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Abstract

The Liangshan prefecture in Sichuan province is an area in China severely affected by the HIV epidemic, with intravenous drug use (IDU) as the main risk factor. No reports on HIV subtypes prevalent in IDUs in Liangshan prefecture could be found. In this study, we have characterized the genotypes of HIV-1 in the IDU

population in Liangshan prefecture and further determined the phylogenetic relationship of the CRF07_BC strains to HIV-1 sequences from the other regions of China, including Xinjiang and Yunnan provinces, to explore the pattern and possible diffusion pathway of HIV-1 in these regions. HIV-1-seropositive drug-naive IDUs identified in Liangshan prefecture, Sichuan province were enrolled in 2009. Full-length *gag* and *pol* genes were amplified by reverse transcription and nested PCR and then sequenced. All of the sequences were subtyped. Phylogenetic trees were constructed using the neighbor-joining and maximum likelihood methods. Divergence times were estimated using a Bayesian molecular clock approach. CRF07_BC was found to be the predominant strain in IDUs in Liangshan prefecture (95.5%). The CRF07_BC strains from Liangshan prefecture were found to be intermixed with those from Yunnan province in phylogenetic trees. The CRF07_BC sequences from Xinjiang province can be grouped into several clusters, suggesting that the expansion of the CRF07_BC epidemic in Xinjiang province was the result of a local epidemic driven by multiple independent introductions in the late 1990s. Only low-level drug-resistant viruses were found in the IDU population. CRF07_BC strains from Liangshan prefecture were more similar to those from Yunnan province than those from Xinjiang province. This finding will contribute to our understanding of the distribution, the evolution, and the potential source of CRF07_BC founder strains, and will also provide useful information for the development of strategies to prevent transmission.

Introduction

HIV DISPLAYS EXTRAORDINARY genetic diversity due to errors in reverse transcription during viral replication and its ability to generate recombinant strains in infected cells.[1–3] Four major groups of HIV-1, M, N, O, and P,

have been identified. Among them HIV-1 group M (HIV-M, major) is responsible for the current pandemic; it can be further classified into nine subtypes and more than 60 circulating recombinant forms (CRFs).[4] HIV-1 infections caused by CRFs have increased substantially in the past decade, and are now responsible for more than 20% of HIV-1 infections worldwide.[5–7] CRF07_BC is one of the most prevalent CRFs in China.[8–13] This CRF was first identified in the intravenous drug use (IDU) population in Xinjiang province in 1997,[8,9] but recent studies have shown that CRF07_BC has been introduced into the men who have sex with men (MSM) population, suggesting that it has spread to different regions of China and across different high-risk groups.[14,15]

Several studies were carried out in China to analyze the transmission and spread of the CRF07_BC strain, resulting in two hypotheses to address the temporal and spatial dynamic of the spread of CRF07_BC in the country. One hypothesis suggests that CRF07_BC originated in Yunnan and spread to Sichuan, Gansu, and finally to Xinjiang province along the drug trade route.[16] The other hypothesis highlighted the important role of Xinjiang province in the CRF07_BC epidemic and proposed that the transmission of both HIV and hepatitis C virus (HCV) from Xinjiang to Yunnan accompanied drug traffic routes.[17] However, both hypotheses were developed based on a small number of CRF07_BC sequences.

Recently, another study analyzing more sequences proposed that CRF07_BC was first introduced into Yunnan province and then into Xinjiang province along drug-transporting routes, and then spread into other provinces of China from Xinjiang.[18] However, a limitation of this study is that very few sequences were obtained and analyzed from Sichuan province, which is

located along the drug-transporting route between Yunnan and Xinjiang province. Analyzing a larger number of CRF07_BC sequences from Sichuan province would be needed to determine whether this diffusion pathway indeed took place.

Liangshan prefecture is an area located in Sichuan province with IDU known to be the main HIV diffusion pathway. It is located along the drug trafficking routes from the "Golden Triangle" to northwest and central China,[19–22] therefore it is not surprising to find a high prevalence of HIV-1 infection in Liangshan prefecture due to IDU.[23–25] The first HIV case in Liangshan prefecture was reported in 1995. In recent years, the incidence of HIV infection has increased significantly. According to a report from the Liangshan prefecture HIV epidemiological survey of the Chinese National Center for AIDS/STD Control and Prevention, of the total number of HIV/AIDS cases in Liangshan prefecture 81.5% were infected in the past 5 years. By the end of June 2009, the cumulative number of individuals infected with HIV in Liangshan prefecture was reported to be 15,700, including 528 AIDS cases and 700 deaths. Injection drug use was the main infection risk factor (unpublished data, China-MSD HIV/AIDS Partnership annual summary meeting, Liangshan prefecture).

Given the recent changing profile of the HIV epidemic with the shift of high-risk behaviors from IDU to sexual contact, it will be important to determine the recent sources of transmission within the region in IDUs, and also to illustrate whether the transmission is occurring within the population or from sources outside by other infected populations. More importantly, there is a need to determine whether the infected IDUs in the region could also serve as a source of transmission to other regions of China. Several studies have reported on the HIV epidemic in IDUs in Liangshan prefecture;

however, most of them focused on behavioral studies and there is so far no data on genetic characterizations of the transmitted HIV-1.

