

Outbreak of a West African Recombinant of HIV-1 in Tashkent, Uzbekistan

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Objectives: This research describes the genetic diversity of HIV-1 in Uzbekistan.

Methods: During 2002 and 2003, blood from HIV-positive patients in Uzbekistan was collected, and part of the proviral *pol* gene and nearly full-length genomes were sequenced and analyzed.

Results: Among 142 Uzbek strains, most clustered genetically with the subtype A strain common in the former Soviet Union. Most of these subtype A–infected drug-naive subjects (65.6%) had an accessory drug resistance mutation, A62V, in the reverse transcriptase gene. Thirteen of the strains (9.2%) clustered with CRF02_AG, an HIV strain common in West Africa. People infected with CRF02_AG were all residents of Tashkent and sampled in 2002. The CRF02_AG strains were monophyletic and probably descended from a single ancestor. Two strains were recombinant between CRF02_AG and subtype A, with each having a different subtype structure. The CRF02_AG and the subtype A elements of the recombinants were monophyletic with Uzbek CRF02_AG and subtype A. New full-length genomes of 12 Uzbek strains suggested that neither the subtype A and nor the CRF02_AG strains in this epidemic were mosaics with other subtypes or circulating recombinant forms.

Conclusion: A genetic analysis of Uzbek HIV strains demonstrated the predominance of subtype A in the epidemic. An outbreak of a West African strain of HIV-1, CRF02_AG, occurred in Tashkent, Uzbekistan in 2002, however. The cocirculation of the 2 strains has resulted in new recombinants that are apparently unique to Uzbekistan.

Key Words: HIV-1 molecular epidemiology, former Soviet Union, injecting drug user epidemic, genetic diversity

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The HIV epidemic in former Soviet Union (FSU) countries is growing faster than it is in any other geographic region of the world, although it began there less than a decade ago.^{1–3} Between 1995 and 2001, the region reported one of the highest annual growth rates in HIV-infected cases; current estimates are that 1.2 to 1.8 million infected persons live in this region.^{2,3} Most transmission occurs among injecting drug users (IDUs) over a broad geographic area from Riga, Latvia in the west to Irkutsk, Russia in the east.^{4,5} An analysis of the molecular epidemiology demonstrates, however, that this is a single interconnected epidemic.^{6,7} Although HIV was initially concentrated in IDU networks, it is now spreading into heterosexual populations in several locations, such as Ukraine and the Russian Federation.^{7–9}

The genetic diversity of HIV-1 is extensive because of error-prone reverse transcription and recombination.¹⁰ Worldwide, HIV-1 can be classified into 9 genetic subtypes and 2 sub-subtypes.¹¹ Recombination between subtypes has generated many circulating recombinant forms (CRFs), with recombinants of defined structure spreading in populations; 17 different CRFs have already been identified.¹¹ The 2 most important in the pandemic are the Southeast Asian CRF01_AE and the West African CRF02_AG.^{12,13}

The HIV-1 genetic subtype infecting people in the FSU is, somewhat surprisingly, subtype A, otherwise common only in Africa.^{7,14,15} This distinguishes the epidemic from that in Europe, where subtype B is most common. Additionally, the strain of subtype A in the FSU is monophyletic, suggesting a common ancestor for all strains; this molecular form of subtype A is called FSU-A.¹⁶ Furthermore, the interstrain genetic diversity in the epidemic is unusually low even though the FSU epidemic has been growing for nearly a decade and covers an enormous geographic area.^{4,6}

The epidemic in Uzbekistan, still nascent, is concentrated among IDU networks, where there was an exponential rise in prevalence between 2001 and 2002.⁷ As such, it is a prototypic “concentrated” epidemic. To date, the genetic subtypes in Uzbekistan have been predominantly FSU-A, with a minority of subtypes B and D.⁷ The current report documents an outbreak of CRF02_AG, a West African virus, in Tashkent, Uzbekistan in 2002.

METHODS

Study Subjects

After receiving informed consent, blood was drawn from HIV-positive patients at the National AIDS Center in

Tashkent, Uzbekistan in 2002 and 2003. Blood was collected in CPT™ vacutainer tubes (Becton Dickinson), and the peripheral blood mononuclear cells (PBMCs) were purified, washed, and then frozen in 95% ethanol at -20°C. Cells were sent to the Navy Medical Research Unit No. 3 (NAMRU-3) in Cairo, Egypt for DNA extraction using QIAamp (QIAGEN, Valencia, CA). Under a different study protocol, samples were also collected from residents of rural villages in Cameroon and processed using the same procedures as were used for Uzbek samples.

