

# Risk Factors of Drug Resistance and the Potential Risk of HIV-1 Transmission of Patients with ART Virological Failure: A Population-Based Study in Sichuan, China

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**Background:** Sichuan Province, in the interior of Southwest China, is the most severe HIV-affected area in China. Few data are currently available for people living with HIV/AIDS (PLWH) with virological failure of antiretroviral therapy (ART). Estimating the HIV-1 drug-resistant spread influencing factors and transmission patterns of the HIV-1 epidemic of PLWH with ART virological failure are critical in Sichuan.

**Methods:** We evaluated the drug-resistant transmission patterns on 5790 PLWH in 2018 with identified pol sequences of the five main HIV-1 subtypes (ie, subtype B, CRF08\_BC, CRF85\_BC, CRF07\_BC, and CRF01\_AE) in Sichuan Province, China. The multivariate logistic regression model was used to explore potential influencing factors of the spread of drug resistance (DR) clustering in the genetic transmission network. Spatial analyses were applied to demonstrate drug-resistant spatial clustering patterns of spatial connections of HIV-1 intercity transmission. Genetic transmission networks were performed by comparing sequences, calculating the pairwise distance, and visualizing the network.

**Results:** There were identified 452 transmission clusters containing 2159 of 5790 patients (37.29%) in the HIV-1 genetic transmission networks. Some clinical and demographic factors (eg, route of transmission, subtype) determined the DR clustering in the genetic transmission networks. The high drug-resistant clustering rates were mainly distributed in the Southern and Northeast of Sichuan Province (eg, Deyang, Neijiang), especially for CRF85\_BC, which showed the highest clustering rate. Some cities had with strong intracity links (eg, Yibin, Neijiang), some cities had with strong transmission links with another city (eg, Ziyang and Guangyuan), 12 of 37 drug resistance mutation sites had a significant difference in the five subtypes ( $P < 0.001$ ).

**Conclusion:** Our findings revealed the HIV-1 drug-resistant spread influencing factors and transmission patterns of PLWH with ART virological failure, which showed regions with high drug-resistant transmission of PLWH may not be a match for regions with severe epidemics in Sichuan, and it provided evidence-based to drug-resistant transmission targeting interventions.

**Keywords:** HIV-1, antiretroviral resistance, transmitted drug resistance, spatial analyses

## Introduction

Since 1981, when the US reported the first case of AIDS, HIV has spread rapidly worldwide, it has been responsible for nearly 76 million infections globally,<sup>1</sup> and approximately 0.85 million PLWH live in China. In view of its harm and

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Received: 2 September 2021  
Accepted: 11 November 2021  
Published: 7 December 2021

Infection and Drug Resistance 2021:14 5219–5233

5219



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prevalence, AIDS affects social security, and has become a major threat to human society, population structure and economic development.<sup>2</sup>

