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Genetic Diversity, Complicated Recombination, and Deteriorating Drug Resistance Among HIV-1-Infected Individuals in Wuhan, China

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Abstract

To identify genotype distribution and drug resistance in people infected by HIV-1 in Wuhan, China, 105 infected people diagnosed with HIV-1 from January to December in 2019 were involved in this study. Ninety-eight gag genes, 101 PR genes, and 98 RT genes were successfully amplified. The phylogenetic analysis results showed that CRF01_AE (38.2%) and CRF07_BC (35.3%) were the two dominant genotypes, followed by CRF55_01B (6.9%), CRF59_01B (2.0%), B (2.0%), B' (2.0%), CRF08_BC (1.0%), CRF80_0107 (1.0%), and unique recombinant form (URF) (11.8%). Most URFs were the recombinants between CRF01_AE and CRF07_BC or CRF07_BC and CRF55_01B. Among the 93 subjects of antiretroviral therapy (ART)-naive, transmitted drug resistance against non-nucleoside reverse transcriptase inhibitors (NNRTIs) was 23.9%, of which V179D/E was the most frequent mutation, accounting for 18.2%. Among the 12 subjects of ART-experienced, drug resistance to first-line regimens developed severely.

Keywords: HIV-1, genotypes, drug resistance, Wuhan city

AIDS is a major infectious disease caused by HIV. In China, the epidemics of HIV-1 is unevenly distributed among different regions and high-risk populations at different periods, which leads to a more complex and diverse situation and more arduous tasks for prevention and control.[1]

According to the data of National Health Commission of China, from January to October in 2019, a total of 131,000 HIV-infected people were newly reported, and 127,000 antiviral treatments have been newly added. The proportion of infected people who met the treatment conditions is 86.6%, and the rate of successful treatment is 93.5%, which certainly is a closer step to achieving ''90-90-90'' goals. By the end of October 2019, there were 958,000 people living with HIV/AIDS. The overall epidemic continues to be at a low level.

Among the main ways in HIV transmission, the intravenous drug use and paid blood donation used to cause widespread HIV epidemics in China. However, with the increased screening of blood usage, restrictions of drug trade, and some other policy interventions in recent years, blood or intravenous transmission is basically blocked, and mother-to-child transmission has been effectively controlled, therefore, sexual transmission has become the dominant transmission route.[2] Among the newly reported infections from January to October in 2019, heterosexual transmission accounted for 73.7%, while homosexual transmission accounted for 23.0%.

Nevertheless, with the extensive application of highly active antiretroviral therapy (HAART), the high genetic variability of HIV and the selective pressure under the antiretroviral drugs have led to the emergence and prevalence of drug-resistant variants, which has seriously affected the effectiveness of clinical treatment. To respond to HIV resistance, the global health community has launched a 5-year plan (2017–2021), which has a detailed plan for the prevention, monitoring, and response to HIV resistance. Countries should strengthen the monitoring of HIV resistance and expand the scope of HAART, meanwhile safely transform to new antiretroviral drugs in first-line and second-line regimens.[3]

Hubei Province is located in central China, bordering Anhui in the east, Chongqing in the west, Jiangxi and Hunan in the south, Henan in the north, and Shaanxi in the northwest. Its capital city, Wuhan, is not only a comprehensive transportation hub in the country but also a central city geographically, industrially, scientifically, and educationally. Since 1995, a certain number of HIV-infected patients have been found in paid blood donors (PBD) in several areas of Hubei Province. Studies have revealed that subtype B' is prevalent in this population.

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From then on, subtype B' has been the most dominant genotypes in a long time.[4] However, limited information about HIV-1 genotypes or drug resistance situations in this region is currently available.

