P-B3

No. 229

CRF01_AE and Subtype B Transmission Networks Cross Over; A New AE-B Recombinant Emerges in Japan

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Background

- The major circulating HIV-1 subtypes in Japan have been **subtype B (B) followed by CRF01_AE (AE),** with prevalences of 86% and 7%, respectively, in newly diagnosed HIV/AIDS cases.
- These two subtypes have distinct epidemiological characteristics; **B predominates in men who have sex with men (MSM), whereas AE is observed mostly in heterosexuals.**
- Transmission networks of these risk populations appear to be crossing over and diffusing. **In our newly diagnosed populations, there have been some cases that the subtypes were not consistent between *pol* and *env* regions**.
- We investigated potential AE-B recombinations and analyzed transmission networks in Japan.

Materials and Methods

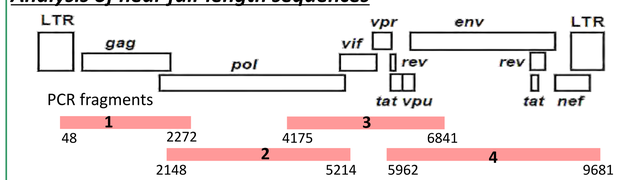
Patients

Newly diagnosed 1070 cases in Nagoya Medical Center from June 1997 to April 2012

Determination of Subtype (MEGA v5.2.1.)
Phylogenetic analysis using the neighbor-joining method (1000 bootstrap replicates)

- *gag* MA (396 bps)
- *pol* PR-RT (1017 bps)
- *pol* IN (864 bps)
- *env* C2V3 (222 bps)

Analysis of near full-length sequences



Determination of breakpoints (Simplot v3.5.1.)
Similarly plotting, Bootscanning, Informative site analysis

Estimation of time generating a CRF by a recombination event (BEAST v1.7.4.)
The time of the most recent common ancestor (tMRCA)

✓This study was conducted according to principles in the Declaration of Helsinki, and was approved by the human subject research committee at Nagoya Medical Center, Japan.
✓ All patients provided written informed consent for the collection of samples and subsequent analyses.

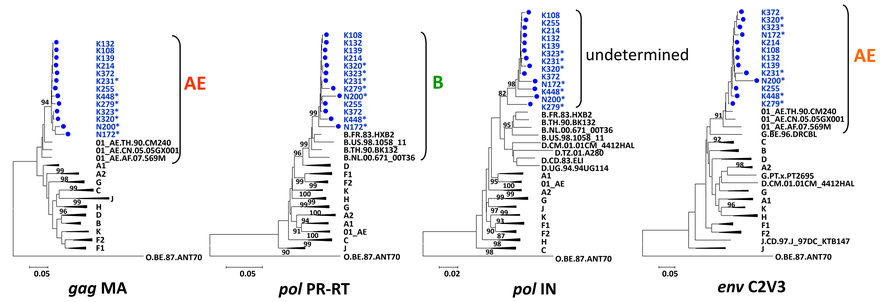
Demographics and clinical characteristics of patients infected with HIV-1 AE-B recombinant form (n=13)

Patient #	Sampling year	Sex	Age	Nationality	Risk factor for infection	CD4 Cell count (Cells/μL)	HIV-1 Viral Load (Copies/mL)
K231*	2003	M	24	Japan	Homo	556	24000
K279*	2003	M	27	Japan	Homo	394	210000
K320*	2004	M	30	Japan	Homo	277	350000
K323*	2004	M	23	Japan	Homo	302	18000
K448*	2005	M	28	Japan	Homo	40	2000000
N172*	2010	M	33	Japan	Bi	37	6200
N200*	2010	M	35	Japan	Hetero	8	397000
K108	2000	M	38	Japan	Homo	273	31000
K132	2001	M	25	Japan	Bi	327	53000
K139	2001	M	27	Japan	Bi	542	33000
K214	2002	M	28	Japan	Homo	365	36000
K255	2003	M	26	Japan	Homo	452	420000
K372	2004	M	28	Japan	Homo	108	11000

*Samples analyzed their near full-length sequences in this study.

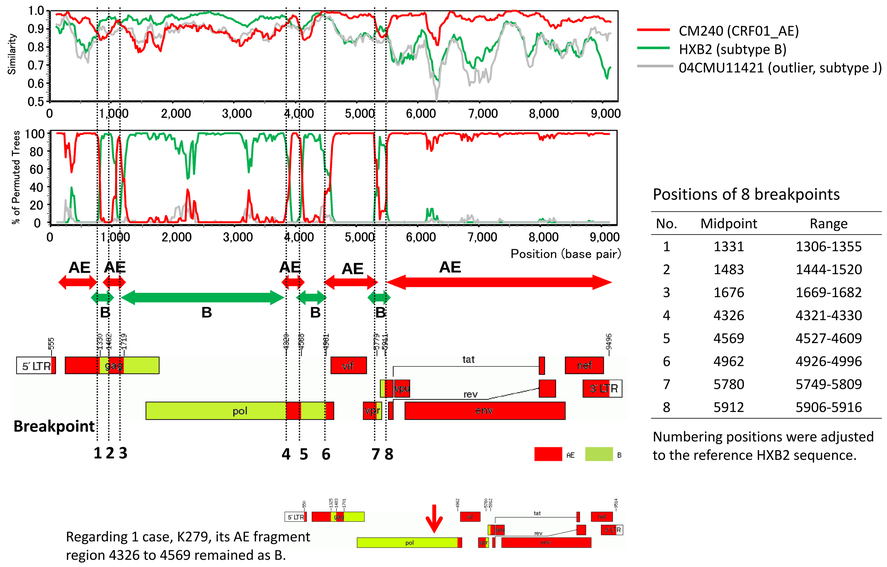
Results

1) Phylogenetic trees of HIV-1 isolates identified in this study



13 cases (blue filled circles and letters) were assumed as AE (*gag*)/B (*pol*)/AE (*env*) recombinant HIV-1. We analyzed 7 cases of them for the near full-length sequences.

