Assessing HIV Transmission Networks in a Thai Cohort with Acute HIV Infection

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BACKGROUND

Bangkok, Thailand has an ongoing HIV epidemic in MSM but phylogenetic studies as to whether this occurs within networks or randomly are lacking. Phylogenetic studies from other metropolitan areas suggest local transmission among networks of MSM with certain characteristics, which might allow for targeted interventions. Phylogenetic studies from other metropolitan areas suggest transmission networks of MSM with certain characteristics, which might allow for targeted interventions. Newly HIV-infected MSM in Bangkok are mostly young men connected to sexually active circles in a multi-risk factor environment, including high partner turnover, stimulant drug use, concurrent STI and undiagnosed HIV infected partners.[1]

The RV254 study in Bangkok enrolls subjects in the earliest stages of HIV infection who are identified through nucleic acid testing (NAT) and sequential immunoassay (IA) testing. Immediate ART is provided and primary endpoints of the study are clinical, immunological and virological characteristics. Baseline drug resistance genotyping of reverse transcriptase (RT) and protease inhibitor (PI) sequences allow the study of clustering in this cohort.

METHODS

Real-time screening of VCT client samples using nucleic acid testing and sequential IA allows for identification of acute HIV infection. Subjects are eligible for study participation if they are in 4thG stages 1 through 3 of acute HIV infection (4thG stage 1: NAT+/4thG immunoassay(IA)-/3rdG IA-; 4thG stage 2: NAT+/4thG IA+/3rdG IA-; 4thG stage 3, NAT+/4thG IA+/3rdG IA+/ Western blot- or indeterminate).[2]

The study collects descriptive data of demographics, behavioral risk factors, number and characteristics of sexual contacts and estimated date of infection through self administered questionnaire, supplemented by physician conducted interviews. Laboratory assessments include CD4+ T-cell count and plasma HIV RNA. Immediate ART is offered at enrollment.

Protease and reverse transcriptase gene sequencing were performed from acid citrate dextrose (ACD) plasma samples at study baseline, prior to start ART. The TRUGENE HIV-1 genotyping assay (Siemens Healthcare Diagnostics, Australia) was employed for the first 66 samples and a validated in-house method was used for the remaining samples. cDNA was generated from plasma RNA using primer RT2955. RT sequences were generated with primers B887-2 and RT-2923 while PR sequences were generated with primers Pr- 1780 and Pr-2172. The generated products were sequenced using BigDye dideoxy dye terminator (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol.

For phylogenetic analysis a multiple alignment of reference strains and sequences of interest were generated using HIVAlign (http://www.hiv.lanl.gov) and were manually edited using Geneious Pro 5.5.5 (Biomatters, Auckland, New Zealand) Molecular Evolutionary Genetics Analysis (MEGA) 5.0 was used to construct a Maximum Likelihood (ML) tree of the sequences of interest and reference HIV-1 subtype strains, designate genetic subtypes and calculate pair-wise genetic distances. A distance of < 1.5% and visual inspections of signature nucleotides were used to identify linked transmission clusters.

HIV-1 subtype was designated using:
- NCBI Genotyping tool
- jpHMM tool at http://jphmm.gobics.de
- HIV BLAST (hiv.lanl.gov)

RESULTS

Real- time screening of 141,233 samples using NAT and sequential IA →330 subjects with acute HIV-1 infection identified →275 enrolled in study RV254 →273 HIV protease gene sequences retrieved from plasma. Of these 273, 93% are MSM and 268 are Thai nationals. Baseline demographic data for these individuals are listed in Table 1.