In this study, a total of 105 patients diagnosed with HIV-1 in Wuhan Jinyintan Hospital from January to December in 2019 were enrolled. Informed consent was obtained from all individual participants enrolled in the study before collecting blood samples. The study was approved by the Ethics Committees of Wuhan Institute of Virology and Wuhan Jinyintan Hospital. Of these individuals, 93 were antiretroviral therapy (ART)-naive and the other 12 were ART-experienced. For each subject, 5 mL of anticoagulant venous blood was collected by the hospital's medical staff in a BD Vacutainer® PPT plasma preparation tube (Becton, Dickinson and Company). After centrifugation at 1,100 *g* for 10 min within 3 h at 25°C, these blood samples were temporarily stored at −80°C. In addition, all the subjects registered corresponding demographic and epidemiological information. The related information is listed in Table 1. The current addresses of 105 subjects were all in Wuhan, including 100 males and 5 females, accounting for 95.2% and 4.8%, respectively. The median age was 32 years, ranging from 17 to 79 years. Among them, the numbers of people aged 17 to 25 and 26 to 35 were the largest, accounting for 26.7% and 32.4%, respectively. In terms of risks factors, the number of people infected by homosexual transmission was 68, accounting for 64.8%; while those infected by heterosexual transmission was 28, accounting for 31.4%; and 9 people had unknown infection routes. As for marital status, there were 63 individuals without spouse, accounting for 60.0%; 33 individuals were married, accounting for 31.4%, and 9 were still unknown. Among 95 individuals with treatment baseline CD4 cell counts, the median was 253 cells/μL ranging from 3 to 691 cells/μL. Among 89 individuals with viral load, the median was 67,746 copies/mL ranging from 0 to 8,789,512 copies/mL.

Table 1. Demographic and Epidemiological Information of 105 Subjects

Characteristics	*Numbers*	*Proportion (%)*
Gender		
Male	100	95.2
Female	5	4.8
Age		
17–25	28	26.7
26–35	34	32.4
36–45	13	12.4
46–55	12	11.4
56–65	10	9.5
>66	8	7.6
Marital status		
Single	63	60.0
Married	33	31.4
Unknown	9	8.6
Risk factors		
Heterosexual	28	26.7
Homosexual	68	64.8
Unknown	9	8.5
Treatment status		
ART-naive	12	11.4
ART-experienced	93	88.6
Viral load (copies/mL)		
0–87, 89, 512	67,746 (median)	—
<10,000	11	12.4
10,000–100,000	50	56.2
>100,000	28	31.5
CD4 cells counts of treatment baseline (cells/μL)		
3–691	253 (median)	—
<200	36	37.9
200–350	25	26.3
≥350	34	35.8

ART, antiretroviral therapy.

Viral RNA was extracted from 140 μL plasma using QIAamp® Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA was synthesized from the extracted viral RNA using PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Japan). The HIV-1 gag genes and partial pol genes encoding PR (protease) and RT (reverse transcriptase) were amplified by nested PCR using KOD-Plus-Neo (TOYOBO, Japan) and 2×TransStart® FastPfu Fly PCR SuperMix (TransGen Biotech, Beijing China). PCR amplification products were identified by 1% agarose gel electrophoresis, visualized under a UV light after staining with AceQ® qPCR SYBR® Green Master Mix (Vazyme Biotech Co., Ltd., Nanjing China) and then sent to Sangon Biotech (Shanghai) Co., Ltd. for sequencing. The amplification and sequencing primers and PCR procedures were described previously.[5] All sequences were submitted to the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to rule out potential laboratory contamination. From a total of 105 samples, 98 *gag* genes (HXB2: nt 790-2292), 101 PR genes (HXB2: nt 2253-2549), and 98 RT genes (HXB2: nt 2550-4229) were successfully amplified. The amplification success rates were all above 90%. Of 95 samples, about 3,400 bp *gag*-PR-RT fragments (HXB2: nt 790-4229) after splicing were obtained. Three samples failed to amplify any of the three genes.

HIV-1 genotype of each subject was identified by phylogenetic analysis using MEGA 7.0 software.[6] Sample sequences and reference sequences were aligned using Clustal W algorithm with minor manual adjustments. All the genotype reference sequences were downloaded from the Los Alamos HIV database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). Neighbor-joining method and Kimura two-parameter model with 1,000 bootstrap replicates were generated to construct the phylogenetic trees, in which bootstrap values higher than 70% were shown at the corresponding nodes. For those sequences identified by the phylogenetic tree as suspected to be unique recombinant forms (URFs), further analysis was performed. According to the results of RIP (https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) and jpHMM (http://jphmm.gobics.de/), the reference sequences of possible parent strains together with sample sequences after being aligned were imported into Simplot 3.5.1 Software to perform a recombination analysis.[7]