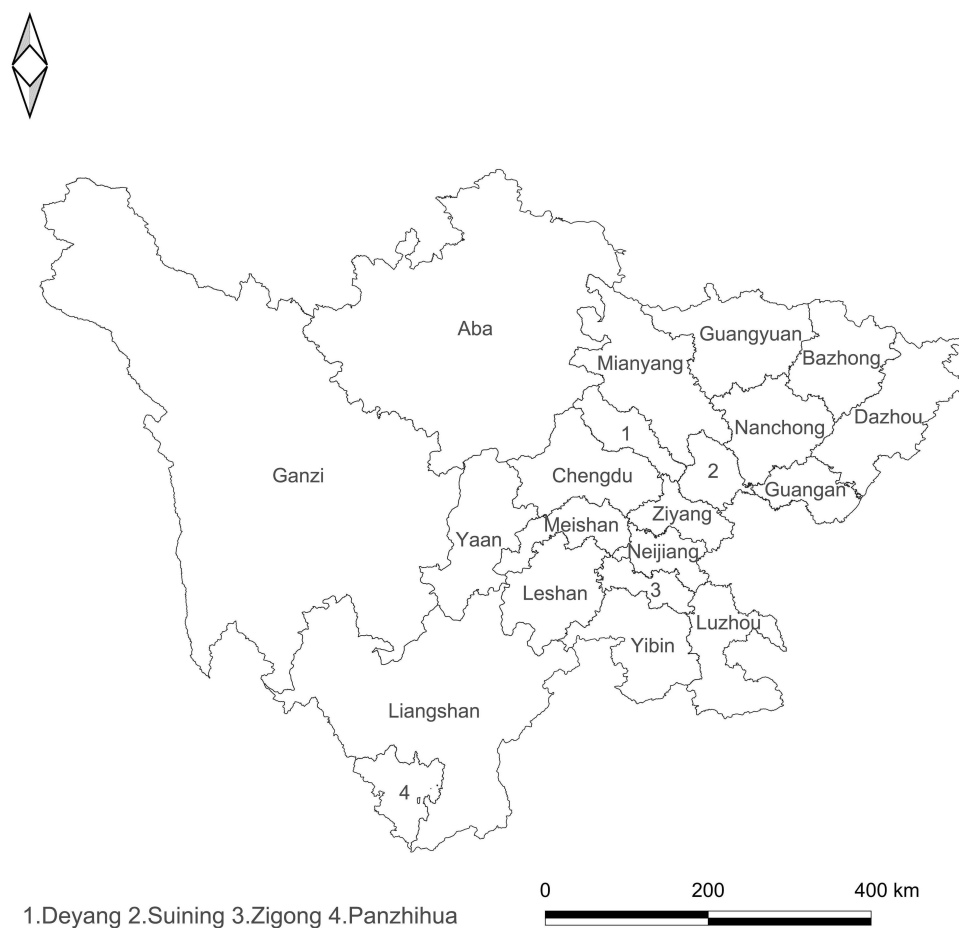
ART is the most effective method of AIDS treatment at present, which is highly effective in stopping reducing the risk of HIV transmissions.<sup>3,4</sup> During antiviral treatment, strains carrying DR gene mutations are screened under drug pressure and accumulate over time, which will eventually reduce the efficacy of antiviral treatment, lead to the failure of antiviral treatment, and even the possibility of DR transmission.<sup>5,6</sup>

China had a massive floating population, studies have revealed that the floating population has become an important factor in the spread of HIV fast spreading bridge crowd and geographic differences may to some extent determine the patterns of HIV transmission.<sup>7,8</sup> HIV genetic-transmission network analysis is an effective strategy for identifying high risk factors,<sup>9</sup> which would be complementary found using traditional epidemiological approaches. Previous studies showed that young men, especially MSM, and some subtypes (eg, CRF55\_01B, CRF01\_AE) may have a higher

transmission risk.<sup>10,11</sup> In addition, reports showed that some demographic differences and HIV-related behaviors (eg, gender differences, ART adherence and ART regimens) lead to the risk of drug resistance with virological failure among PLWH, which were reported among PLWH in some countries, such as China,<sup>12–14</sup> Uganda,<sup>15</sup> Malawi,<sup>16</sup> Cameroon.<sup>17</sup> Yuan had reported that DR transmission in MSM with virological failure was concentrated in urban cities.<sup>18</sup>

Sichuan province (Figure 1) is in the interior of Southwest China, adjacent to Yunnan and Guizhou in the South and Tibet in the West. Sichuan has a population of approximately 85 million people, and is a developing area, Sichuan Province is the most severely HIV affected area in China.<sup>19</sup> The universal coverage of ART led to a significant decline in morbidity and mortality among PLWH.<sup>20</sup> However, the prevalence of virological failure on ART was high in Sichuan.<sup>14</sup>

Therefore, this study aimed to establish the HIV-1 drug-resistant spread influencing factors of PLWH with ART virological failure and evaluated the transmission



**Figure 1** The distribution of people living with HIV/AIDS recruited in this study.

patterns of the HIV-1 epidemic of PLWH with ART virological failure in Sichuan.

## Methods

### Study Subjects

Sichuan is located in the southwest of China, where the epidemic of HIV-1 is most serious in China, especially in central, eastern, and southern regions. Between January 2018 and December 2018, 7011 whole blood samples were collected from participants experiencing treatment failure in Sichuan Province. The study subjects were selected based on the following criteria: (1) receiving confirmatory HIV diagnosis, (2) receiving ART for at least 6 months; (3) being diagnosed as a virological failure in ART (namely HIV RNA level >1000 copies/mL), because gene sequencing was conducted in this population according to Chinese “National Guideline for Detection of HIV/AIDS (2020)”. The study subjects were excluded if they had used ART outside national guidelines or were missing treatment regimen information.