Genotyping

Partial *pol* polymerase chain reaction (PCR) amplification from PBMC DNA was performed on a convenience sample of 142 using a nested strategy. The first-round amplification was done with 2 primers: Pro5F (5'-AGAAATTG CAGGGCCCCTAGGAA) and RT3474R (5'-GAATCTCT CTGTTTTCTGCCAG), using Amplitaq Gold (Applied Biosystems, Foster City, CA) and 2 mM of MgCl⁺⁺ in a total volume of 50 μL. The second-round amplification was completed using the following 2 primers: Pro3F (5'-AGAICAGAGCCAACAGCCCCACCA) and ProRT (5'-TTCCCCACTAACTTCTGTATGTCATTGACA). Both amplification reactions began with a "hot start" to activate the polymerase, followed by 45 cycles for 30 seconds at 94°C, 30 seconds at 55°C, and 1.5 minutes at 55°C. The amplicon contained the coding sequences for the protease protein and part of the reverse transcriptase (RT) protein, corresponding to nts 2253 through 3266 on HXB-2 (Genbank Accession No. K03455). Nearly full-length genome sequences were also amplified, and all were sequenced in the Applied Biosystems 3100 automated sequencer using Big Dye terminators (Applied Biosystems).^{17,18}

Analysis

A multiple alignment of the newly derived protease/RT sequences and full-length genome sequences with selected reference sequences was constructed, consisting of 1069 nts and 9914 nts, respectively. Phylogenetic trees were generated, and the consistency of branching order was evaluated using SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE modules of the Phylogeny Inference Package (V3.52c).¹⁹ Recombinant analysis used Simplot, version 3.4,²⁰ and alignment examination determined precise breakpoints.^{13,21} After breakpoint identification, each segment was extracted and analyzed phylogenetically to confirm the assignment of subtype; breakpoint locations were designated relative to HXB-2

(Genbank Accession No. K03455). The Kimura 2-parameter method was used to calculate pairwise genetic distances after removal of hypermutated strains. Analysis of the protease-RT sequences for mutations that might lead to resistance to antiretroviral drugs was also performed.²²

Nucleotide Sequence

Sequences from Uzbekistan were submitted to Genbank under accession numbers AY845716 through AY845857 (partial *pol*) and AY829203 through AY829214 (nearly full length) and those from Cameroon under AY847362 through AY847453 (partial *pol*).

RESULTS

There were 142 partial *pol* sequences of HIV-1 from Uzbekistan and 12 nearly full-length genome sequences. Most of the samples were collected in 2002 (72.5%) and were from male subjects (90.6%), IDUs (84.8%), and residents of the capital, Tashkent (64.6%) (Table 1). Phylogenetic analysis of the sequences revealed that the strains were mostly subtype A (88.0%) and clustered with 97BL006 from Belarus (Fig. 1).²³ This phylogenetic cluster of subtype A (FSU-A) has only been found in the FSU and not yet in any part of Africa (see Fig. 1).¹⁸ In addition to the subtype A strains, a significant minority were CRF02_AG (9.2%), a CRF common in West and West Central Africa and unexpected in Uzbekistan.^{7,12}

There were also 2 subtype B strains and 2 strains (02UZ813, 02UZ678) that clustered neither with other strains nor with each other. These strains proved to be recombinants between CRF02_AG and FSU-A. 02UZ813 was predominantly CRF02_AG, whereas UZ678 was predominantly subtype A (Fig. 2). The subtype analysis showed that the subtype A portions of the recombinants were monophyletic with FSU-A, supporting the hypothesis that the recombinants were formed locally.

Isolated secondary antiretroviral resistance mutations were found in some patient sequences but none that would confer resistance. One, A62V, is a secondary RT mutation that does not confer drug resistance by itself but is common among the subtype A-infected subjects; 82 (65.6%) had this mutation.²⁴ These strains are also monophyletic within subtype A in the phylogenetic analysis and thus are descended from a single ancestor (see Fig. 1).