The phylogenetic analyses were based on the *gag*, PR, RT genes, and *gag*-PR-RT fragments of 102 samples (Fig. 1) showed that CRF01_AE and CRF07_BC were the two dominant genotypes in Wuhan, accounting for 38.2% and 35.3%, respectively; and the other genotypes were as follows: CRF55_01B, 6.9%; Subtype B/B', 4.0%; CRF59_01B, 2.0%; CRF08_BC, 1.0%; and CRF80_0107, 1.0%. In addition, there were 12 URF, accounting for 11.8%. To further analyze these URFs, a bootscan analysis was used in Simplot 3.5.1 Software. According to the analysis results in Figure 2, it was found that

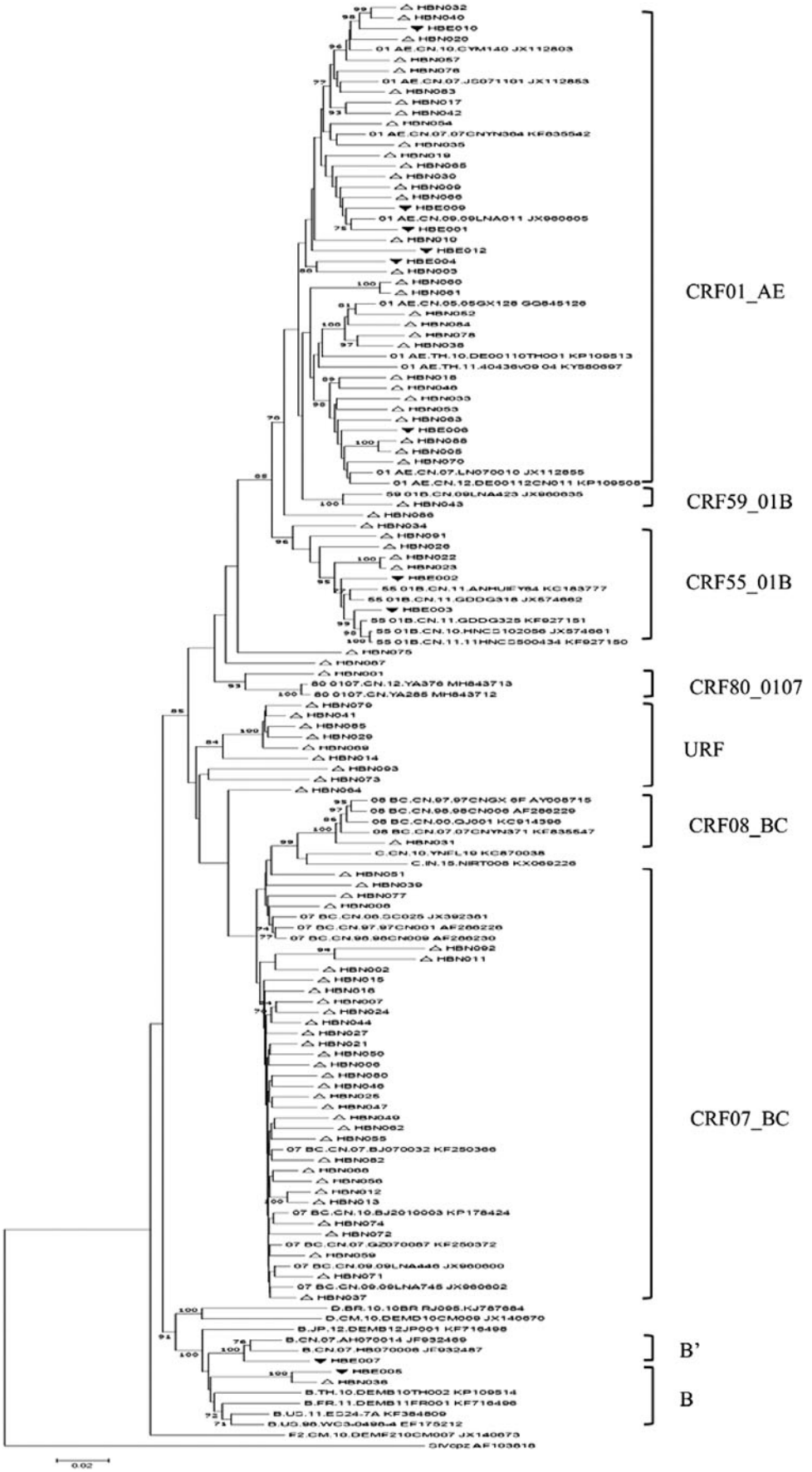

FIG. 1. Neighbor-joining phylogenetic tree based on gag-PR-RT gene was generated using Kimura two-parameter model with 1,000 bootstrap replicates. The scale bar indicated 0.02 genetic distance. ''△'' stands for ART-naive and ''▼'' stands for ART-experienced in this study. ART, antiretroviral therapy. Figure 1 can be viewed in greater detail online.