Among the 7011 PLWH, pol sequence information was successfully exported from 5926 (84.5%) samples. After eliminating duplicate samples and other HIV-1 subtype sequences, 5790 PLWH with pol sequences of the five main HIV-1 subtypes (ie, subtype B, CRF08\_BC, CRF85\_BC, CRF07\_BC, and CRF01\_AE) were finally included in this study.

### Ethics Statement

The use of anonymous clinical/demographic and sequence data was reviewed, and the study protocol was approved by the Ethics Committee of the Sichuan Center for Disease Control and Prevention. The study was conducted following the Helsinki Declaration of 1964.

### Laboratory Tests

About 5 mL of venous blood was extracted to test the CD4<sup>+</sup> T cell. Flow cytometry was used to quantify the CD4<sup>+</sup> T cells in the local CDCs. The plasma samples were isolated from each participant and preserved in a -80°C freezer.

### Amplification, Sequencing, and HIV-1 Genetic Transmission Network Analysis

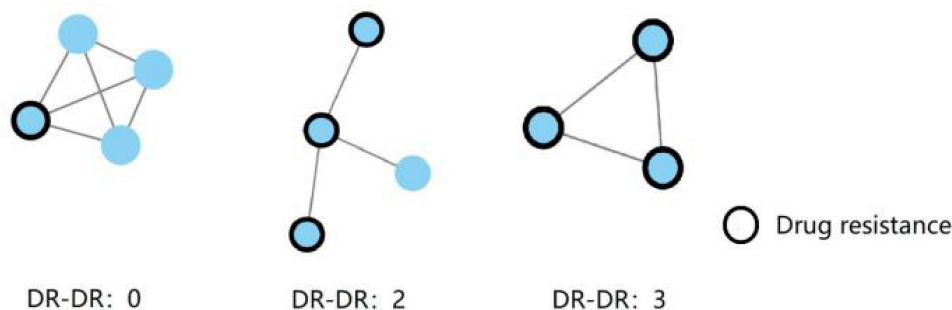
The viral nucleic acid was obtained from 200 mL plasma of PLWH by extraction machines (MagNA Pure LC 2.0 system, Roche, Branchburg, NJ). Similar to the previous reports, sequences were generated from the HIV-1 pol. Briefly, the

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to amplify the full-length protease gene in the pol region and the first 300 codons of the reverse transcriptase gene. The PCR products were dealt with electrophoresis with 1% agarose gel, and the amplified positive products were purified and sequenced by Beijing Genomics Research Center Ltd. The detailed amplification and sequencing were as previously described.

The results of the HIV gene sequence were spliced, cleaned and edited by software sequencer 4.9, then the results of the splicing were corrected by bioedit software, and compared with the Los Alamos HIV database reference sequence. It should be explained that to avoid interference caused by ART, 62 DRMs were removed from all sample sequences by comparing reference subtype standard-strain sequences,<sup>21</sup> considering our study focusing on the effect of DRMs on network characteristics, the length of the total sequence was 876 bp. Then, the phylogenetic tree was constructed by kimura 2 parameter model by neighbor joining method of mega 6.0 software, and 1000 times of evolution tree were constructed again. Bootstrap was used to construct the phylogenetic tree 1000 times, the results showed that the accuracy of genotyping was verified by the value of more than 70%, and the genotyping was determined.

The molecular transmission network was constructed by grouping the subtypes of the strains. The approximate maximum likelihood tree (ML) with different subtypes as the external group was constructed by fasttree 3.0. The nucleotide substitution model was GTR + G + I, and the node (branch point) value of the evolutionary tree was calculated using the SH test embedded in the software. The constructed mL evolutionary tree was introduced into cluster picker 1.2.5. The spread clusters were extracted according to bootstrap value >95% and gene distance < 3%, and the sequence in the cluster was included in the subsequent analysis. The gene distance between the clusters was calculated using the software hyphy 2.2.4 in TN93 model, and the relationship between the two sequences was determined by the minimum gene distance method. As the gene distance when the number of clusters is the most, it is the gene threshold of the molecular network. The visualization of communication network is realized using cytoscape.