The distribution of samples by subtype as well as demographic and risk factor information is shown in Table 1. The only male subject who reported having had sex with another man (MSM) was infected with subtype B. Neither sex

TABLE 1. Demographic and Risk Factor Distribution of Uzbek Study Subjects

	Total n (%)	Year		Sex		Risk Factor			City	
		2002 n (%)	2003 n (%)	M n (%)	F n (%)	IDU n (%)	Heterosexual	MSM n (%)	Tashkent n (%)	Other n (%)
Subtype A	125 (88.0)	87 (69.6)	38 (31.2)	112 (91.1)	11 (8.9)	82 (84.6)	15 (12.4)		50 (60.2)	33 (40.0)
CRF02	13 (9.2)	13 (100)	—	10 (83.3)	2 (16.7)	11 (91.7)	1 (8.3)		10 (100)	
A/CRF02	2 (1.4)	2 (100)	—	2 (100)	—	1 (100)			2 (100)	
Subtype B	2 (1.4)	1 (50)	1 (50)	2 (100)	—	1 (50)		1 (50)		1 (100)
Total	142	103 (72.5)	39 (27.5)	126 (90.6)	13 (9.4)	95 (84.8)	16 (14.3)	1 (0.9)	62 (64.6)	34 (35.4)

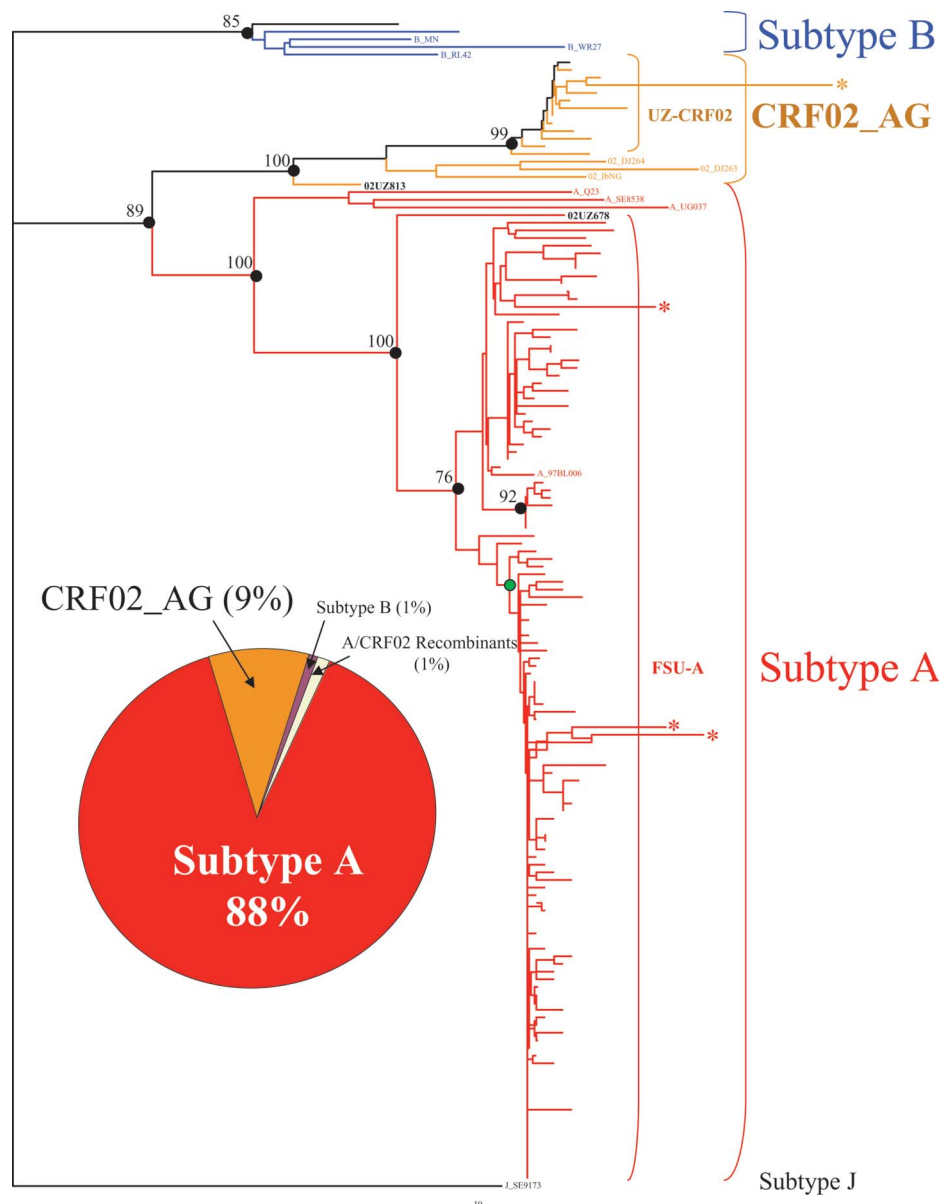


FIGURE 1. Phylogenetic analysis of 142 partial *pol* sequences of HIV-1 from Uzbekistan. A neighbor-joining phylogenetic tree was built, and significant bootstrap values (>70%) were placed next to the nodes. An open circle marks the node containing all the strains with the A62V mutation. The genetic distance corresponding to the lengths of the branches is shown by the scale below. Subtype B (blue), CRF02_AG (orange), and subtype A (red). Reference samples are those named, preceded by the subtype; 2 of the study samples (black) are also named. Asterisks indicate hypermutated strains. Inset: Pie chart of the subtype distribution of samples.

