HIV-2 CRF01_AB: First Circulating Recombinant Form of HIV-2

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Background: Five HIV-2–seropositive cases were recently identified in Japan, outside the HIV-2 endemic area of West Africa. To clarify the molecular epidemiology of HIV-2 in Japan, we analyzed sequences of these cases in detail.

Methods: HIV-2 genetic groups were determined by *gag* and *env* sequences. For suspected recombinant isolates, the genetic structure was determined by full-length genomic analyses. To understand the history and evolution of HIV-2 recombinant isolates, we estimated the time of most recent common ancestor by Bayesian Markov chain Monte Carlo method.

Results: Three isolates were determined as recombinants of groups A and B, and their mosaic genome structures were identical with that of 7312A, a recombinant isolate reported in 1990 from Côte d'Ivoire. Our 3 isolates and 7312A fulfilled the criteria for determining a circulating recombinant form (CRF). These isolates were verified by the Los Alamos HIV sequence database as the first CRF of HIV-2, HIV-2 CRF01_AB. The mean time of most recent common ancestor of CRF01_AB was estimated as between 1964 and 1973, several decades after the estimated emergence of HIV-2.

Conclusions: We recently identified HIV-2 CRF01_AB cases in Japan. This ectopic observation of the virus outside its original endemic area suggests an ongoing global spread of HIV-2 CRF01_AB.

Key Words: circulating recombinant form, CRF01_AB, HIV-2, molecular epidemiology

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INTRODUCTION

One million people worldwide are infected with HIV-2. The distribution of HIV-2, unlike the global epidemic of HIV-1, is still mainly restricted to West Africa and several European and Asian countries.[1–4] HIV-2 has been characterized as less pathogenic than HIV-1,[5–11] with more than 75% of HIV-2–infected cases remaining asymptomatic throughout their clinical course.[4] HIV-2 can be genetically classified into 8 groups, A to H, which have equivalent genetic distances to those of HIV-1 groups but not subtypes, with groups A and B circulating in the human population.[12–16] In addition, 2 different AB recombinants (7312A and 510-03) have been identified in West Africa,[12,13,17–19] but their circulation has not been identified to date.

In Japan, only 2 HIV-2–infected cases have been reported, but both were infected abroad.[20,21] Inside the country, there has been no evidence of HIV-2 transmission and circulation. Here we report 5 HIV-2–infected cases recently identified in Japan. Of these 5 cases, 3 were shown by full-length genomic analysis to be infected with the same type of recombinant virus determined to be the first circulating recombinant form (CRF) of HIV-2.

METHODS

HIV-2 Samples and Quantification of HIV Plasma Viral Loads

Among 843 HIV/AIDS cases registered at the Nagoya Medical Center (NMC), Japan from 1994 to 2008 (for demographic characteristics, see **Table, Supplemental Digital Content 1**, http://links.lww.com/QAI/A49), 5 cases (3 males and 2 females) were diagnosed serologically as HIV-2 infected. To better understand the molecular epidemiology of HIV-2 infection in Japan, we analyzed the HIV-2 genetic groups of the 5 cases.

Plasma HIV-1 viral loads were measured by the Cobas Amplicor HIV-1 monitor test v1.5 (Roche Diagnostics, Tokyo, Japan) or the Cobas TaqMan HIV-1 test (Roche Diagnostics),

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whereas plasma HIV-2 viral loads were measured by an in-house quantification assay, the Poisson quantification method described elsewhere.[22,23] In brief, total RNA was extracted from 500 μL of plasma sample using the QIAamp UltraSens Virus Kit (QIAGEN, Tokyo, Japan). Reverse transcription (RT) and nested polymerase chain reaction (PCR) (RT-nested PCR) were performed using serially diluted RNA samples, and HIV-2 viral loads were statistically calculated using results from samples diluted to near the endpoint. (For details of RT-nested PCR reaction mixtures and thermal programs, see **Table, Supplemental Digital Content 2**, http://links.lww.com/QAI/A50).