We determined the characteristics of the network, including nodes (individuals in the network), edges (the link between two nodes, representing the potential transmission relationship between the two individuals), degrees (the number of edges linking one node to other nodes), network sizes (the number of individuals in a cluster), and



**Figure 2** Resistance-drug transmission sketch map.

clusters (groups of linked sequences). Drug-resistant individuals in the network are shown in [Figure 2](#).

## Data Collection

PLWH was screened and selected from the basic information system for AIDS Prevention and Control of the Sichuan Provincial Center for Disease Control and Prevention (CDC). Retrospective data were exported from their medical records in the system, including demographic characteristics (ie, ethnic, sex, age, and marital status), and HIV/AIDS-related characteristics (ie, route of HIV transmission, CD4<sup>+</sup> T cell counts after diagnosis, disease stage, and sexually transmitted disease infection status).

## Statistical Analysis

Univariate logistic regression analysis was used to identify risk factors associated with DR transmission in the genetic transmission network, and independent variables were drug-resistant mutation individuals in the study. Each DR subject was linked to two or more subjects, indicating the presence of the individuals with transmission risk in the network<sup>22</sup> (1 = had more than 2 degrees in the genetic transmission network, 0 =  $\leq 1$  degree). Frequencies of demographic data (such as sociodemographic and AIDS-related characteristics) as the dependent variables were calculated. Multivariate logistic regression model was used to further test the significance of all micro-significant (P-value <0.1) variables in univariate logistic regression model (P-value <0.05). Statistical analysis was performed in SPSS 25.

## HIV DR Transmission Network Among Cities

The network data were visualized using QGISv3.10 (QGIS.org (2020). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.org>). In the intensity matrix and flow graph,

and the propagation network is visualized with different colors. Each PLWH is assigned to the city in which he/she lived and connected to other PLWH with infectious relationships through aside. The number of infectious diseases is represented by the color grading in the intensity matrix, and the darker color indicates that the two regions are more closely related to HIV infection. The graph shows the flowchart of the transmission network between cities, which is composed of edges representing HIV transmission links and edges with drug-resistant mutation links. The size of the circle represents the propagation intensity in the city, and the line width is measured by the spatial connection intensity consistent with the value of the spatial intensity matrix.

## The Connection Degree of Drug-Resistant Mutation Sites in Subtypes

In each subtype virus cluster, the ratio of the number of links with common drug resistance mutation sites (DRMs) to the total number of total links in the network was analyzed. Significant differences in categorical variables were analyzed using chi-square tests. P values <0.05 indicated statistical significance.

## Results

### Characteristics of Transmission Networks

We used the HIV-1 transmission network to explore the risk associated with the potential transmission among PLWH with virological failure. Among the collected samples, 5926 were successfully sequenced, 5790 sequences were obtained after eliminating repeated sequences. HIV-1 transmission network was constructed and is shown in [Figure 3](#). The most total number of clusters calculated threshold for each gene distance of the five main HIV-1 subtypes is 0.01,

