤1.5%.

## METHODS

### ANALYSIS OF HIV ENV SEQUENCES

HIV *env* sequences were analyzed by next generation sequencing (NGS) for partners who acquired HIV during the study (seroconverters), the corresponding enrolled index participants, and other indexes in *pol* sequence clusters. Trees included consensus *env* study sequences, non-study *env* sequences from each country, and background *env* sequences. Participants were classified as "linked" if their *env* sequences formed a distinct monophyletic cluster with bootstrap support ≥90%.

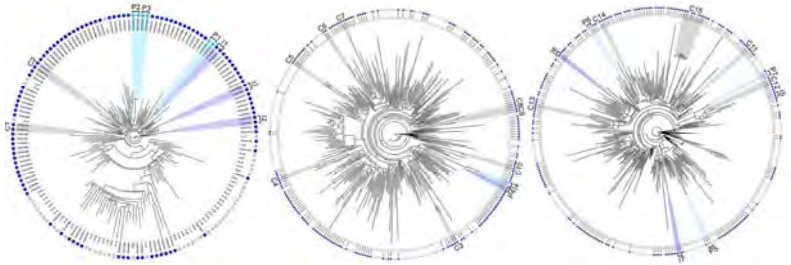
## RESULTS

*Pol* sequences were obtained for 467 (98.7%) of 473 index samples and samples from all seven seroconverters; 22 sequences were excluded from analysis due to high sequence ambiguity or intersubtype recombination. HIV subtypes were A1 (Ukraine) and CRF01_AE (Indonesia and Vietnam). Eighteen *pol* clusters were identified that included 2-7 sequences per cluster (Table 1, Figure 1).

Genetic linkage was evaluated using *env* sequences generated by NGS (Figures 2). Fourteen groups of linked infections were identified, including two index-partner pairs (IP1 and IP4), one partner-partner pair (PP), nine index-index pairs (C1, C2, C5-C8, C10, C11, C13), one group of three indexes (C12), and one group of seven indexes (C15). Only two of seven index-partner pairs were classified as genetically linked. Demographic and behavioral characteristics of participants with genetically-linked HIV infections are shown in Table 2.

**Figure 1.** Phylogenetic analysis of *pol* sequences from each study site.

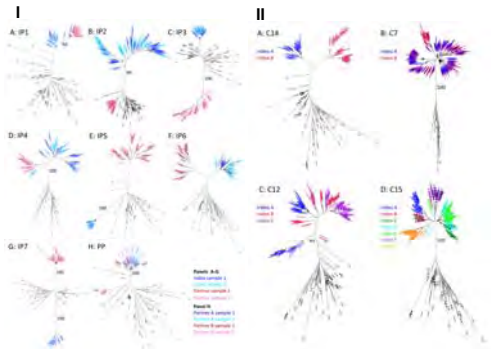
Blue dots: study sequences; grey dots: background sequences. Purple highlight: index sequences from index-partner pairs (I1-I7); blue highlights: partner sequences from index-partner pairs (P1-P7); grey highlights: sequences from index-index clusters (C1-C15).



Indonesia Ukraine Vietnam

## RESULTS

**Figure 2.** Phylogenetic trees of *env* sequences from index-partner pairs (Panel A) and index-index clusters (Panel B).




**Panel I:** *Env* phylogenetic trees from seven seroconversion cases (index-partner pairs, A-G) and one partner-partner pair (H). **Panel II**: Representative *env* phylogenetic trees for index-index clusters: unlinked case (A); linked case (B); cluster involving three indexes (C); and cluster involving seven indexes (D). Each tree includes non-study HIV *env* sequences (black branches).

**Table 1.** Characteristics of *pol* clusters.

| Cluster type | Cluster ID | Study site | Cluster size | Median pol pairwise genetic distance, % | Linkage status (NGS, env) |
|---|---|---|---|---|---|
| Index-Partner | IP1 | Indonesia | 2 | 0.31 | Linked |
| | IP4 | Ukraine | 2 | 0.4 | Linked |
| Partner-Partner | PP | Indonesia | 2 | 0.08 | Linked |
| Index-Index | C1 | Indonesia | 2 | 0.0 | Linked |
| | C2 | Indonesia | 2 | 0.0 | Linked |
| | C3 | Ukraine | 2 | 1.23 | Data N/A |
| | C4 | Ukraine | 2 | 0.54 | Unlinked |
| | C5 | Ukraine | 2 | 1.18 | Linked |
| | C6 | Ukraine | 2 | 1.33 | Linked |
| | C7 | Ukraine | 2 | 0.0 | Linked |
| | C8 | Ukraine | 2 | 0.93 | Linked |
| | C9 | Ukraine | 2 | 1.26 | Unlinked |
| | C10 | Ukraine | 2 | 0.38 | Linked |
| | C11 | Vietnam | 2 | 1.41 | Linked |
| | C12 | Vietnam | 3 | 0.33 | Linked |
| | C13 | Vietnam | 2 | 1.19 | Linked |
| | C14 | Vietnam | 2 | 1.02 | Unlinked |
| | C15 | Vietnam | 7 | 0.16 | Linked |

**Table 2.** Characteristics of participants with linked HIV infections.

| Cluster type | Cluster ID | Sex | Age | Marital status | Number of injection partners | Number of sexual partners | Ed. |
|---|---|---|---|---|---|---|---|
| Index-Partner | IP1 | F/M | 22/28 | Married/Married | no data, 1 | 1, 1 | S/H |
| | IP4 | F/F | 31/33 | Married/Married | no data, 2-4 | 1, 0 | S/H |
| Partner-Partner | PP | M/M | 29/36 | Single/Married | no data | ≥2, 1 | H/H |
| Index-Index | C1 | M/M | 27/41 | Single/Single | ≥5, 2-4 | 0, 1 | S/H |
| | C2 | M/M | 34/35 | Single/Single | 2-4, 2-4 | 0, 1 | H/H |
| | C5 | M/F | 31/33 | Married/Married | 2-4, 1 | 1, 1 | S/H |
| | C6 | M/F | 36/40 | Married/Married | 2-4, ≥5 | 1, 1 | S/S |
| | C7 | F/M | 30/40 | Single/Single | 2-4, ≥5 | 1, 1 | H/H |
| | C8 | M/M | 35/39 | Single/Married | 1, 2-4 | 0, 1 | H/S |
| | C10 | F/M | 34/39 | Married/Married | 2-4, 2-4 | 1, 1 | S/H |
| | C11 | M/M | 41/45 | Single/Married | 2-4, 2-4 | 0, 0 | S/P |
| | C12 | All M | 23-43 | All single | 1 or 2-4 | 0 or 1 | All S |
| | C13 | M/M | 29/36 | Married/Single | ≥5, 1 | 1, 0 | S/P |
| | C15 | All M | 24-42 | Single or married | 1 or 2-4 | 0, 1 or ≥2 | All S |

F: female; M: male; Ed: education; H: higher; S: secondary; P: primary.

## CONCLUSIONS

- Complex patterns of sequence clustering and genetic linkage were observed, with up to seven participants in a cluster.
- Five (71.4%) of seven injection partners acquired HIV infection from a source other than the corresponding index participant.
- These findings suggest that a comprehensive HIV prevention program that includes interventions for both HIV-infected and HIV-uninfected individuals may be most effective in this population.

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