Genetic and temporal dynamic analyses have been important tools with which to study genetic diversity and phylodynamics. This has been used to reconstruct the history of the HIV epidemic and has provided important information to aid in the development of strategies for the prevention of HIV-1 transmission. Genetic analysis in the determination of the HIV genotype is a key to identifying the distribution of HIV-1 infection in the population and can potentially link this to the source of transmission. Therefore in an attempt to determine the potential source of transmission in the region, in this study we have characterized the HIV-1 strains in IDUs in Liangshan prefecture based on the full-length HIV-1 gag to determine the distribution of HIV-1 subtype infections in our study population and its relationship to HIV viral genotypes from other regions of China. We have also determined the viral pol gene sequence to investigate the existence of drug-resistant variants in the population.

Materials and Methods

Study subjects and specimens

HIV-positive drug-naive individuals were randomly recruited from the local HIV/AIDS sentinel surveillance sites in Liangshan prefecture with informed consents. The $CD4^+$ T cells counts were determined using flow cytometry and reagents provided by Becton Dickinson Biosciences (San Jose, CA) with fresh whole blood specimens before the plasma was separated; the plasma was then stored at $-80°C$; until use. The Ethical Review Board, Science and Technology Supervisory Committee at the Beijing Institute of Microbiology

and Epidemiology approved the study.

HIV-1 RNA extraction, amplification, and sequencing

Viral RNA was extracted from 500 µl of HIV-1-positive plasma specimens using a high pure viral RNA kit (Roche, Basel, Switzerland). Viral full-length gag and pol genes were amplified separately after reverse transcription and nested polymerase chain reaction (PCR) as described previously.[26] Positive PCR products were sequenced by the Beijing Liuhe Huada gene technology company (BGI, Beijing, China) with a variety of internal specific primers (available on request) after purification.

Genotyping and phylogenetic analysis of HIV-1 sequences

The sequenced fragments were edited and assembled as described.[27] To check for potential contamination, the sequences obtained were compared to all known sequences in the HIV sequence database and all previous sequences identified in our laboratory using the Basic Local Alignment Search Tool (BLAST) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences with 100% identity were considered to be contaminated. The HIV genotype was determined using the national center for biotechnology information viral genotyping tool (www.ncbi.nih.gov/projects/genotyping/formpage.cgi).

This was carried out by combining both gene fragments from the same isolate and then further confirmed by phylogenetic analysis with reference sequences using the neighbor-joining method in MEGA 5.0[28] and the maximum likelihood method in PhyML3.0.[29] For maximum likelihood analysis, the nucleotide substitution model was first selected with jModelTest software,[29,30] SPR was used for tree searching, and the

reliability of topologies was estimated by performing bootstrap analysis with 104 replicates. The possible intersubtype mosaicism was screened with the online Recombination Identification Program (version 3.0; http://hiv-web.lanl.gov) and further confirmed by the online software jpHMM-HIV (http://jphmm.gobics.de/).

Bayesian Markov chain Monte Carlo (MCMC) evolutionary analysis

MCMC analysis was fulfilled step by step as described.[31] First, all of recombinant sequences detected by using RDP software were removed. Second, the dataset was balanced to maximize the "clock-likeness" of the data. To do this, a similar number of sequences from each time point and as many time points as possible were selected. Samples from the most overrepresented time points (2009 year) were randomly removed. Third, Path-O-Gen software was used to test the existence of an actual molecular clock signal by observing the regression between root-to-tip divergence and sampling data. Fourth, the nucleotide substitution model was selected by using jModelTest software. Fifth, the best demographic model was selected using Bayes Factors criteria. Sixth, phylogenies were inferred using BEAST v.1.7.5 with models selected above and other default parameters. Simulations were run until convergence (i.e., an effective sample size greater than 200 for all parameters). The first 10% of the output was used as a burn-in. The results were summarized using Tracer v.1.5. Maximum clade credibility (MCC) trees were summarized using TreeAnnotator v.1.6.2, and the final trees were edited using Figtree v.1.3.1.[32]

Drug resistance analysis

Drug resistance mutations (DRMs) profiles and antiretroviral susceptibility were determined using the World Health Organization 2009 list of

mutations for surveillance of TDR as implemented in the Calibrated Population Resistance tool (v.5.0 beta)[33,34] (http://hivdb.stanford.edu).

Results

Characteristics of participants for the study

A total of 502 participants residing in Liangshan prefecture (Fig. 1) who were confirmed as having HIV in 2007 and 2008 were enrolled in the study in 2009 (Table 1). Most (97.4%) were infected through intravenous drug use. The average age of the participants was 31.1 years old (ranging from 3 to 62 years). The Yi ethnic minority is the main ethnicity in our study population. The CD4 T cell counts varied widely, ranging from 22 to 1,446 cells/μl. Among the 502 participants, 489 subjects were infected by HIV through IDU and were subjected to further detailed analysis.

View larger version

FIG. 1. Geographic location of Liangshan prefecture in China and area information of reference sequences. Map of China highlighting Liangshan prefecture, Sichuan province. Provinces containing reference sequences for CRF07_BC phylogenetic analysis are labeled in color according to that used in the maximum likelihood (ML) tree and maximum clade credibility (MCC) tree. Color images available online at www.liebertpub.com/aid

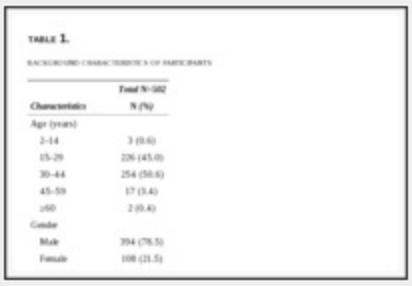

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TABLE 1. BACKGROUND CHARACTERISTICS OF PARTICIPANTS

Prevalence of HIV-1 subtypes

From 489 participants, 392 full-length gag genes (80.2%) and 284 full-length pol genes (58.1%) were successfully obtained. Among them, both gag and pol genes were identified from 280 samples (57.3%). Based on their sequences, the subtype variants from 401 participants (82.0%) were determined. The other samples failed to amplify either gene fragment possibly due to low viral load and/or sequence variations at the primer binding sites. A Basic Local Alignment Search Tool (BLAST) search showed no evidence of sample contamination. Analysis of sequences showed that all the gene structures were normal with the proper open reading frames.