nor transmission risk factors distinguished those having subtype A from those with CRF02_AG, but both populations were primarily male (90.4%) and IDUs (85.3%) (see Table 1). Among the 15 patients with CRF02_AG or its recombinants, only 1 reported travel (to Russia) and none reported contact with foreigners. CRF02_AG was significantly concentrated in samples collected in 2002 ($P < 0.05$), however, and from patients reporting Tashkent as their residence ($P < 0.025$). The data suggest that the CRF02_AG outbreak began in 2002 among IDUs in Tashkent.

A comparison between CRF02_AG strains from this outbreak and CRF02_AG strains from rural Cameroon, with predominantly heterosexual transmission, showed significantly less variation among the Uzbek strains (0.8%) than among the Cameroon strains (5.1%) ($P < 0.001$; Fig. 3) and no statistical

difference from the diversity that exists among the subtype A strains (1.4%). The intersubject genetic distances between CRF02_AG strains from Uzbekistan, where injecting drug use is the predominant risk factor, were significantly lower than those between strains from sub-Saharan Africa.

A phylogenetic analysis of 12 selected full-length genomes from Uzbekistan consisted of 8 that were subtype A, 3 that were CRF02_AG, and 1 that was hypermutated subtype B (Fig. 4). Careful recombinant analysis of 11 of the strains demonstrated that all of the subtype A strains were entirely CRF02_AG and that all the CRF02_AG strains were entirely CRF02_AG. Up to this time, 3 full-length examples of FSU-A existed, 1 each from Belarus, Russia, and Ukraine; now, there are 11. In addition, the 3 full-length sequences of the CRF02_AG from Uzbekistan should make the epidemic easier to track.