Genomic DNA Sequencing

HIV-2 proviral DNAs were purified from peripheral blood mononuclear cells using the DNA blood mini kit (QIAGEN). To determine HIV-2 genetic groups, *gag* (777 bps: 1163 to 1939 according to SIVmac239) and *env* (454 bps: 7300 to 7753) gene fragments were amplified by nested PCR using LA Taq polymerase (Takara Bio, Shiga, Japan) and previously reported[13,24] primers: gagA, gagB, gagC, and gagF for *gag*, and PFD1, LTR9574, EB2, and EB5 for *env*. To determine full-length genomic sequences, 4 DNA fragments containing (1) 5′ long terminal repeat (LTR) (915 bps: 31 to 945), (2) *gag* to *nef* genes (9122 bps: 899 to 10020), (3) 3′ LTR (791 bps: 9463 to 10252), and (4) the joining point of the circular 2 LTR form (597 bps: 10085 to 10279 and 1 to 402) were amplified by nested PCR using 8 primer pairs (see **Table, Supplemental Digital Content 3**, http://links.lww.com/QAI/A51). The following PCR program was used: denaturation (2 minutes at 94°C) followed by 40 cycles of PCR (94°C: 15 seconds, 60°C: 30 seconds, and 70°C: 1 minute/1000 bps). Sequencing was performed using a 3730 DNA Analyzer (Applied Biosystems, Tokyo, Japan).

Phylogenetic Tree Analysis and Determination of Recombinant Genome Structures

Multiple sequence alignment was performed using CLUSTAL W, and genetic distances were calculated based on the maximum composite likelihood model using MEGA software v4.[25] Phylogenetic trees were constructed using the neighbor-joining method.

Complete full-length genomic sequences of 4 HIV-2 group A strains (ALI, BEN, CAM2CG, and UC2), 3 HIV-2 group B strains (D205, EHO, and UC1), and SIVmac239, (a rhesus macaque-adapted simian immunodeficiency viral isolate) were used as reference sequences. After realigning the sequence set, recombinant breakpoints were determined by similarity plotting, bootscanning, and informative site analysis using SimPlot software, v3.5.1.[26]

Estimated Times of the Most Recent Common Ancestors

Evolutionary rates, chronological phylogenies, and other evolutionary parameters were estimated from 17 full-length or near full-length HIV-2/SIV genomic sequences (see **Table, Supplemental Digital Content 4**, http://links.lww.com/QAI/A52) using the Bayesian Markov chain Monte Carlo (MCMC) method implemented in BEAST v1.4.8.[27] The alignment data for the full-genome sequences were processed into 2 subsets consisting of sequences corresponding to the group A or B region of HIV-2 AB-recombinant virus. Bayesian MCMC analyses were performed using a relaxed molecular clock model.[28] The nucleotide substitution model was evaluated by the hierarchical likelihood ratio test using PAUP v4.0 beta[29] with MrModeltest (Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University), and the general time-reversible model[30] was adopted with both invariant sites and gamma-distributed site heterogeneity for 4 rate categories. The coalescent model used in the analyses was a logistically growing population because the population size of HIV-2 seemed constant in the early phase followed by exponential growth in the recent period.[31] Each Bayesian MCMC analysis was run for 40 million states and sampled every 10,000 states. Posterior probabilities were calculated with a burn-in of 4 million states and checked for convergence using Tracer v1.4. The posterior distribution of the substitution rate obtained from the heterochronous sequences was subsequently incorporated as a prior distribution for the evolutionary rate of HIV-2 genome regions A and B, thereby adding a timescale to the phylogenetic histories of the HIV-2 strains and enabling the times of most recent common ancestor (tMRCAs) to be estimated.[32]

Accession Numbers

Nucleotide sequences have been registered as #AB499685 to AB499695 in the DNA databank of Japan.

TABLE 1. Demographic and Clinical Characteristics of Patients Diagnosed as HIV-2 Infected

Patient #	Year	Sex	Age (Yrs)	Nationality	Risk Factor for Infection	$CD4^+$ Cell Count (Cells/μL)	HIV-1 Viral Load (Copies/mL)*	HIV-2 Viral Load (Copies/mL)	Western Blot†		Opportunistic Infections
									HIV-1	HIV-2	
NMC307	2004	M	28	Nigerian	Hetero	241	<50	350,000	I	P	Tuberculosis
NMC678	2007	F	28	Japanese	Hetero	883	<50	ND	I	P	—
NMC716	2007	M	36	Nigerian	Hetero	4	<50	680,000	I	P	Candidiasis
NMC786	2008	M	38	Ghanaian	Hetero	1	<40	60,000	N	I	Candidiasis, CMV infection
NMC842	2008	F	34	Japanese	Hetero	110	<40	25,000	N	P	—

*Detection limits of Cobas Amplicor HIV-1 monitor v1.5 and Cobas TaqMan HIV-1 tests were 50 and 40 copies/ml, respectively.
†New LAV Blot I and II kits (Bio-Rad Laboratories, Tokyo, Japan) were used.
CMV, cytomegalovirus; F, female; Hetero, heterosexual contact; I, intermediate; M, male; ND, not detected; N, negative; P, positive.