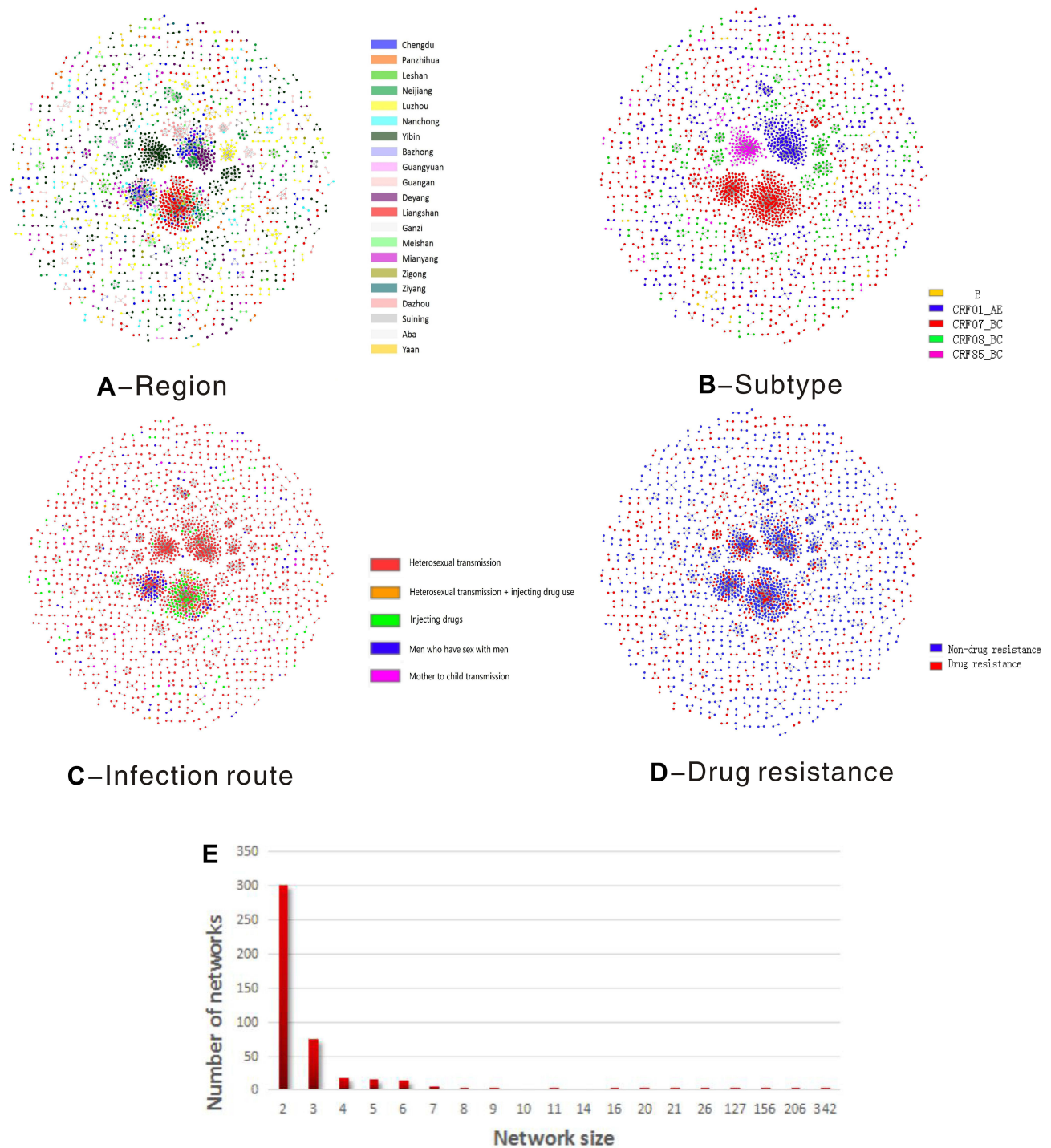
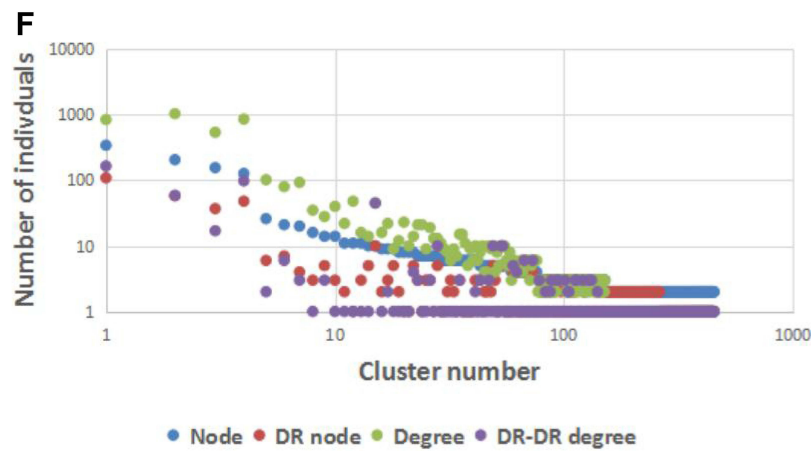


Figure 3 Continued.

cluster picker identified 452 transmission clusters containing 2159 of 5790 patients (37.29%). Four hundred and fifty-two clusters were observed with the number of sequences per cluster ranging from 2 to 342. Of these clusters, 301 clusters had only two linked PLWH (one link), and 151 clusters had  $\geq 2$  potential transmission partners.