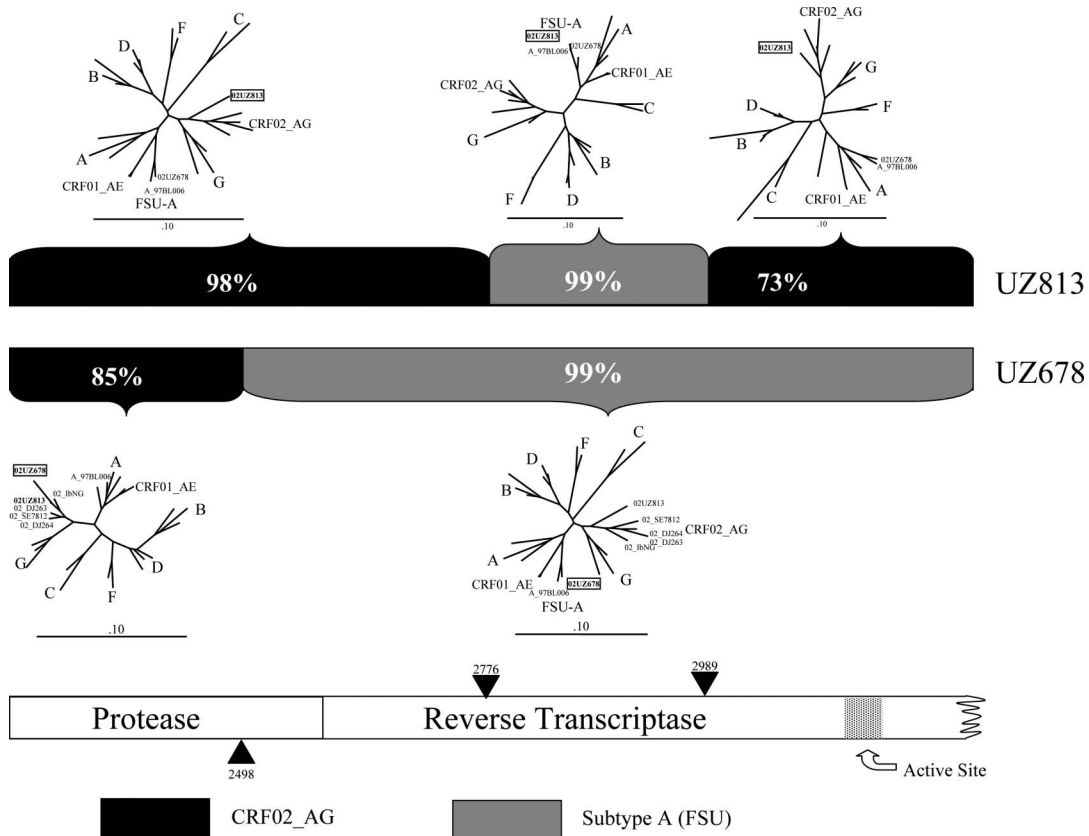


FIGURE 2. Subtype structure of 2 recombinants of subtype A and CRF02_AG. Subtype structure was determined by bootscanning, distance scanning, and visual inspection for informative sites.^{13,21} Confirmatory phylogenetic analyses for each segment are shown with bootstrap support for genetic assignment. The location of the coding region for protease and the coordinates for recombinant breakpoints (relative to HXB2) are shown below. Subtype A, gray; CRF02_AG, black.

DISCUSSION

The Uzbek HIV epidemic is dominated by the same subtype A found in other countries of the FSU—FSU-A. A similar finding based on partial env sequences has been previously reported, strongly suggesting that the most common genetic form in Uzbekistan is FSU-A.⁷ A significant proportion of the subtype A strains in this analysis (65.6%) carried an accessory resistance mutation which, in conjunction with other mutations, would confer resistance to nucleoside RT inhibitors.²⁴ The strains with this mutation form a monophyletic cluster, evidence of a point source introduction.

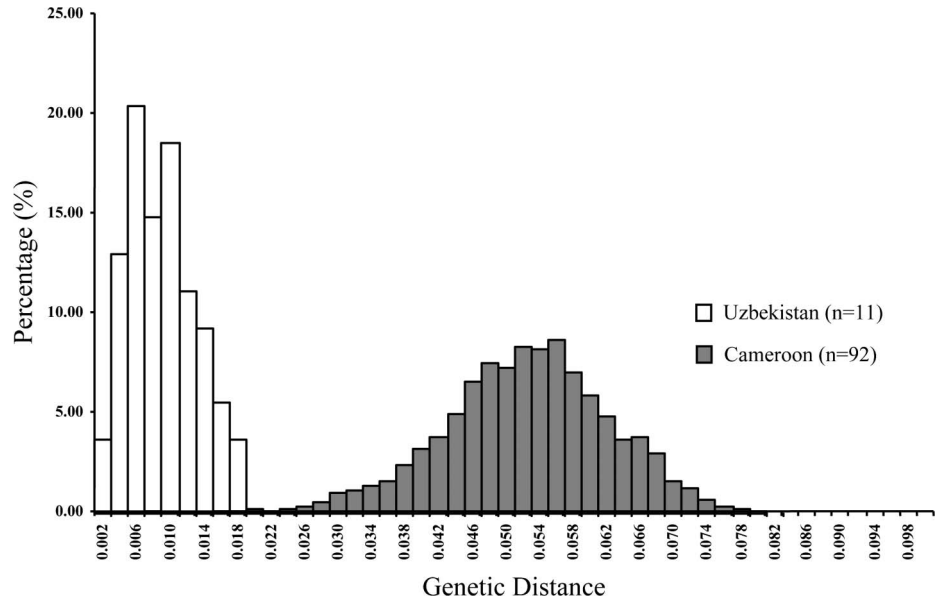
In this study of HIV-infected patients, a significant minority (9.2%) were infected with CRF02_AG, a recombinant common in West and West Central Africa.¹² CRF02_AG has also spread to a small number of people in Estonia, Korea, Taiwan, Ecuador, and Israel, but most of these epidemics have been associated with self-contained transmission clusters.^{25,26} The outbreak of CRF02_AG in Uzbekistan may have been a single contained transmission cluster at the time of this report, but recent evidence suggests further spread (M. D. Saah, personal communication, 2004). It is possible that the strain is, in essence, a molecular marker for 1 particular drug-using network. The interconnectedness of drug-using networks in