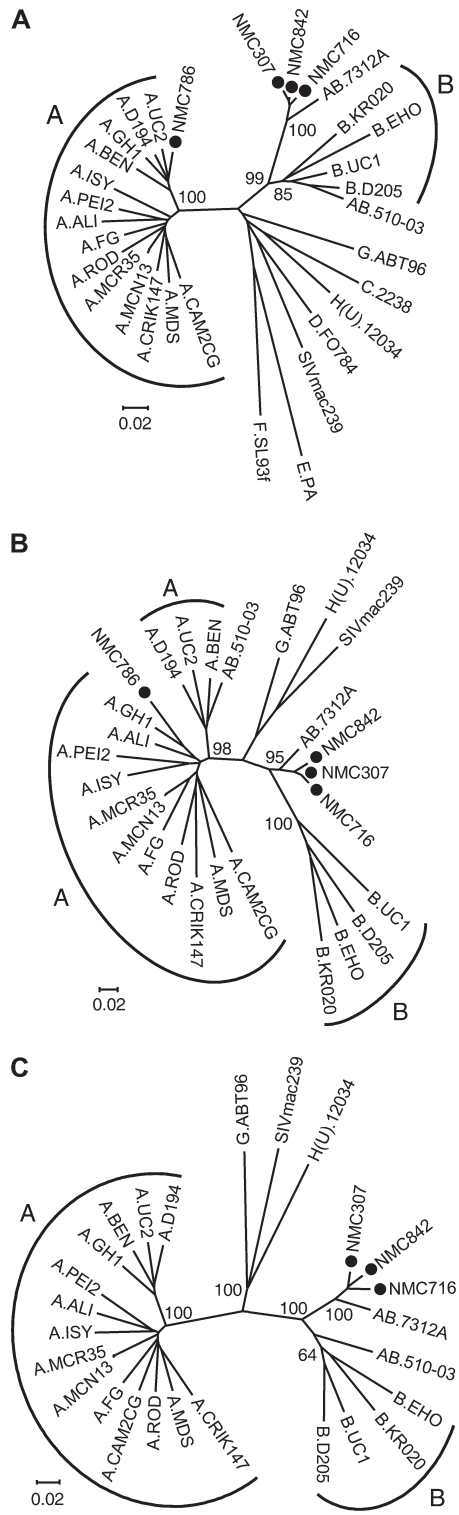

FIGURE 1. Phylogenetic tree analyses of HIV-2 isolates identified in this study. Phylogenetic tree analyses are shown using the following: A, HIV-2 *gag* gene sequences (bps: 1163 to 1939 in the reference SIVmac239 sequence); B, *env* gene sequences (bps: 7300 to 7753); and C, full-length or near full-length genomic sequences. Phylogenetic trees were constructed by the neighbor-joining method. Bootstrap values were calculated by 1000 analyses and are shown at the major tree nodes. Scale bar represents 0.02 nucleotide substitutions per site. Each reference HIV-2 strain is represented by its genetic group and name. HIV-2 isolates identified in this study (NMC307, NMC716, NMC786, and NMC842) are shown by filled circles.

RESULTS

HIV-2 Infection Confirmed by Nucleotide Amplification in Four AIDS Cases

Profiles of 5 HIV-2–seropositive cases are summarized in Table 1. The 3 males were from West African countries, a major endemic area for HIV-2, and suspected as seropositive before arriving in Japan. However, 2 females, both Japanese, were suspected to be recently infected within Japan based on their interviews. All their risk factors were heterosexual contacts, and no personal connection was confirmed among any of these cases. Thus, these 5 cases were independently infected with HIV-2 on different occasions. Notably, 4 cases (NMC307, NMC716, NMC786, and NMC842) were found at advanced stage AIDS with low $CD4^+$ cell counts and high HIV-2 viral loads, accompanied by opportunistic infections (Table 1). One case (NMC678) was found at an asymptomatic stage with high $CD4^+$ cell count and undetectable viremia. HIV-1 RNAs were undetectable in all 5 cases, indicating that they were infected by HIV-2 alone.