2) Determinations of breakpoints of HIV-1 AE-B recombinants



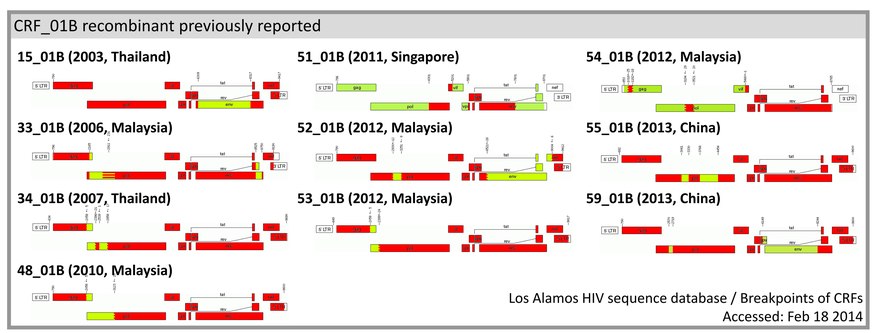
Regarding 1 case, K279, its AE fragment region 4326 to 4569 remained as B.

Positions of 8 breakpoints

No.	Midpoint	Range
1	1331	1306-1355
2	1483	1444-1520
3	1676	1669-1682
4	4326	4321-4330
5	4569	4527-4609
6	4962	4926-4996
7	5780	5749-5809
8	5912	5906-5916

Numbering positions were adjusted to the reference HXB2 sequence.

6 cases shared an identical recombination structure of the AE-B strains, which differs from any previously reported types. They were designated as **CRF 69_01B** by the Los Alamos HIV sequence database.



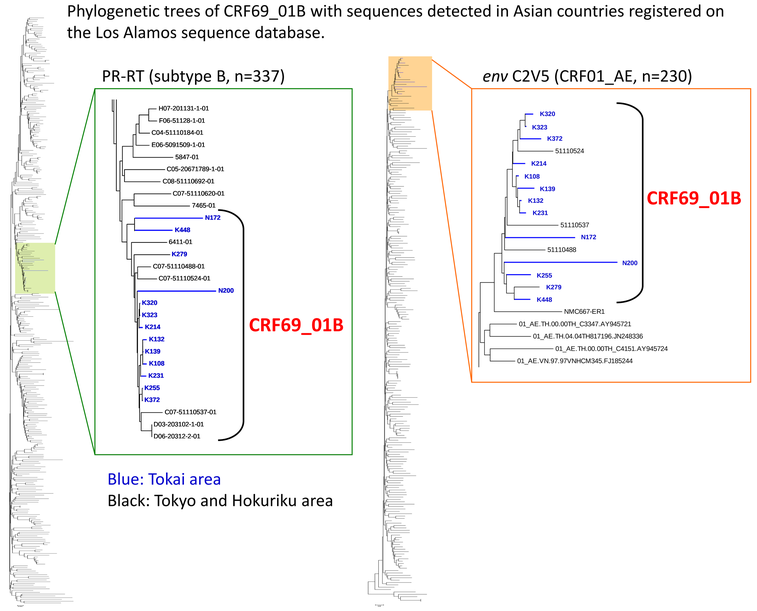
3) The time of the ancestral recombination event originating in CRF69_01B

Bayesian MCMC evolutionary parameters for HIV-1 novel CRF in Japan

Regions (according to HXB2)	Substitution rate	Coefficient of Variation	tMRCA of the CRF (txy)	tMRCA of the related group (tx or ty)
pol PR-RT (2253-3269)	2.6 (3.3 - 2.5)	0.48 (0.37-0.58)	1997.2 (1994.3-1999.9)	1993.6 (1989.1-1997.7)
env C2V5 (6895-7653)	10.7 (12.0 - 9.3)	0.41 (0.33-0.48)	1997.5 (1995.7-1998.12)	1993.8 (1990.2-1996.11)

Nucleotide substitution rates are expressed in 10-3 substitutions per year per site. 95% highest posterior densities are shown in parentheses.

Phylogenetic trees of CRF69_01B with sequences detected in Asian countries registered on the Los Alamos sequence database.



tMRCAs for *pol* PR-RT region (B) and *env* C2V5 region (AE) were estimated both from 1993 to 1997. The clade of CRF69_01B in *pol* region was constructed with sequences isolated in Japan only. A similar conclusion was obtained in *env* region.

Conclusions

- A new recombinant strain composed of AE and B, **CRF69_01B**, was identified in Japan.
- tMRCA analysis revealed that **CRF69_01B emerged between 1993 and 1997**, soon after AE was introduced from neighboring countries in mid 1990s.
- Phylogenetic analyses indicated that the AE and B fragments of CRF69_01B are genetically close to AE and B in Japanese heterosexual and MSM populations, respectively. This suggests that **the recombination seems to take place in the Japanese populations.**
- Previous epidemiological data describing distinct socio-epidemiological characteristics for Japanese MSM and heterosexual populations, our data indicate **crossing over between the two populations**.
- The knowledge of epidemic strains is important for diagnosis and treatment of HIV/AIDS, it should be monitored through continuous surveillance of HIV-1 subtypes.