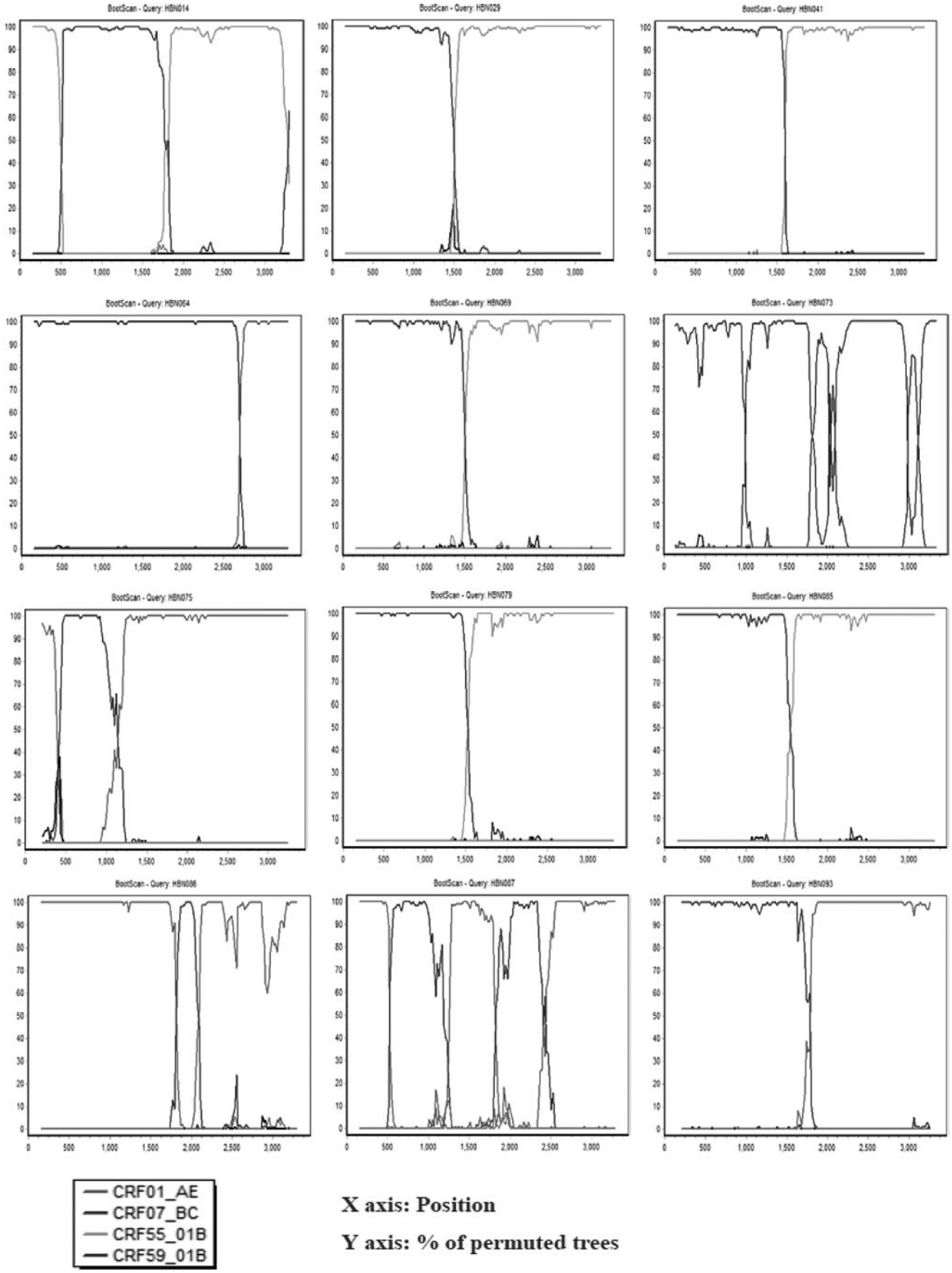

FIG. 2. Bootscan analyses were performed using Simplot 3.5.1 software of 12 URFs identified in this study with 300 bp windows and 20 bp step. Reference sequence of subtype D or subtype F was used as outgroup. X axis represented nucleotide positions of gag-PR-RT sequence obtained from each individual and Y axis represented the bootstrap values in permuted trees. URF, unique recombinant form.