The First Circulating Recombinant Form Discovered in HIV-2: HIV-2 CRF01_AB

HIV-2 genetic groups were determined by both *gag* and *env* sequences. We were successful in analyzing 4 AIDS cases, however, we failed to amplify these 2 genes and analyze in asymptomatic case NMC678. One isolate (NMC786) was clearly classified into group A in phylogenetic tree analysis (Fig. 1A, B). On the other hand, isolates NMC307, NMC716, and NMC842 formed an independent cluster with a reference AB recombinant isolate 7312A (Fig. 1A, B). To better understand the detailed genomic structures of the 3 suspected AB recombinants, full-length genomic sequences of the 3 cases were analyzed. In the phylogenetic tree with full-length or near full-length reference sequences (Fig. 1C), NMC307, NMC716, NMC842, and 7312A formed an independent cluster with a high bootstrap value of 100%, suggesting these 4 isolates are the same type of AB-recombinant virus.

We next compared their genomic structures. As shown in Fig. 2A, similarity plotting and bootscanning analyses revealed that the recombinant breakpoints of our 3 isolates perfectly matched those of 7312A. This finding was supported by subregion phylogenetic analyses (Fig. 2B). In conclusion, NMC307, NMC716, and NMC842 are AB-recombinant forms with a mosaic genome structure identical to that of 7312A, demonstrating that they are the same type of HIV-2 AB-recombinant form.

The minimum requirement for declaring a new CRF, as proposed by the Los Alamos HIV sequence database in 1999, is at least 3 cases with no direct linkage, accompanied with near full-length sequences.[33,34] These CRF nomenclature