The network illustrates the risk-specific distribution of PLWH with ART virological failure. To explore the geographic dimension of HIV-1 transmission, among all 21 cities in Liangshan (16.67%), Yibin (15.28%), Neijiang (11.25%), Luzhou (10.33%) showed the largest number of PLWH, followed by Chengdu (7.87%), Deyang



**Figure 3** HIV-1 transmission clusters. **(A)** Region-associated transmission clusters. Different colors represent different cities. **(B)** Genetic subtype-associated transmission networks. Different colors represent different subtypes: Orange: B subtype; blue: CRF\_01AE; red: CRF\_07BC; green: CRF\_08BC; pink: CRF\_85BC. **(C)** Infection route and region-associated transmission clusters. Different colors represent different infection routes: red: heterosexual transmission (Hetero); Orange: sexual transmission and injection drug users (IDUs); green: IDUs; blue: men who have sex with men (MSM); pink: mother to child transmission. **(D)** Drug resistance associated genetic transmission networks. Blue: CRF\_01AE; red: CRF\_07BC **(E)** Size of HIV-1 transmission clusters among the PLWH, size represents the number of individuals in a cluster. **(F)** Distribution of HIV-1 transmission degree. The node represents individuals in the network; degree represents the number of edges that link one node to other nodes.

(7.69%). CRF07\_BC accounted for 1107 (51.27%) nodes, CRF01\_AE accounted for 566 (26.22%) nodes, CRF08\_BC accounted for 290 (13.43%) nodes, CRF85\_BC accounted for 171 (7.92%) nodes, and B subtype accounted for only 25 (1.16%) of the clustering nodes. Of the 452 networks, of which among the infection routes, 80.9% of cases occurred among heterosexual transmission, 6.4% among men who have sex with men (MSM), 9.4% among injection drug users (IDUs), 1.4% among sexual transmission and IDUs, and 1.6% among the mother to child transmission. In the network, 761 (35.25%) DR sequences were observed in the transmission network, among these, 647 subjects (13.36%) were linked to other DR sequences (DR-DR) (Figure 3D).

In the clusters within the molecular network, the largest four clusters accounting for 127 (5.88%), 156 (7.23%), 206 (9.54%) and 342 (15.84%) of nodes, respectively, the transmission route with the highest clustering rate was the heterosexual transmission, IDUs and MSM, moreover we found that drug injection and heterosexual contact were often cross-linked Figure 3C). Furthermore, there was one large cluster in the network ( $n = 127$  persons), which mainly belonged to Yibin city (CRF85\_BC) sequences. CRF07\_BC has two large clusters that mainly belonged to Liangshan and Chengdu. Moreover, the largest CRF01\_AE clusters reported the PLWH in the cities of Deyang, Neijiang, Chengdu, Dazhou were cross-linked. Additionally, the clusters were more linked within the city (Figure 3A).

The network clustering rate of drug-resistant PLWH in 21 cities in Sichuan Province is shown in Table 1. The total clustering rate in Sichuan Province was 37.29% (2159/5790), and the total drug resistance clustering rate was 13.14% (761/5790). The clustering rate of Deyang (69.17%, 166/240) and Neijiang (61.99%, 243/392) was much higher than the average clustering rate, while the clustering rate of Liangshan (20.88%, 360/1724) was the lowest in the whole province. Nanchong (24.84%, 38/153) and Neijiang (24.74%, 97/392) had the highest drug resistance clustering rate in the province, while the drug resistance clustering rate in five cities (ie, Chengdu, etc.) was only 7%.

## Risk Factors of Drug-Resistant Clustering

Links (degrees) between each (node) for the subjects (nodes) ranged from 1 to 178. A total of 2443 PLWH were DR-associated individuals, 761 DR individuals (35.25%) in the transmission network, 373 of 2433 drug-resistant PLWH showed DR-associated transmission links  $\geq 2$ . Table 2 shows the sociodemographic and AIDS-related characteristics of the DR participants with ART virological failure.