According to the National Center for Biotechnology Information (NCBI) viral genotyping tool, 383 CRF07_BC (95.5%) strains, 12 CRF08_BC (3.0%) strains, 3 CRF01_AE (0.7%) strains, and 3 subtype C (0.7%) strains were identified from 401 positive samples. The subtype of each strain was further confirmed by phylogenetic analysis using the reference sequences representing subtypes A–D, F–H, J, K, CRF01_AE, CRF07_BC, and CRF08_BC (www.hiv.lanl.gov) (Fig. 2). Our results thus suggest that CRF07_BC is the dominant strain that is circulating in Liangshan prefecture.

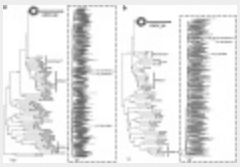

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FIG. 2. Phylogenetic tree analysis. Neighbor-joining trees were created with the full length *gag* **(a)** and *pol* **(b)** genes of HIV-1 sequences from the intravenous drug use (IDU) population in Liangshan prefecture and a selection of reference sequences of subtype A–D, F–H, J, K, CRF01_AE, CRF07_BC, and CRF08_BC (*black dots*, http://hiv-web.lanl. gov/). The lengths of the *gag* and *pol* genes of HIV-1 sequences obtained in the study were 1,503 and 3,056 base pairs using HXB2 as the genomic calibrator. Each reference sequence is labeled with the HIV-1 subtype, followed by the sequence name. The bootstrap values supporting the branch (more than 70%) are indicated at the corresponding nodes of the tree. The scale bars representing genetic distances are listed below.

Phylogenetic relationship among CRF07_BC strains prevalent in Liangshan prefecture and in other areas in China

Investigation of the geographic relationship between Liangshan CRF07_BC strains and those from other areas will be useful in determining the temporal dynamics of the CRF07_BC strain. For this purpose, the gag and pol gene fragments derived from same variant were first assembled for phylogenetic analysis to obtain the strongest phylogenetic signal. All CRF07_BC sequences covering the full-length gag and pol regions were downloaded from the HIV sequence database, including six from Xinjiang, one from Taiwan, five from Sichuan, three from Liaoning, one from Hebei, one from Guangxi, and five with the areas of origin uncertain. Considering that fewer sequences were obtained from other areas as compared to Liangshan, 86 CRF07_BC sequences (12 from Beijing, 19 from Guangxi, 1 from Henan, and 54 from Yunnan) obtained from another cohort in our laboratory were also added into the dataset. All sequences were aligned together along with four subtype C references as the outgroup. A maximum likelihood (ML) tree was constructed using the GTR+I+G model of nucleotide substitution.

In the phylogenetic tree, two clusters containing 10 or more sequences were identified; one is composed of sequences from Guangxi, Hebei, Yunnan, and Liaoning provinces (Cluster I) and the other is composed of sequences from Guangxi province (Cluster II), suggesting that HIV from specific populations in different regions is clustered (Cluster I) while others are unique for that region (Cluster II). Most sequences from Liangshan prefecture were distributed evenly in the phylogenetic tree and intermixed with those from Yunnan province, and only several small clusters containing three to seven sequences were identified.

Xinjiang province is considered a key area for the phylogenetic study of the CRF07_BC strain and has been proposed to be the source of the strain. However, few sequences coving full-length gag and pol segments can be found. To include CRF07_BC strains from Xinjiang province into the phylogenetic analysis, a new dataset containing short CRF07_BC sequences was constructed. For this purpose, all CRF07_BC sequences with geographic information were downloaded from the HIV sequence database (www.hiv.lanl.gov/content/index).

Sequences from different areas were separated and manually selected in order to maximize the length and the number of segments analyzed. Based on this criteria, 8 sequences from Beijing, 20 from Guangxi, 7 from Hebei and Henan, 8 from Yunnan, 8 from Liaoning, 1 from Taiwan, and 290 from Xinjiang spanning 1,473 bp of the gag gene (from 817 to 2,289 according to the HXB2 calculator) were selected. The distribution of Liangshan sequences in the ML tree was similar as shown in trees constructed with full-length gag–pol sequences (Fig. 3). Several large clusters were identified containing sequences isolated in Xinjiang province, suggesting that the CRF07_BC epidemic in Xinjiang province might be a result of multiple

introductions from Sichuan or Yunnan province.