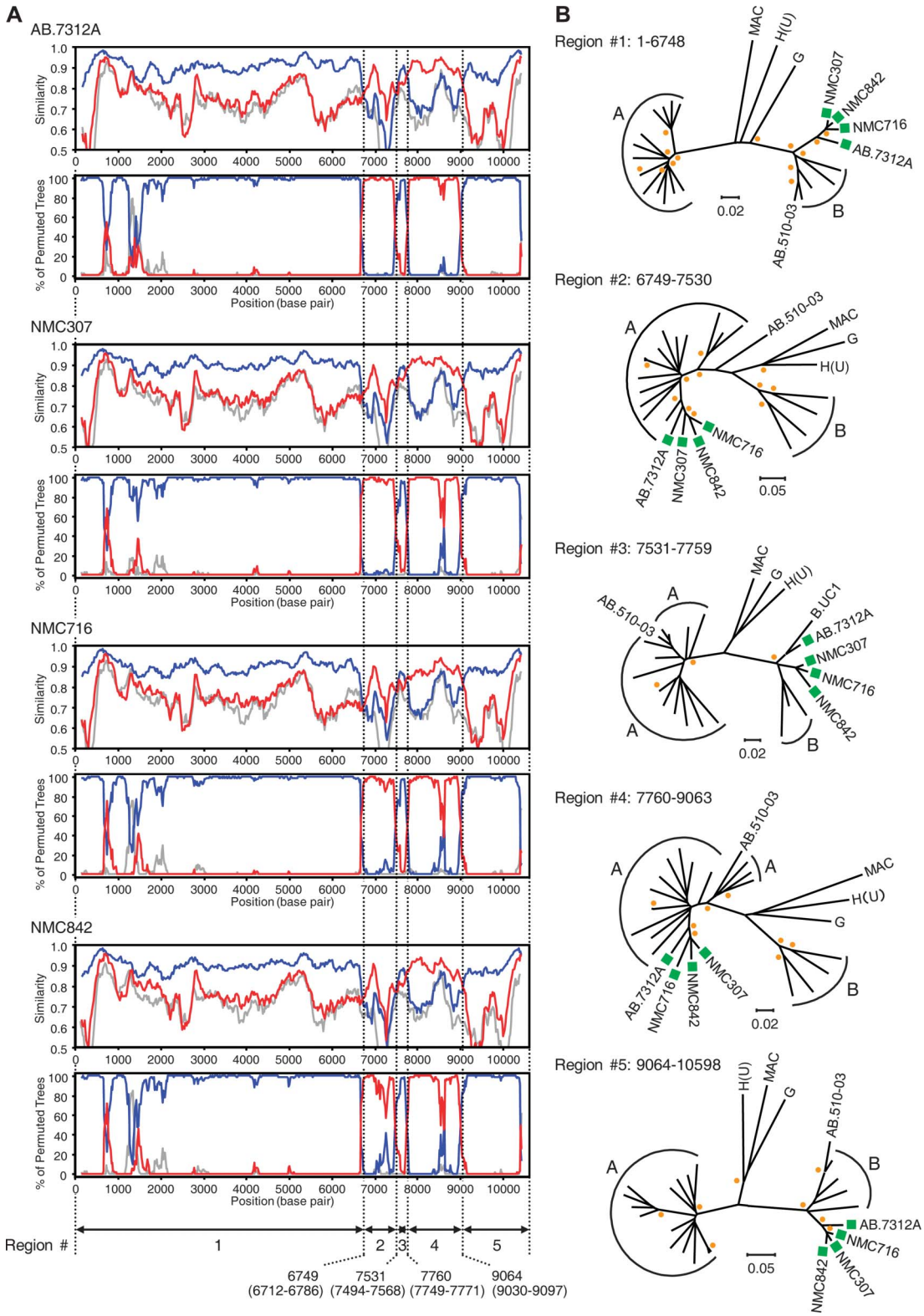

FIGURE 2. Determination of mosaic genome structures of HIV-2 AB recombinants. A, Similarity plotting (top) and bootscanning (bottom) data for each case of AB.7312A, NMC307, NMC716, and NMC842. Plots for consensus group A, consensus group B, and SIVmac239 are shown in red, blue, and gray, respectively. Both similarity plotting and bootscanning were performed with window and step sizes of 300 and 20 nucleotides, respectively. Bootscanning was performed using the neighbor-joining algorithm with 500 replicates. Each position of the 4 recombinant breakpoints is represented in the aligned sequence data set as the midpoint and

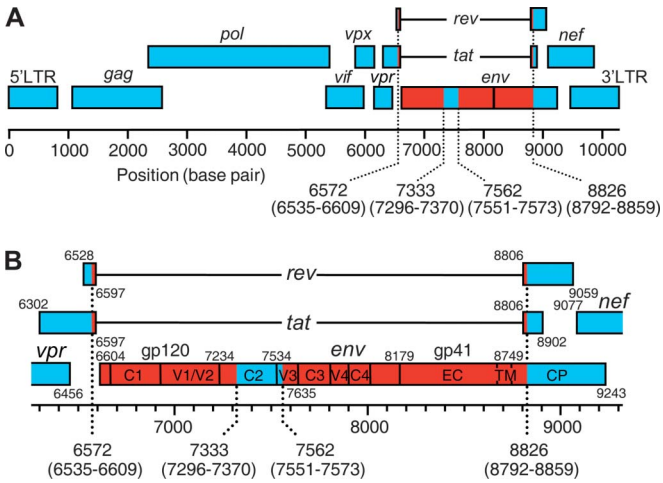

FIGURE 3. Schematic drawings for the genomic structure of HIV-2 CRF01_AB. A, Whole genomic structure; and B, Details around the *env* gene are represented. Regions belonging to group A and B are shown in red and blue, respectively. Numbering positions were adjusted to the reference SIVmac239 sequence.[35,36] Each position of 4 recombinant breakpoints is represented as the midpoint and range. C, constant region; CP, cytoplasmic domain; EC, extracellular domain; gp, glycoprotein; TM, transmembrane domain; V, variable region.

requirements are perfectly fulfilled with full-length genomic sequence information for 4 cases independently infected on different occasions with the AB recombinant identified by us and others.[12,13,19] Our data were carefully reviewed by editors of the Los Alamos HIV sequence database and confirmed as the first CRF discovered in HIV-2. They decided that the least confusing and most consistent way to name this new strain was to call it HIV-2 CRF01_AB.

The genomic structure of CRF01_AB is shown in Fig. 3. Interestingly, all 4 recombinant breakpoints of the CRF were located near or within the *env* gene (Fig. 3A). Further detailed analysis revealed that CRF01_AB possessed a chimeric gp120 containing a backbone of group A and a partial C2V3 fragment of group B and a chimeric gp41 containing extracellular and transmembrane domains of group A and a cytoplasmic domain of group B (Fig. 3B).

CRF01_AB Emerged Approximately in the Mid 20th Century

To estimate the time of CRF01_AB emergence, the time of the most recent common ancestor (tMRCA) of the recombinant was calculated by the Bayesian MCMC method. The mean substitution rates per year for the group A and B regions were estimated as 2.22×10^{-3} and 1.64×10^{-3}, respectively (Table 2), and the mean tMRCAs for groups A and B were estimated from 1921 to 1929, and from 1909 to 1948, respectively (Table 3). Similar results[31,37] validate our estimations. Finally, the mean tMRCA of CRF01_AB was estimated from 1964 to 1973. As the emergent times for groups A and B were estimated in the early 20th century, several decades seem to have been required for CRF01_AB to emerge. Concerning the geographical origin of the recombinant form, 3 of 4 isolates (7312A, NMC307, and NMC716) were identified in West Africans from Côte d'Ivoire and Nigeria. As these 2 countries were reported as sites of an epidemic in HIV-2 group A and B strains,[38,39] the most likely geographical origin of CRF01_AB is the south coastal area of West Africa.