CRF01_AE was predominant at 86% (234)(N), followed by subtype B 8% (21) and CRF01/B 6% (15). A West African CRF02_AG, a South African C and a B/C strain (B Thai and Indian C) were also identified. 13/21 B strains clustered with Thai B'. 5 non-Thai participants had 3 CRF01_AE, 1 subtype B infections (p=0.28).
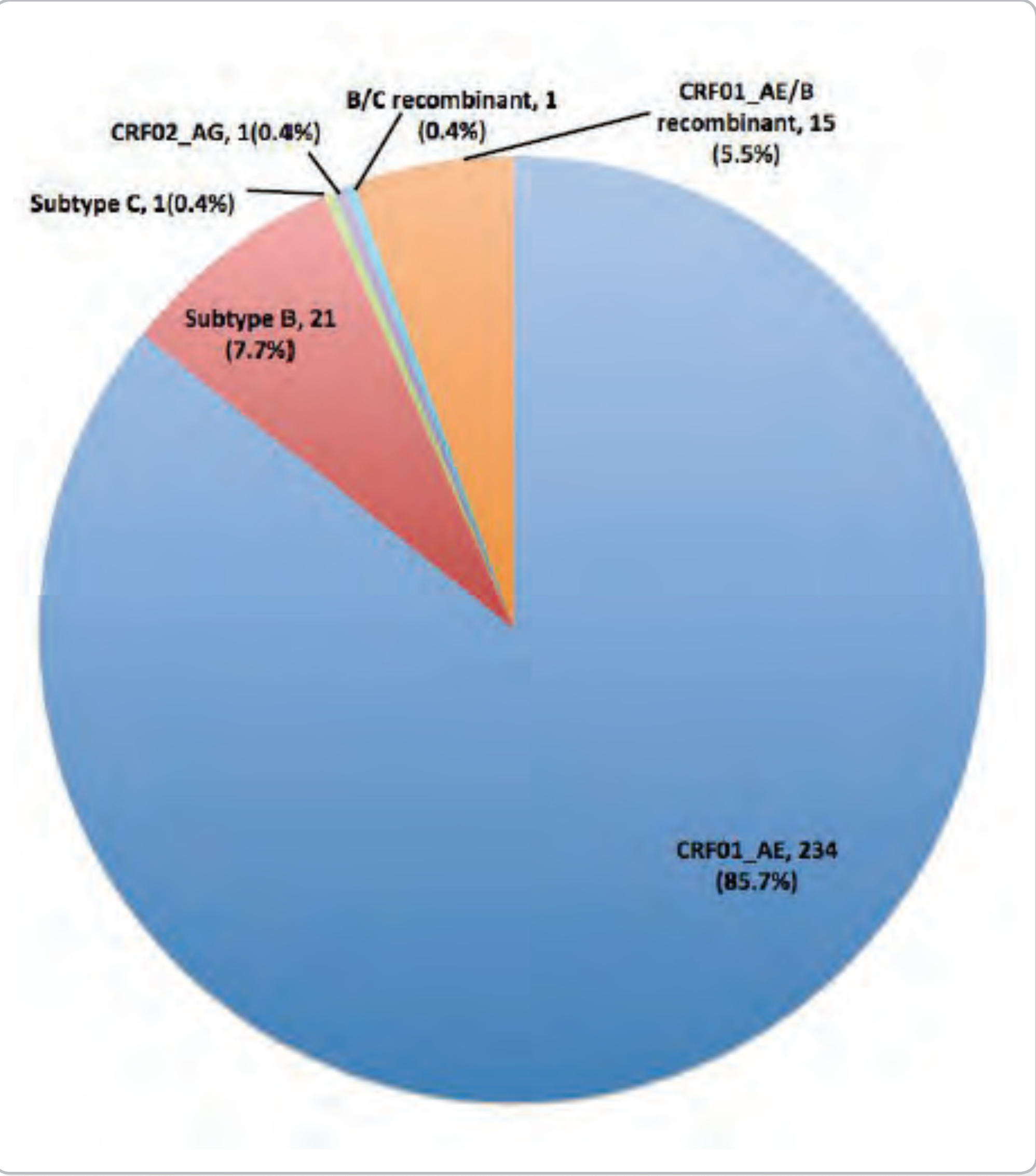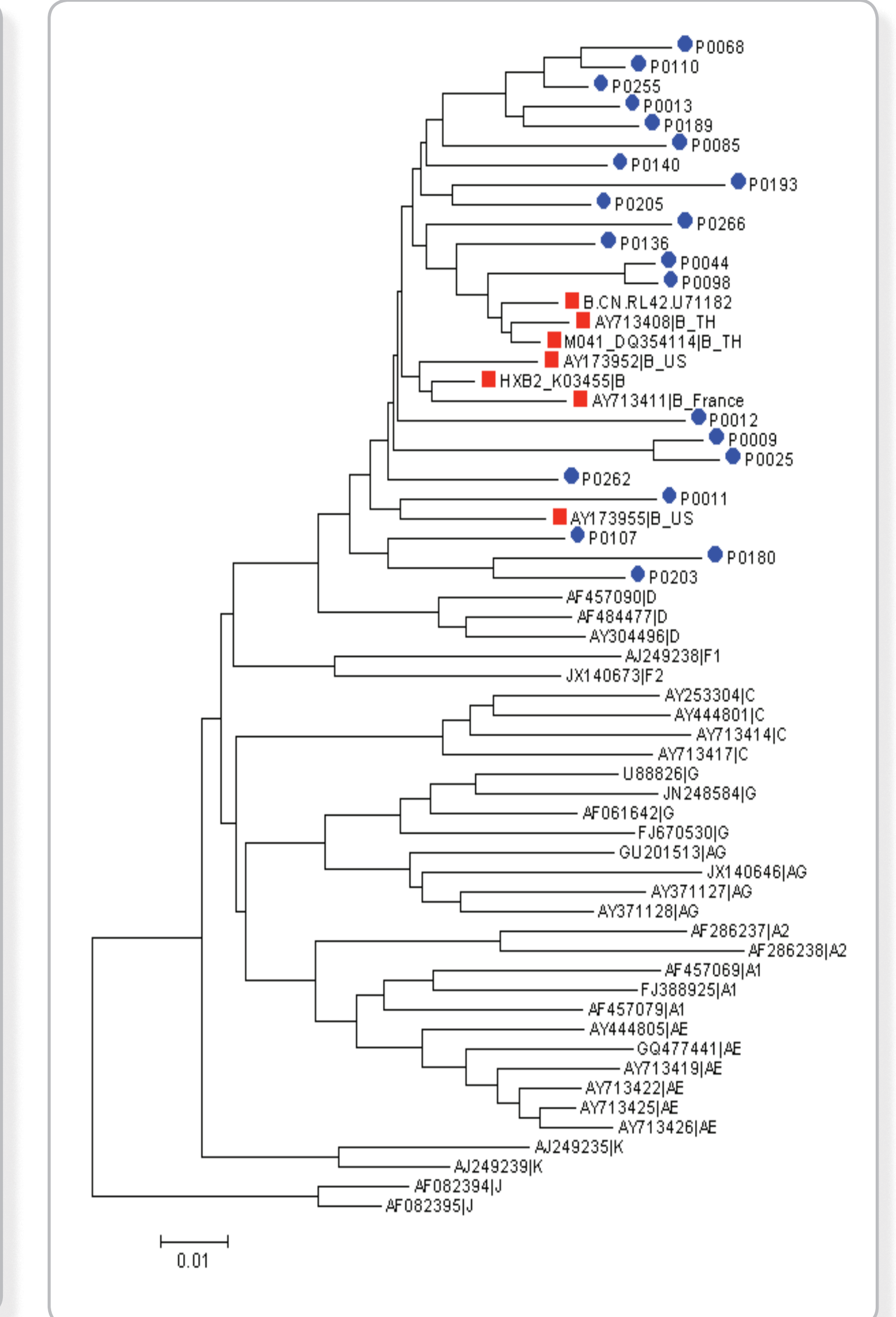
Figure 1.