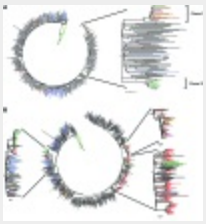

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FIG. 3. Evolutionary relationship between Liangshan HIV-1 CRF07_BC sequences from the IDU population and those from other areas in China depicted on a maximum likelihood tree of the full-length *gag–pol* gene **(A)** and partial *gag* gene **(B)**. **(A)** Full-length *gag–pol* sequences representing CRF07_BC from several areas were identified in the laboratory or obtained from the database (http://hiv-web.lanl.gov/) and used for comparison with the sequences generated in this study. Each reference sequence is labeled using the same color used in Fig. 1. The branch support values (more than 70%) are indicated at the corresponding nodes of the tree. The subtree containing clusters is illustrated at the right of the original tree. **(B)** The ML tree based on partial CRF07_BC *gag* sequences representing CRF07_BC from several areas is constructed to illustrate the evolutionary relationship between strains from Liangshan prefecture and Xinjiang province. The subtree containing clusters is illustrated accordingly. Color images available online at www.liebertpub.com/aid

Estimated timeline of the expansion of CRF07_BC in China

The final dataset for MCMC analysis was set up using 151 CRF07_BC sequences selected from the phylogenetic analysis dataset containing the 1,473-bp length gag gene (Fig. 4). The general time reversible (GTR) nucleotide substitution model, strict clock model, and Bayesian skyline demographic model were selected for MCMC analysis. In the MCC tree, nine clusters were observed. Among of them, eight clusters (Clusters 2–9) were composed of sequences from Xinjiang and the other one (Cluster 1) was composed of sequences from four other provinces or cities (Beijing, Hebei, Liaoning, and Guangxi). The tMRCAs of CRF07_BC were similar to those in previous studies.[16] All of the Xinjiang clusters were introduced into the area at the end of 1990s (between 1996 and 1999), which is similar to the time that CRF07_BC was introduced into Taiwan.[16] Cluster V was founded in the early 2000s, which is later than the other clusters.

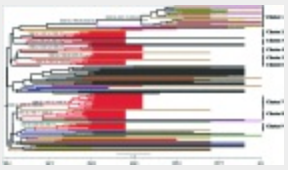

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FIG. 4. Maximum clade credibility trees of the HIV-1 CRF07_BC sequences from China based on the partial *gag* gene. All of the sequences are labeled using the same colors used in Fig. 1. The dates of MRCA and posterior probability are labeled beside each node. Color images available online at www.liebertpub.com/aid

Drug-resistant analysis

The 284 full-length pol genes obtained in the study were screened on line for the presence of transmitted drug-resistant mutations (see Materials and Methods). Because most of the drugs used for AIDS therapy in China are still limited to reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs), the RTI and protease transmitted drug-resistant mutations were analyzed separately. So far only 4 of the 284 strains under investigation showed major mutations (D67N, D103N, G190A, and T215I separately), which is known to be associated with drug resistance to RTIs (Table 2). The overall prevalence of mutations conferring resistance to RTIs was 1.4%.

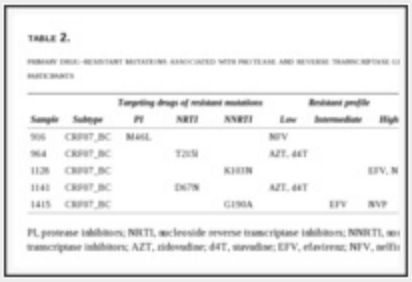

TABLE 2.

Sample	Subtype	Targeting drugs of resistant mutations: PI	NRTI	NNRTI	Resistant profile: Low	Intermediate	High
916	CRF07_BC	M46L			NFV		
964	CRF07_BC		T215I		AZT, 4AT		
1128	CRF07_BC			K103N			EFV, N
1141	CRF07_BC		D67N		AZT, 4AT		
1415	CRF07_BC			G190A		EFV	NVP

PI, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, no transcriptase inhibitors; AZT, zidovudine; 4AT, stavudine; EFV, efavirenz; NFV, nelfi

See full table

TABLE 2. PRIMARY DRUG-RESISTANT MUTATIONS ASSOCIATED WITH PROTEASE AND REVERSE TRANSCRIPTASE GENES AMONG TREATMENT NAÏVE PARTICIPANTS

To investigate protease-transmitted drug-resistant mutations, the amino acid sequence of each strain was compared to the subtype B consensus amino acid sequence using the published HIV drug resistance software tools from the International Aids Society USA (IAS-USA) and only a single major

mutation (M46L) was seen in one sequence from our collected viral sequences. The overall prevalence of mutations conferring resistance to PIs was 0.35%.

Discussion

Sichuan province is one of the epicenters of the HIV/STI epidemic in China. By 2008, there were 19,375 HIV infections reported in Sichuan province, which ranked sixth among all provinces/autonomous regions in China.[35] IDU was the primary diffusion pathway of HIV in Sichuan (75.6%).[36] Liangshan is the largest autonomous prefecture of the ethnic minority, the Yi nationality in China, with 46.5% of the population belonging to the Yi ethnic group. Among this group, cultural traditions lead to more frequent casual sexual behavior. Thus, heterosexual transmission from persons infected through IDU may become a major HIV transmission risk for the general population in the Liangshan prefecture. We believe this is the first large-scale molecular epidemiological study to analyze the spread of HIV-1 in IDUs in Liangshan prefecture, Sichuan province. CRF07_BC was shown to be the dominant strain. The detailed sequence information of CRF07_BC strains prevalent in Liangshan prefecture, Sichuan province will provide clues for the analysis of CRF07_BC temporal dynamics in China.