DISCUSSION

In this study, we identified 3 HIV-2 AB recombinants with the same recombination pattern as 7312A, an isolate reported in Côte d'Ivoire in 1990.[12,13,19] These 4 isolates are determined as the first CRF of HIV-2, named CRF01_AB. It is noteworthy that all 3 of our cases infected with CRF01_AB were found at the AIDS stage. Considering that more than 75% of HIV-2–infected cases have a prognosis of remaining asymptomatic throughout their lifetimes[4] and that few HIV-2–seropositive cases were reported in Japan in the last 2 decades, 3 HIV-2 cases in the AIDS stage infected with the same CRF and identified in the past 5 years is highly unusual. Regarding the incubation periods for AIDS development in the 3 cases, not much information was available except for NMC842. This case was found to be seronegative for HIV-1/2 when tested in 2000. Thus, this case seems to have developed AIDS at most within 8 years, same as the median incubation period for AIDS development in HIV-1 infections (7.7–12.3 years).[40–45] As for the other 2 cases (NMC307 and NMC716), they developed AIDS at 28 and 36 years old (Table 1), which is significantly younger than age 65, reported as the peak of death by HIV-2 infections.[46,47] Though the number of cases identified is still small, we are concerned that the CRF01_AB might have acquired higher pathogenicity through recombination and adaptation to humans. As shown in Figure 3B, CRF01_AB has a recombination in the C2V3 region, the site of the major determinant for anti-envelope host immune responses and a functional domain for the chemokine receptor–binding site. The chimeric structure in the C2V3 region may confer advantages in host immune escape and viral replication capacity.

According to tMRCA analysis of the 4 isolates, CRF01_AB is estimated to have emerged sometime between 1964 and 1973. Interestingly, the mean tMRCA of the 3 isolates collected at NMC was estimated from 1982 to 1995 (Table 3), a later estimate than that of the 4 isolates, suggesting ongoing selection and evolution of CRF01_AB through transmission which has been taking place from the era of the 7312A isolate to the NMC isolates.

In conclusion, we report here the first CRF of HIV-2, CRF01_AB. Although national borders worldwide have

range (bottom). B, Subregion phylogenetic tree analyses. Phylogenetic trees were individually constructed by the neighbor-joining method using 5 subregion sequences. The HIV-2 isolates identified in this study (NMC307, NMC716, and NMC842) and AB.7312A are shown by green filled squares. Bootstrap values were calculated from 1000 analyses, and values greater than 95% are shown as orange dots at tree nodes. Scale bar represents 0.02 or 0.05 nucleotide substitutions per site. MAC, SIVmac239.

TABLE 2. Parameters in Bayesian MCMC Analysis for HIV-2/SIV Phylogenetic Inferences

	Substitution Rate Per Year		Coefficient of Variation		Population Size	
Data Set	**Mean**	**95% HPD**	**Mean**	**95% HPD**	**Mean**	**95% HPD**
Group A Region	2.22×10^{-3}	6.86×10^{-4}–3.68×10^{-3}	0.173	0.076–0.293	405.2	98.3–830.2
Group B Region	1.64×10^{-3}	5.99×10^{-4}–2.87×10^{-3}	0.269	0.170–0.395	341.2	93.3–668.9
Combined*	1.87×10^{-3}	6.39×10^{-4}–3.32×10^{-3}	0.235	0.088–0.382	357.9	93.3–709.2

*Combined data were produced from the 2 subsets, "group A region" and "group B region," using a LogCombiner program.
HPD, highest posterior density.

TABLE 3. Estimated TMRCAs of Monophyletic Clades in the HIV-2/SIV Lineage

	Group A region		Group B region		Combined	
Data set	**Mean**	**95% HPD**	**Mean**	**95% HPD**	**Mean**	**95% HPD**
Clade						
NMC isolates*	1982	1960–1996	1995	1987–2002	1990	1974–2002
CRF01_AB†	1964	1933–1985	1973	1956–1986	1971	1949–1986
Group A	1921	1864–1963	1929	1882–1964	1927	1879–1964
Group B	1909	1837–1962	1948	1915–1973	1934	1879–1973
HIV-2/SIV	1818	1670–1923	1821	1697–1930	1822	1693–1926

*This clade consisted of our 3 CRF01_AB isolates: NMC307, NMC716, and NMC842.
†This clade consisted of all 4 CRF01_AB isolates: 7312A, NMC307, NMC716, and NMC842.
HPD, highest posterior density;
SIV, simian immunodeficiency virus.

become more porous than ever, it is still surprising that the same recombinant strain was harvested in Japan, an island nation remote from the original endemic area, West Africa. This ectopic observation of the virus outside its endemic area suggests an ongoing global spread of HIV-2 CRF01_AB.