Figure 2.

33 Subjects (31 CRF01_AE and 2 B) formed 14 clusters as represented in Figure 3. There are 11 clusters of 2, 2 clusters of 3, and 1 cluster of 5 individuals. With the exception of one cluster of subtype B, all clusters represent only Thai MSM infected with CRF01_AE. Only 2 clusters are known to be linked. 30 of the 33 clustering individuals live in the Bangkok area. Table 1 shows that subjects who clustered were not significantly different from non–clustering subjects, including age, 4thG stage, HIV subtype, plasma HIV RNA level, days since exposure and number of sexual partners preceding enrollment. There were more students among the clustering subjects than among the non-clustering subjects. Median (range) time between infections was 590 (0-1211) days for the clusters of 2, 158 (84-277) days for the clusters of 3, and 144 (43-194) days for the cluster of 5 subjects.
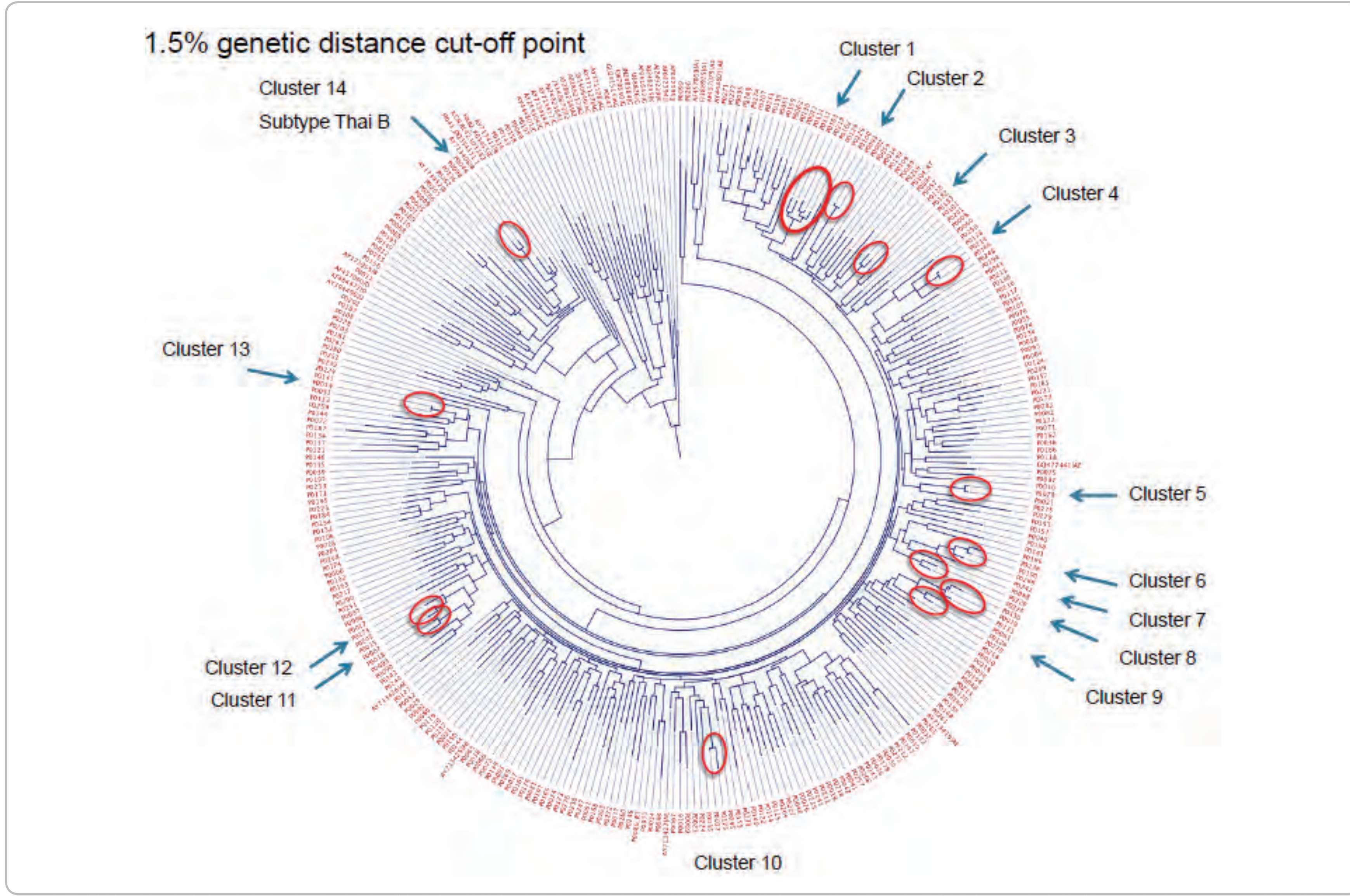
Figure 3.