different countries of the FSU is not well understood, and the spread of this particular CRF02_AG strain might reveal some of those connections.

The presence of 2 unique recombinants of CRF02_AG and FSU-A suggests that 2 drug networks, 1 with CRF02_AG and 1 with FSU-A, may have overlapped to some extent. Unique recombinants are usually found in people who have been dually infected with both genetic forms; sampling from PBMC DNA in 1 such person over time has revealed either or both parental forms and a haphazard distribution of recombinants.²⁷ The close genetic relationship that each of the Uzbek recombinants has to existing Uzbek strains argues for a local origin of the recombinants in Tashkent, Uzbekistan.

Low intersubject genetic diversity characterizes HIV-1 from IDU epidemics in many countries and subtypes.²⁸ The low genetic diversity of the A epidemic in the FSU is, by far, the largest and the most enduring of these epidemics.⁶ It has been spreading since 1996 and now covers an immense territory. The Tashkent CRF02_AG outbreak in IDUs also has low diversity. HIV-1, like other retroviruses, is notoriously mutagenic, making approximately 1 error per replication cycle,¹⁰ with an estimated 10¹⁰ progeny virions produced each day in an untreated HIV patient²⁹; the mechanism by which

FIGURE 3. Histogram of the distribution of the intersubject pairwise genetic distances among CRF02_AG samples from Cameroon (gray) and Uzbekistan (white). Using the Kimura 2-parameter method of distance computation, all intersubject pairwise genetic distances were computed and the mean calculated. The proportion of the population in every distance interval is plotted.



viral populations in IDUs have remained relatively unchanged is difficult to understand and deserves more thorough and systematic investigation.

The outbreak of CRF02_AG in Tashkent demonstrates the rapid spread of new viruses into drug-using networks of the FSU. When a new strain enters a drug network, it begins to recombine with extant strains and to create new recombinants unique to that drug network.³⁰ Unfortunately, drug networks

provide the ideal means for rapidly spreading HIV within core groups and represent an emerging threat for the introduction of novel or recombinant strains into larger heterosexually active communities.

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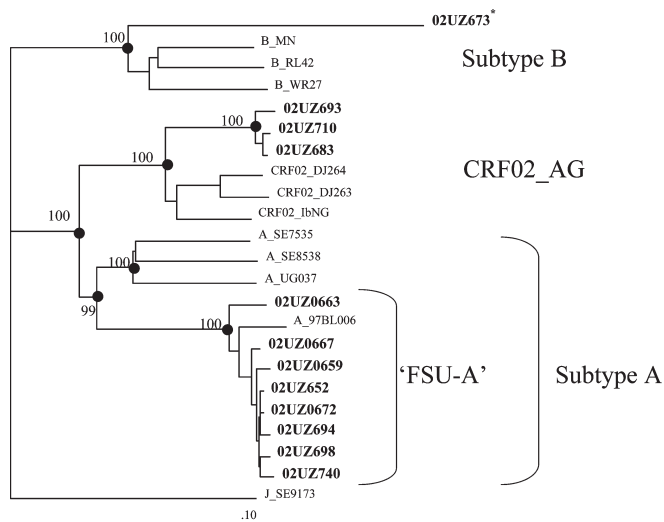


FIGURE 4. Phylogenetic analysis of 12 nearly complete genomes of HIV-1 from Uzbekistan. A neighbor-joining phylogenetic tree was built, and significant bootstrap values were placed next to the nodes. An open circle marks the node containing all the strains with the A62V mutation; an asterisk indicates that the strain that was hypermutated. The genetic distance corresponding to the lengths of the branches is shown by the scale below. Reference samples are those named, preceded by the subtype.

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