TABLE 2. SUMMARY OF DRUG RESISTANCE IN ANTIRETROVIRAL THERAPY-EXPERIENCED POPULATION

			Drug resistance mutations			*Drug susceptibility prediction*				
Sample ID	*Genotype*	*Treatment regimen*	*NRTIs*	*NNRTIs*	*Accessory mutations*	*AZT*	*3TC*	*TDF*	*EFV*	*NVP*
HBE001	CRF01_AE	TDF/3TC/EFV	K65R	K103N/V106M	—	S	IR	HR	HR	HR
HBE002	CRF55_01B	TDF/3TC/EFV	K65R/M184V	V106M/G190A	V179E	S	HR	IR	HR	HR
HBE003	CRF55_01B	TDF/3TC/EFV	L74I/M184V	K103N/P225H	A98G/V179E	S	IR	S	HR	HR
HBE004	CRF01_AE	TDF/3TC/EFV	K65R	Y188L	V179D	S	IR	HR	HR	HR
HBE005	B	AZT/3TC/NVP	M41L/L74I/M184V/L210WT215Y	K103N	E44A/A62V K238N	HR	HR	HR	HR	HR
HBE006	CRF01_AE	TDF/3TC/EFV	K65R	K103N/G190S	A62V	S	IR	HR	HR	HR
HBE007	B’	Unknown	K65R/M184V	K103N/Y181C	A62V	S	HR	IR	HR	HR
HBE009	CRF01_AE	Unknown	M184V	V106M/F227L	V179D	S	HR	S	HR	HR
HBE010	CRF01_AE	Unknown	K65R/M184V	Y181C	V108I/N348I	S	HR	IR	IR	HR
HBE012	CRF01_AE	TDF/3TC/EFV	L74I/M184VT215Y	K103N/P225H	—	IR	HR	S	HR	HR

HR, high-level resistance; IR, intermedia resistance; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitor; S, susceptible.

these URFs were recombined between CRFs. Among all the samples, five samples demonstrated parent strains as CRF01_AE and CRF07_BC, namely HBN064, HBN075, HBN086, HBN087, and HBN093, and their recombination breakpoints and patterns were various. Six samples were composed of CRF07_BC and CRF55_01B. Another five samples, that is, HBN029, HBN041, HBN069, HBN079, and HBN085, shared the very similar recombination patterns. HBN014 was different from the above five. Besides, HBN073 was likely to be the recombinant of CRF07_BC and CRF59_01B.

Analysis of drug resistance mutations (DRM) was based on Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/). The transmitted drug resistance (TDR) mutation survey was carried out in the ART-naive population. For the 93 individuals with ART-naive, 91 PR genes and 88 RT genes were successfully amplified. In PR region, it was demonstrated that there was no major resistance mutation but an accessory resistance mutation site Q58E (1, 1.1%). Moreover, other mutations were identified, which appeared to be quite frequent, including L10I/V (9, 9.9%), K20R (21, 23.1%), A71V/T (16, 17.6%), and V82I (11, 12.1%). As regard to the RT region, 20 samples (23.9%) were identified to have DRMs against non-nucleoside reverse transcriptase inhibitors (NNRTIs), and the most prevalent site was V179D/E (16, 18.2%), followed by K103N/S (2, 2.3%), V106I/M (2, 2.3%), and E138A (1, 1.1%). These mutations against NNRTIs were all single mutations per individual.

The acquired drug resistance (ADR) mutation survey was carried out in the ART-experienced population. For the 12 ART-experienced individuals, 10 PR genes and 10 RT genes were successfully amplified. In PR region, no major resistance mutation or accessory resistance mutation was found. However, the results showed that every subject had at least one mutation both against nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs. Table 2 summarizes the HIV-1 genotypes, medication regimens, drug resistance mutation sites against NRTIs and NNRTIs in RT region, and susceptibility to different antiretroviral drugs.