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REFERENCES

1. Marlink R. Lessons from the second AIDS virus, HIV-2. *AIDS*. 1996;10: 689–699.
2. Schim van der Loeff MF, Aaby P. Towards a better understanding of the epidemiology of HIV-2. *AIDS*. 1999;13:S69–S84.
3. Bock PJ, Markovitz DM. Infection with HIV-2. *AIDS*. 2001;15:S35–S45.
4. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. *Trends Microbiol*. 2008;16:588–595.
5. Whittle H, Morris J, Todd J, et al. HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS*. 1994;8:1617–1620.
6. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science*. 1994;265: 1587–1590.
7. Kanki PJ, Travers KU, MBoup S, et al. Slower heterosexual spread of HIV-2 than HIV-1. *Lancet*. 1994;343:943–946.
8. Adjorlolo-Johnson G, De Cock KM, Ekpini E, et al. Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. *JAMA*. 1994;272:462–466.
9. Ota MO, O'Donovan D, Alabi AS, et al. Maternal HIV-1 and HIV-2 infection and child survival in The Gambia. *AIDS*. 2000;14:435–439.
10. O'Donovan D, Ariyoshi K, Milligan P, et al. Maternal plasma viral RNA levels determine marked differences in mother-to-child transmission rates of HIV-1 and HIV-2 in The Gambia. MRC/Gambia Government/ University College London Medical School working group on mother-child transmission of HIV. *AIDS*. 2000;14:441–448.
11. Schim van der Loeff MF, Jaffar S, Aveika AA, et al. Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS*. 2002;16:1775–1783.
12. Gao F, Yue L, White AT, et al. Human infection by genetically diverse SIV_{SM}-related HIV-2 in West Africa. *Nature*. 1992;358:495–499.
13. Gao F, Yue L, Robertson DL, et al. Genetic diversity of human immunodeficiency virus type 2: evidence for distinct sequence subtypes with differences in virus biology. *J Virol*. 1994;68:7433–7447.
14. Chen Z, Luckay A, Sodora DL, et al. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. *J Virol*. 1997;71:3953–3960.
15. Yamaguchi J, Devare SG, Brennan CA. Identification of a new HIV-2 subtype based on phylogenetic analysis of full-length genomic sequence. *AIDS Res Hum Retroviruses*. 2000;16:925–930.
16. Damond F, Worobey M, Campa P, et al. Identification of a highly divergent HIV type 2 and proposal for a change in HIV type 2 classification. *AIDS Res Hum Retroviruses*. 2004;20:666–672.
17. Ndembi N, Abraha A, Pilch H, et al. Molecular characterization of human immunodeficiency virus type 1 (HIV-1) and HIV-2 in Yaoundé, Cameroon: evidence of major drug resistance mutations in newly diagnosed patients infected with subtypes other than subtype B. *J Clin Microbiol*. 2008;46:177–184.
18. Yamaguchi J, Vallari A, Ndembi N, et al. HIV type 2 intergroup recombinant identified in Cameroon. *AIDS Res Hum Retroviruses*. 2008; 24:86–91.
19. Robertson DL, Hahn BH, Sharp PM. Recombination in AIDS viruses. *J Mol Evol*. 1995;40:249–259.
20. Kusagawa S, Imamura Y, Yasuoka A, et al. Identification of HIV type 2 subtype B transmission in East Asia. *AIDS Res Hum Retroviruses*. 2003; 19:1045–1049.
21. Utsumi T, Nagakawa H, Uenishi R, et al. An HIV-2-infected Japanese man who was a long-term nonprogressor for 36 years. *AIDS*. 2007;21: 1834–1835.
22. Kato S, Hanabusa H, Kaneko S, et al. Complete removal of HIV-1 RNA and proviral DNA from semen by the swim-up method: assisted