To explore factors associated with DR-associated transmission links  $\geq 2$ , we investigated the influence of 13 groups of dependent variables on DR transmission by using a logistic regression model (Table 2). For the univariate analysis, age, occupation, marital status, ethnicity, education level, route of transmission, latest CD4+ T cell counts, stage of disease, history of sexually transmitted diseases, time of ART, subtype were significantly

**Table I** Urban Distribution of the Individuals Clustering of DR

City	Number of Infections	Clusters	Clustering Rate %	Clusters of DR	Clustering Rate of DR (%)
Chengdu	524	170	32.44%	37	7.06%
Deyang	240	166	69.17%	55	22.92%
Leshan	170	76	44.71%	26	15.29%
Suining	70	28	40.00%	7	10.00%
Ziyang	151	32	21.19%	14	9.27%
Mianyang	250	86	34.40%	39	15.60%
Yaan	125	41	32.80%	16	12.80%
Meishan	139	48	34.53%	18	12.95%
Yibin	595	330	55.46%	113	18.99%
Zigong	90	27	30.00%	8	8.89%
Neijiang	392	243	61.99%	97	24.74%
Luzhou	471	223	47.35%	94	19.96%
Bazhong	54	17	31.48%	8	14.81%
Nanchong	153	69	45.10%	38	24.84%
Dazhou	239	78	32.64%	17	7.11%
Guangyuan	53	23	43.40%	4	7.55%
Guangan	183	75	40.98%	23	12.57%
Panzhihua	144	60	41.67%	15	10.42%
Liangshan	1724	360	20.88%	130	7.54%
Aba	12	4	33.33%	1	8.33%
Ganzi	11	3	27.27%	1	9.09%
Total	5790	2159	37.29%	761	13.14%

associated with DR-associated transmission links  $\geq 2$  ( $P < 0.05$ ). However, no statistically significant difference was found in terms of the gender and initial treatment regimen ( $P > 0.05$ ).

Multivariate logistic regression analyses showed that individuals with  $\geq 2$  links were more likely to be a history of sexually transmitted diseases (OR: 1.69, 95% CI: 1.15–2.48), IDUs (OR: 1.70, 95% CI: 1.05–2.76) and CRF85\_BC subtype (OR: 2.86, 95% CI: 1.81–4.51). Additionally, employed (OR: 0.70, 95% CI: 0.51–0.97), AIDS-sufferer being involved in clinical stage (OR: 0.69, 95% CI: 0.53–0.89), ART time  $> 36$  years (OR: 0.66, 95% CI: 0.47–0.92) subjects were associated with lower likelihood of DR-associated transmission links  $\geq 2$ .

## The Geographic Dimension of HIV-I DR Transmission Links

We have constructed spatial explicit transmission routes among PLWH with virological failure at the city scale (Figure 4). The strength of transmission links between cities was diverse. Specifically, the intensity matrix shows that the

pattern of DR-DR links in the network is different from general transmission links.

In terms of general transmission links, among all 21 cities in Sichuan, Yibin had the strongest intracity links, followed by Neijiang, Liangshan and Deyang, while major cities which had cross-regional HIV-1 transmission were Chengdu Neijiang, Liangshan, Guang'an and Mianyang. The results showed that most cities had strong intracity links (eg, Neijiang and Yibin), and some cities also showed strong transmission links with other cities (eg, Mianyang and Chengdu) (Figure 4A).

In terms of DR-DR links, except for a few cities that have only intracity links such as Dazhou and Bazhong, Leshan (16/17), Yibin (126/134), and Neijiang (138/148) ranked the top three in terms of the proportion of intracity transmission links in all DR-DR links. As for the absolute quantity, Neijiang showed the largest number of local transmissions. For the cross-regional DR-DR link strength ratio, Ziyang (22/23) ranked first, followed by Guangyuan (12/13) and Mianyang (38/49). Totally, DR-DR links were