IDU is one of the major risk factors for HIV infection, which has led to approximately 5–10% of HIV infections worldwide.[37] In China, the number of drug users has consistently increased in the past decade. In 1997, the number of registered drug users was 70,000. In 2005, the number has increased to 1.16 million. However, the actual number could be as high as 3.5 million. Furthermore, the prevalence of HIV infection among drug users

had also increased from 35% in 1995 to 49% in 2004. Among the 780,000 people living with HIV/AIDS in China, 16.9% were infected through IDU (2011).[38] Although the ratio is currently not as high as for sexually transmitted individuals, most of the IDUs in China also engaged in high-risk sexual behaviors that led to a rapid spread of HIV between IDUs and other populations.[39] Between 34% and 72% of the female IDUs in China were reported to have commercial sex partners and 31.8% have nonregular sex partners; both groups had inconsistent condom usage.[40–42]

Several studies reported that many IDUs were also MSM in China[43,44] and to obtain drugs, some IDUs were also likely to trade sex for money,[45] which facilitated HIV-1 transmission from IDUs to other populations via heterosexual or MSM transmission (from male IDUs to MSM).[46] A number of international reports have pointed out that the overlap between injecting drug use behaviors and sexual risk behaviors is the main driving force of the HIV epidemic in China.[47,48] Thus, surveillance of the HIV epidemic in IDUs would be helpful for predicting the spread of the HIV epidemic in the general population.

There is still controversy about the hypothesis concerning the temporal dynamics of CRF07_BC in Asia. In this study, CRF07_BC strains from Liangshan prefecture and Yunnan province dispersed among all CRF07_BC strains in the phylogenetic tree; these are different from Xinjiang strains. Xinjiang CRF07_BC sequences were grouped into several clusters with a large genetic distance from other sequences and high bootstrap values. This suggests that multiple introductions of CRF07_BC strains into Xinjiang province occurred. The MCMC analysis further proved the results and demonstrated that most of the introductions happened at the end of the

1990s. Our data refuted the contention that CRF07_BC was first introduced into Xinjiang province and supported the contention that it was introduced into Yunnan and Sichuan provinces before being introduced into Xinjiang province. More sequences from Gansu, Ningxi, and Shanxi provinces, which are between Sichuan and Xinjiang provinces, will be helpful in absolutely demonstrating the temporal and geographic migration of CRF07_BC in China.

Highly active antiretroviral therapy (HAART) has been widely employed in China since the start of the "Four Free and One Care" policy in 2003. With the widespread use of HAART, HIV-1 drug resistance has become an issue in some areas of China. For example, in 2005, a high ratio of HIV drug-resistant mutations (20.8%) was reported in patients (n=124) treated with ART in Henan province, central China.[49] In the IDU population, the data from the Chinese National (HIVDR) Surveillance and Monitoring Network showed that 20.8% of individuals undertaking first line drugs experienced virological failure.[50] Here we found that a low level of drug-resistant strains was found in Liangshan, suggesting that the ART program in Liangshan is functioning well.

According to the WHO HIVDR Threshold Surveillance protocol, our study population was below the threshold of 5%, suggesting that there are well-functioning ART programs in Liangshan prefecture and the transmission of RTI and protease mutant viruses is still minimal. However, with the recent scale up of free HAART in Liangshan and the spread of HIV among different high-risk populations, drug resistance surveillance will still be needed in the region.

In summary, we have carried out a comprehensive survey and genetic

characterization of the HIV strains that are prevalent in the IDU population in Liangshan prefecture, which we believe is the most complete analysis to date. We found that CRF07_BC is still highly dominant in the IDU population, even 20 years after its initial identification in the first outbreak in China. High genetic diversity was found when compared to strains prevalent in other areas. A close relationship was identified between these strains and those from Yunnan province, and relatively low levels of drug-resistant mutations were found. Phylogenetic analysis showed that CRF07_BC strains should be introduced into Xinjiang province following drug traffic from Yunnan province, through Liangshan prefecture of Sichuan, Gansu, and Qinghai provinces. The detailed CRF07_BC sequence information will provide basic information for designing intervention strategies and for HIV vaccine design.

Sequence Data

The gene sequences were deposited in GenBank with the following accession number: KJ820145–KJ820408, KP234525-KP234916, and KP234917-KP235200.

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Author Disclosure Statement

No competing financial interests exist.