reproduction technique using spermatozoa free from HIV-1. *AIDS*. 2006; 20:967–973.
23. Kinai E, Hanabusa H, Kato S. Prediction of the efficacy of antiviral therapy for hepatitis C virus infection by an ultrasensitive RT-PCR assay. *J Med Virol*. 2007;79:1113–1119.
24. Damond F, Loussert-Ajaka I, Apetrei C, et al. Highly sensitive method for amplification of human immunodeficiency virus type 2 DNA. *J Clin Microbiol*. 1998;36:809–811.
25. Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol*. 2007;24: 1596–1599.
26. Lole KS, Bollinger RC, Paranjape RS, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol*. 1999;73: 152–160.
27. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol*. 2007;7:214.
28. Drummond AJ, Ho SY, Phillips MJ, et al. Relaxed phylogenetics and dating with confidence. *PLoS Biol*. 2006;4:e88.
29. Wilgenbusch JC, Swofford D. Inferring evolutionary trees with PAUP*. *Curr Protoc Bioinformatics*. 2003;Chapter 6:Unit 6.4.
30. Rodríguez F, Oliver JL, Marín A, et al. The general stochastic model of nucleotide substitution. *J Theor Biol*. 1990;142:485–501.
31. Lemey P, Pybus OG, Wang B, et al. Tracing the origin and history of the HIV-2 epidemic. *Proc Natl Acad Sci U S A*. 2003;100:6588–6592.
32. Pybus OG, Drummond AJ, Nakano T, et al. The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach. *Mol Biol Evol*. 2003;20:381–387.
33. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature proposal. In: Kuiken CL, Foley B, Hahn B, et al, eds. *Human Retroviruses and AIDS 1999*. Los Alamos, NM: Los Alamos National Laboratory; 1999:492–505.
34. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature proposal. *Science*. 2000;288:55–56.
35. Calef C, Mokili J, O'Connor DH, et al. Numbering positions in SIV relative to SIVMM239. In: Kuiken C, Foley B, Hahn B, et al, eds. *HIV Sequence Compendium 2001*. Los Alamos, NM: Los Alamos National Laboratory; 2001:171–181.
36. Lin G, Bertolotti-Ciarlet A, Haggarty B, et al. Replication-competent variants of human immunodeficiency virus type 2 lacking the V3 loop exhibit resistance to chemokine receptor antagonists. *J Virol*. 2007;81: 9956–9966.
37. Wertheim JO, Worobey M. Dating the age of the SIV lineages that gave rise to HIV-1 and HIV-2. *PLoS Comput Biol*. 2009;5:e1000377.
38. Pieniazek D, Ellenberger D, Janini LM, et al. Predominance of human immunodeficiency virus type 2 subtype B in Abidjan, Ivory Coast. *AIDS Res Hum Retroviruses*. 1999;15:603–608.
39. Zeh C, Pieniazek D, Agwale SM, et al. Nigerian HIV type 2 subtype A and B from heterotypic HIV type 1 and HIV type 2 or monotypic HIV type 2 infections. *AIDS Res Hum Retroviruses*. 2005;21:17–27.
40. Hessol NA, Koblin BA, van Griensven GJ, et al. Progression of human immunodeficiency virus type 1 (HIV-1) infection among homosexual men in hepatitis B vaccine trial cohorts in Amsterdam, New York City, and San Francisco, 1978–1991. *Am J Epidemiol*. 1994;139:1077–1087.
41. Veugelers PJ, Page KA, Tindall B, et al. Determinants of HIV disease progression among homosexual men registered in the Tricontinental Seroconverter Study. *Am J Epidemiol*. 1994;140:747–758.
42. UK Register of HIV Seroconverters Steering Committee. The AIDS incubation period in the UK estimated from a national register of HIV seroconverters. *AIDS*. 1998;12:659–667.
43. Pezzotti P, Galai N, Vlahov D, et al. Direct comparison of time to AIDS and infectious disease death between HIV seroconverter injection drug users in Italy and the United States: results from the ALIVE and ISS studies. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1999;20:275–282.
44. Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action. Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. *Lancet*. 2000;355:1131–1137.
45. Morgan D, Mahe C, Mayanja B, et al. HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? *AIDS*. 2002;16:597–603.
46. Poulsen AG, Aaby P, Larsen O, et al. 9-year HIV-2-associated mortality in an urban community in Bissau, west Africa. *Lancet*. 1997;349:911–914.
47. Berry N, Jaffar S, Schim van der Loeff M, et al. Low level viremia and high CD4% predict normal survival in a cohort of HIV type-2-infected villagers. *AIDS Res Hum Retroviruses*. 2002;18:1167–1173.