In the present study, it was confirmed that CRF01_AE and CRF07_BC were the two main dominant genotypes, which are basically consistent with the results of the latest molecular epidemiological survey in China.[8] CRF01_AE is mainly popular among sexually transmitted people. CRF07_BC is formed by recombination of two subtypes B and C. It was first found in drug users in Yunnan Province, which has gradually spread to sexually transmitted people, including MSM.[9] CRF55_01B accounted for 6.9%, which was first reported in 2013 in MSM population in Shenzhen, China. It is recombined of CRF01_AE and subtype B. CRF55_01B has been found prevalent to varying degrees in other regions in China. In addition, two cases of CRF59_01B (2.0%) and one case of CRF80_0107 (1.0%) were identified. Both two CRFs have never been reported in Hubei Province before. There were only two cases (2.0%) of the B’ subtype that was previously prevalent in PBD in this region.

After analyzing the 12 cases of URF (11.8%), it was clarified that these URFs were all recombined between CRFs. Five samples exhibited parent strains of CRF01_AE and CRF07_BC, which are the dominant genotypes in most areas of China, with a higher probability of recombination and the recombination patterns varying a lot. For example, CRF79_0107 and CRF80_0107 (a case found in this study) were CRFs formed by CRF01_AE and CRF07_BC. In addition, six

samples showed parent strains of CRF01_AE and CRF55_01B. Out of these six samples, five samples had very similar recombination pattern. Efforts will be made to successfully amplify the rest of virus genomes of the five samples subsequently, because this recombination pattern may become a new CRF. Considering that with the increasing trend of CRF55_01B in different regions of China, the probability of recombination with other genotypes was also gradually increased. In recent years, some URFs recombined between CRF07_BC and CRF55_01B have been reported one after another.[10,11] From these results, it can be seen that in Wuhan, where multiple genotypes existed at the same time, the recombination situation has become more and more complicated, which undoubtedly poses a huge challenge to the control of HIV transmission and treatment.

According to the analysis results of drug resistance mutations, among the ART-naive population mutations, the overall TDR rate was 23.9%. Notably, the most frequent mutation V179D/E (16, 18.2%) is a polymorphic accessory NNRTIs-selected mutation, which contributes to the low-level reductions in susceptibility to each of the NNRTIs. A recent study has demonstrated that the natural presence of V179E was found in all but one of the 228 patients infected with CRF55_01B,[12] which is consistent with our results. Apart from V179D/E, the occurrence rates of K103N/S and V106M are both 2.3%. Both two mutations can cause high resistance of NVP and EFV in the current first-line regimen in China. Therefore, it is necessary to increase the intensity of drug resistance test before treatment, and if conditions are permitted, it can be given priority to treatment that does not contain NNRTIs. Among ART-experienced population, it was found that the resistance to the drugs of first-line regimen is very serious. Each person carries at least two major drug resistance mutations. It should be recommended to replace their current regimen by the second-line regimen as soon as possible.[13] In all individuals, no major drug resistance mutations were identified in the protease gene. It is probably because the medication basically follows the first-line therapy plan of China's AIDS treatment manual, that is, two NRTIs and one NNRTIs, and there is no major drug resistance mutation in the protease against PIs.

In summary, this study conducted a systematic analysis of HIV-1 genotype distribution, recombination, and drug resistance mutations in Wuhan City. These results will shed light on a more comprehensive understanding of HIV-1 epidemiology in Wuhan, even the whole country, and it may provide important guidance and suggestions for a more effective treatment.

Sequence Data

The GenBank accession numbers of the nucleotide sequences are MT561465-MT561494 and MT582810-MT583076.

Authors' Contributions

Y.Z., Y.C., L.R., and R.Y. conceived and designed the experiments; Y.L., Y.Z., H.P., and L.H. collected the samples; Y.Z., Y.L., and W.W. performed the experiments; Y.Z. analyzed the data; Y.Z., L.R., and R.Y. wrote and edited the article. All authors have read and approved the final article.

Author Disclosure Statement

No competing financial interests exist.

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