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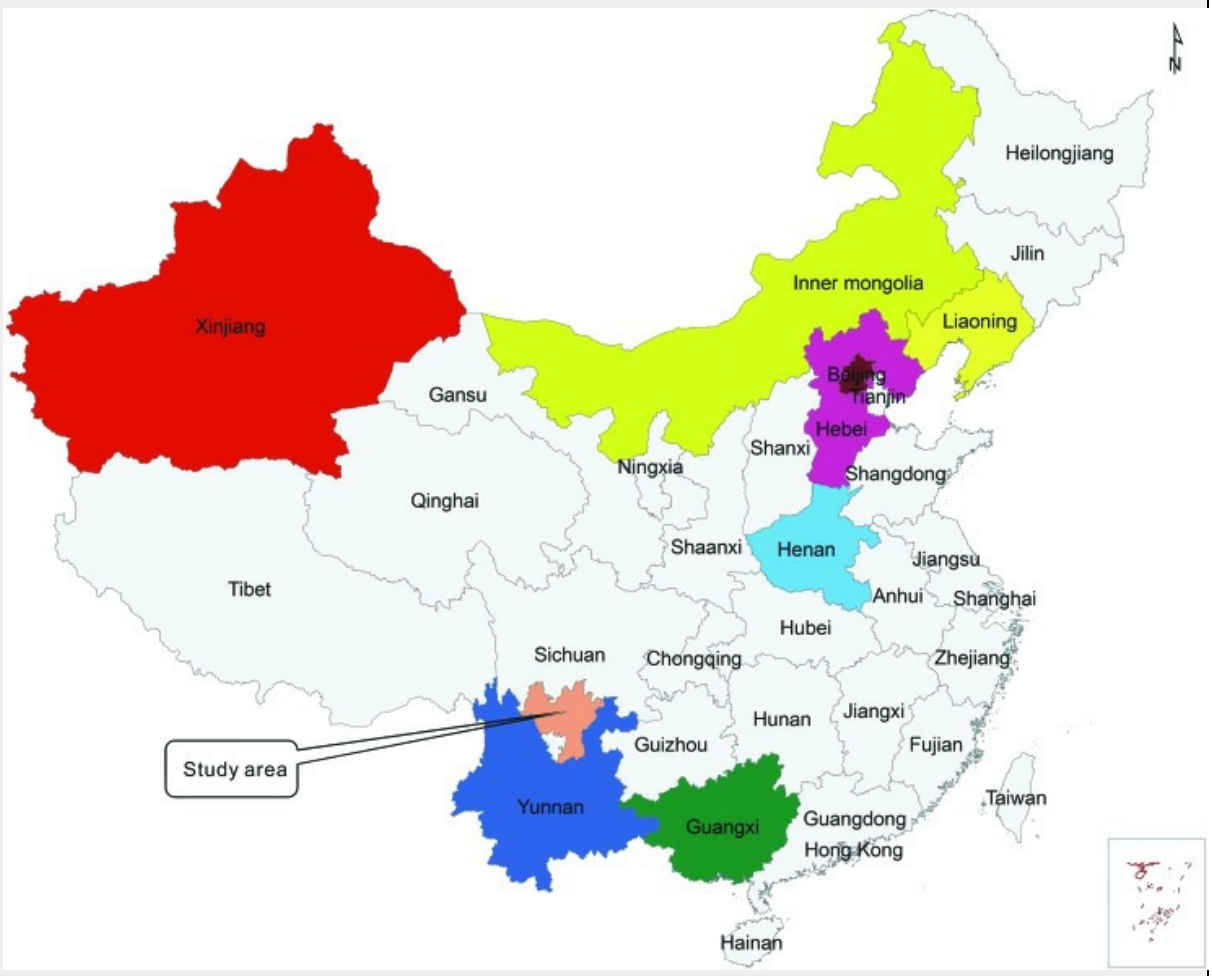

FIG. 1.

Geographic location of Liangshan prefecture in China and area information of reference sequences. Map of China highlighting Liangshan prefecture, Sichuan province. Provinces containing reference sequences for CRF07_BC phylogenetic analysis are labeled in color according to that used in the maximum likelihood (ML) tree and maximum clade credibility (MCC) tree. Color images available online at www.liebertpub.com/aid

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TABLE 1.

BACKGROUND CHARACTERISTICS OF PARTICIPANTS

Characteristics	Total N=502 N (%)
Age (years)	
2–14	3 (0.6)
15–29	226 (45.0)
30–44	254 (50.6)
45–59	17 (3.4)
≥60	2 (0.4)
Gender	
Male	394 (78.5)
Female	108 (21.5)
Ethnic	
Han ethnic	21 (4.2)
Bai minority ethnic	59 (11.8)
Yi minority ethnic	420 (83.7)

Unknown	2 (0.4)
Route of transmission	
Heterosexual	4 (0.8)
IDU	489 (97.4)
Mother to children	1 (0.2)
Unknown	8 (1.6)
$CD4^+$ count (cells/μl)	
≤200	44 (8.8)
201–350	112 (22.3)
351–500	158 (31.5)
≥501	188 (37.5)

IDU, intravenous drug use.

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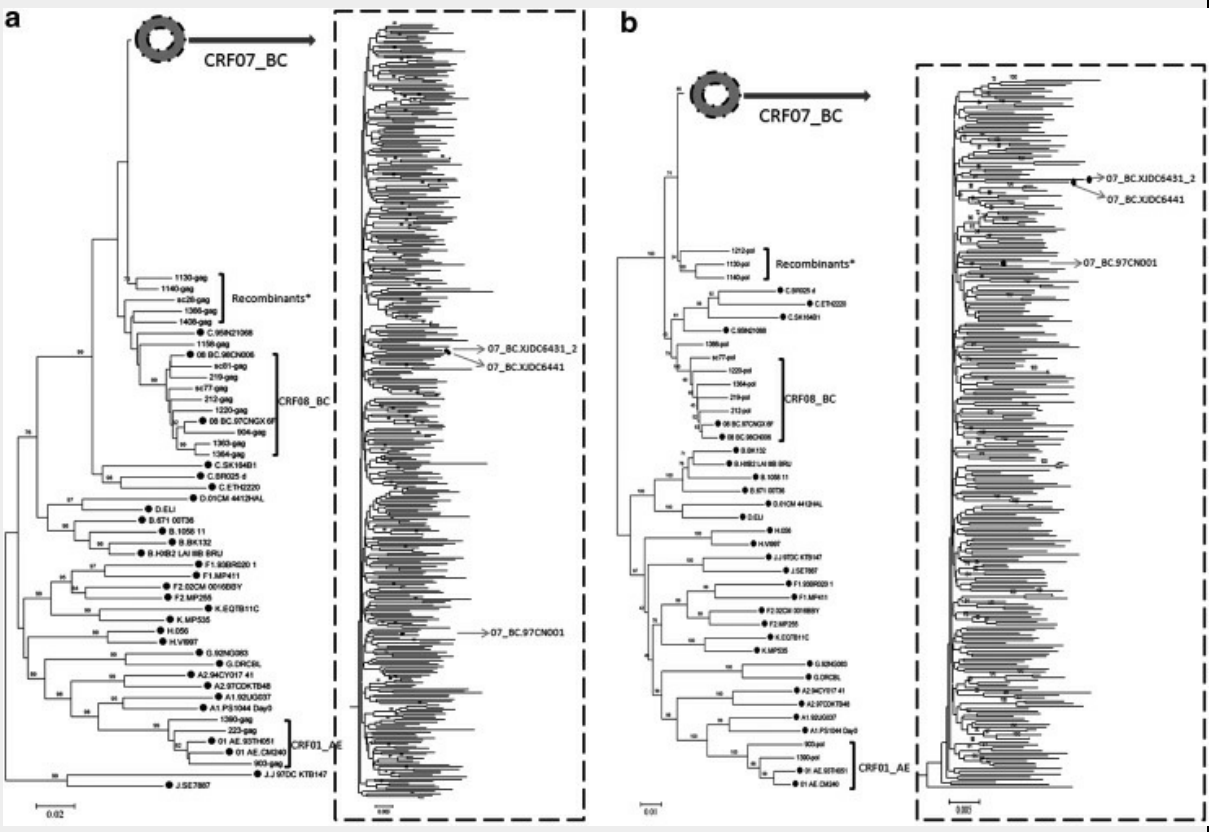