Table 1.

Characteristics	Overall (n=273)	Linked transmission (n=33)	Non-Linked transmission (n=240)	p-values
Age, Median (IQR)	27 (23 - 32)	26 (22 - 30)	27 (23 – 33)	0.24
Gender, n(%)				0.37
Male	262 (96)	33 (100)	229 (95)	
Female	11 (4)	-	11 (5)	
MSM, n (%)	254 (93)	33 (100)	221 (92)	0.14
Education, n(%)				0.82
Primary school/lower	8 (4)	-	8 (4)	
Secondary school	64 (28)	10 (32)	54 (28)	
Diploma	13 (6)	1 (3)	12 (6)	
Bachelor degree/higher	139 (62)	20 (65)	119 (62)	
Family income (USD/month)	1061 (606 – 1818)	1515 (606 -3030)	1061 (606 -1818)	0.27
Thai	268 (98)	33 (100)	235 (98)	1.0
Occupation				0.03
Not working	28 (10.3)	3 (9.1)	25 (10.5)	
Student	68 (25.0)	12 (36.4)	56 (23.4)	
Private business/Factory employee	101 (37.1)	14 (42.4)	87 (36.4)	
Soldier/police	2 (0.7)	1 (3.0)	1 (0.4)	
Government officer	20 (7.4)	2 (6.1)	18 (7.5)	
Sex worker	9 (3.3)	1 (3.0)	8 (3.4)	
Others	44 (16.2)	-	44 (18.4)	
4thG stage				0.13
1	36 (13)	4 (12)	32 (13)	
2	93 (30)	15 (45)	68 (28)	
3	154 (56)	14 (42)	140 (58)	
HIV-subtype				0.57
CRF01_AE	234 (85.7)	31 (93.9)	203 (85)	
CRF02_AG	1 (0.4)	-	1 (0.4)	
B	21 (7.7)	2 (6.1)	19 (7.9)	
C	1 (0.4)	-	1 (0.4)	
CRF01_AE/B recombinant	15 (5.5)	-	15 (6.3)	
B/C recombinant	1 (0.4)	-	1 (0.4)	
Days since HIV exposure	18 (14 - 25)	17 (13 - 20)	19 (14 - 25)	0.06
Alcohol use during HIV Exposure	64 (23)	7 (21)	57 (24)	0.83
Drug used during HIV Exposure	58 (21)	8 (24)	50 (21)	0.65
Number of sexual partner in the past month, median (Min-Max)	2 (1 – 20)	2 (1 – 20)	2 (1 – 15)	0.28
CD4 T cells (cells/μL)	371 (269 - 504)	362 (304 - 420)	378 (266.5 - 506.5)	0.62
HIV RNA in blood (log10copies/mL)	5.8 (5.2 - 6.7)	5.7 (5.3 - 6.7)	5.8 (5.2 - 6.7)	0.76

CONCLUSIONS

The predominance of CRF01_AE sequence clusters in our cohort of mostly MSM suggests local MSM transmission networks. The majority of Bangkok MSM with acute HIV infection in our cohort do not cluster, suggesting that numerous sexual transmission networks contribute to the ongoing epidemic of HIV among MSM in the city. The presence of HIV-1 strains from other regions indicates global transmission networks.