FIG. 2.

Phylogenetic tree analysis. Neighbor-joining trees were created with the full length *gag* **(a)** and *pol* **(b)** genes of HIV-1 sequences from the intravenous drug use (IDU) population in Liangshan prefecture and a selection of reference sequences of subtype A–D, F–H, J, K, CRF01_AE, CRF07_BC, and CRF08_BC (*black dots*, http://hiv-web.lanl. gov/). The lengths of the *gag* and *pol* genes of HIV-1 sequences obtained in the study were 1,503 and 3,056 base pairs using HXB2 as the genomic calibrator. Each reference sequence is labeled with the HIV-1 subtype, followed by the sequence name. The bootstrap values supporting the branch (more than 70%) are indicated at the corresponding nodes of the tree. The scale bars representing genetic distances are listed below.

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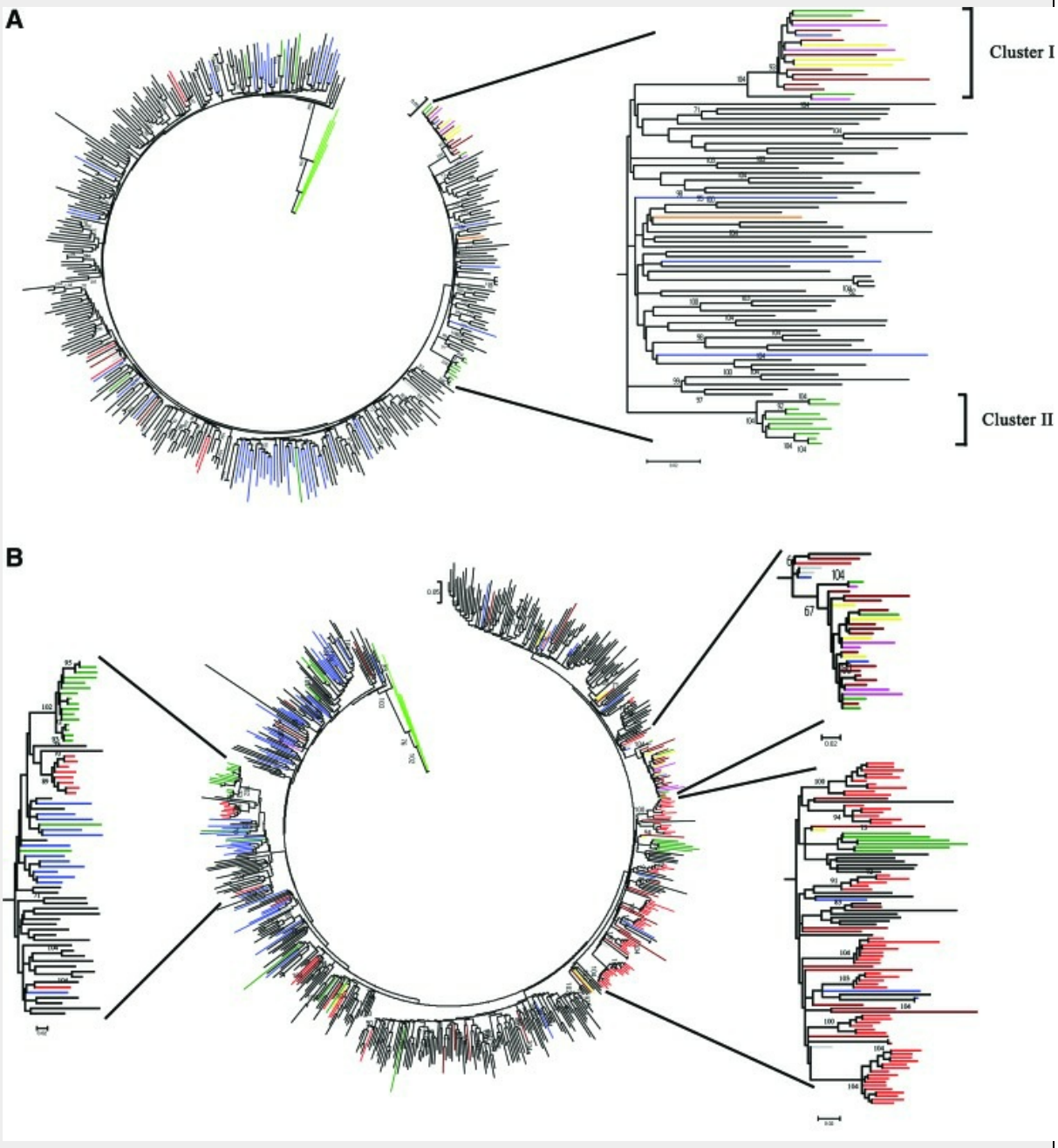

FIG. 3.

Evolutionary relationship between Liangshan HIV-1 CRF07_BC sequences from the IDU population and those from other areas in China depicted on a maximum likelihood tree of the full-length *gag–pol* gene **(A)** and partial *gag* gene **(B)**. **(A)** Full-length *gag–pol* sequences representing CRF07_BC from several areas were identified in the laboratory or obtained from the database (http://hiv-web.lanl. gov/)

and used for comparison with the sequences generated in this study. Each reference sequence is labeled using the same color used in Fig. 1. The branch support values (more than 70%) are indicated at the corresponding nodes of the tree. The subtree containing clusters is illustrated at the right of the original tree. **(B)** The ML tree based on partial CRF07_BC *gag* sequences representing CRF07_BC from several areas is constructed to illustrate the evolutionary relationship between strains from Liangshan prefecture and Xinjiang province. The subtree containing clusters is illustrated accordingly. Color images available online at www.liebertpub.com/aid

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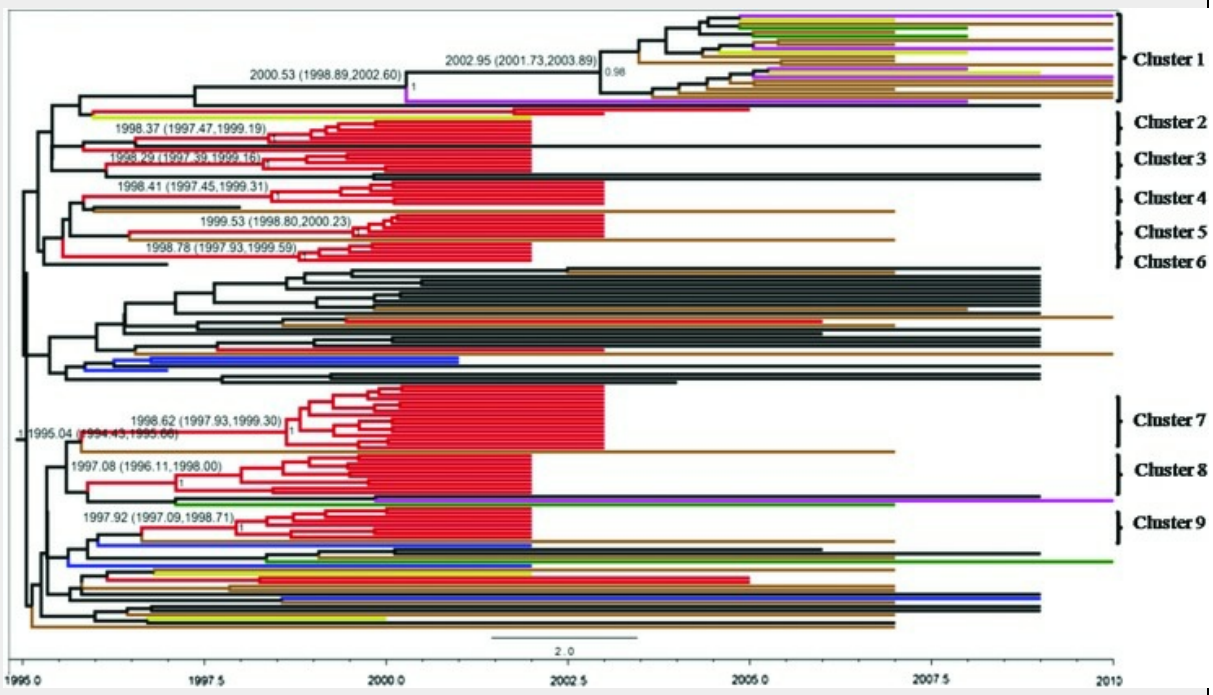

FIG. 4.

Maximum clade credibility trees of the HIV-1 CRF07_BC sequences from China based on the partial *gag* gene. All of the sequences are labeled using the same colors used in Fig. 1. The dates of MRCA and posterior probability are labeled beside each node. Color images available online at www.liebertpub.com/aid

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TABLE 2.

PRIMARY DRUG-RESISTANT MUTATIONS ASSOCIATED WITH PROTEASE AND REVERSE TRANSCRIPTASE GENES AMONG TREATMENT NAÏVE PARTICIPANTS

		Targeting drugs of resistant mutations			Resistant profile		
Sample	Subtype	PI	NRTI	NNRTI	Low	Intermediate	High
916	CRF07_BC	M46L			NFV		
964	CRF07_BC		T215I		AZT, d4T		
1128	CRF07_BC			K103N			EFV, NVP
1141	CRF07_BC		D67N		AZT, d4T		
1415	CRF07_BC			G190A		EFV	NVP

PI, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, nonnucleoside reverse transcriptase inhibitors; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; NFV, nelfinavir; NVP, nevirapine.

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