**Table 2** Risk Factors of Drug Resistant Nodes with Drug Resistance-Associated Transmission Links  $\geq 2$ 

Variables	Total	%	$\geq$ Link2	%	OR	AOR
<b>Gender</b>						
Male	1771	72.79	278	15.70	1	
Female	662	27.21	95	14.35	0.90 (0.70~1.16)	
<b>Age (years)</b>						
$\leq 14$	90	3.70	3	3.33	1	1
15–49	1464	60.17	156	10.66	3.46 (1.08~11.06)*	2.07 (0.32~13.45)
$\geq 50$	879	36.13	214	24.35	9.33 (2.92~29.81)***	4.27 (0.64~28.34)
<b>Occupation</b>						
Farmers	1559	64.08	261	16.74	1	1
Workers	63	2.59	13	20.63	1.29 (0.69~2.41)	1.68 (0.87~3.23)
Employed	656	26.96	75	11.43	0.64 (0.49~0.85)**	0.70 (0.51~0.97)*
Students/Children	108	4.44	7	6.48	0.35 (0.16~0.75)**	2.37 (0.66~8.51)
Retired	47	1.93	17	36.17	2.82 (1.53~5.19)**	1.79 (0.93~3.44)
<b>Marital status</b>						
Married or living With spouse	1298	53.35	193	14.87	1	1
Single	562	23.10	64	11.39	0.74 (0.54~1.00)*	1.09 (0.75~1.58)
Divorced/widowed	573	23.55	116	20.24	1.45 (1.13~1.88)**	1.18 (0.93~1.55)
<b>Ethnicity</b>						
Han	1773	72.87	307	17.32	1	1
Yi	639	26.26	63	9.86	0.52 (0.39~0.70)***	0.70 (0.42~1.16)
Others	21	0.86	3	14.29	0.80 (0.23~2.72)	0.93 (0.26~3.37)
<b>Education level</b>						
Illiteracy	566	23.26	74	13.07	1	1
Primary school	939	38.59	184	19.60	1.62 (1.21~2.17)**	1.06 (0.75~1.49)
Middle school	619	25.44	77	12.44	0.95 (0.67~1.33)	0.76 (0.50~1.15)
High school or technical secondary school	219	9.00	26	11.87	0.90 (0.56~1.44)	0.83 (0.46~1.49)
Junior college or above	90	3.70	12	13.33	1.02 (0.53~2.00)	1.09 (0.49~2.42)
<b>Route of transmission</b>						
Hetero	1752	72.01	296	16.89	1	1
MSM	195	8.01	27	13.85	0.79 (0.52~1.21)	1.38 (0.82~2.31)
IDUs	323	13.28	44	13.62	0.78 (0.55~1.09)	1.70 (1.05~2.76)*
Sexual and IDUs	56	2.30	2	3.57	0.18 (0.44~0.75)*	0.39 (0.09~1.70)
MCT	107	4.40	4	3.74	0.19 (0.07~0.52)**	0.28 (0.43~1.80)
<b>Latest CD4+ T cell counts (cells/<math>\mu</math>L)</b>						
$\leq 200$	885	36.37	143	16.16	1	1
200~500	1402	57.62	216	15.41	0.95 (0.75~1.19)	1.07 (0.83~1.39)
$> 500$	146	6.00	14	9.59	0.55 (0.31~0.98)*	0.71 (0.38~1.34)
<b>Stage of disease</b>						
HIV	811	33.33	148	18.25	1	1
AIDS	1622	66.67	225	13.87	0.72 (0.58~0.91)**	0.69 (0.53~0.89)**
<b>History of sexually transmitted diseases except for HIV</b>						
Never	2237	91.94	331	14.80	1	1
Ever	196	8.06	42	21.43	1.57 (1.10~2.25)*	1.69 (1.15~2.48)**

(Continued)



Table 2 (Continued).

Variables	Total	%	≥Link2	%	OR	AOR
<b>ART related information Time of ART, years</b>						
≤12	378	15.54	73	19.31	I	I
12~36	956	39.29	173	18.10	0.92 (0.68~1.25)	0.88 (0.64~1.22)
>36	1099	45.17	127	11.56	0.55 (0.40~0.75)***	0.66 (0.47~0.92)*
<b>Initial treatment regimen</b>						
First-line treatment	2390	98.23	370	15.48	I	I
Second-line treatment	43	1.77	3	6.98	0.41 (0.13~1.33)	0.72 (0.21~2.42)
<b>Subtype</b>						
CRF07_BC	1320	54.25	172	13.03	I	I
CRF01_AE	688	28.28	102	14.83	1.16 (0.89~1.51)	1.01 (0.75~1.37)
CRF08_BC	256	10.52	53	20.70	1.74 (1.24~2.45)**	1.29 (0.88~1.87)
CRF85_BC	107	4.40	46	42.99	5.03 (3.32~7.62)***	2.86 (1.81~4.51)***
B	62	2.55	0	0.00		

Notes:\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

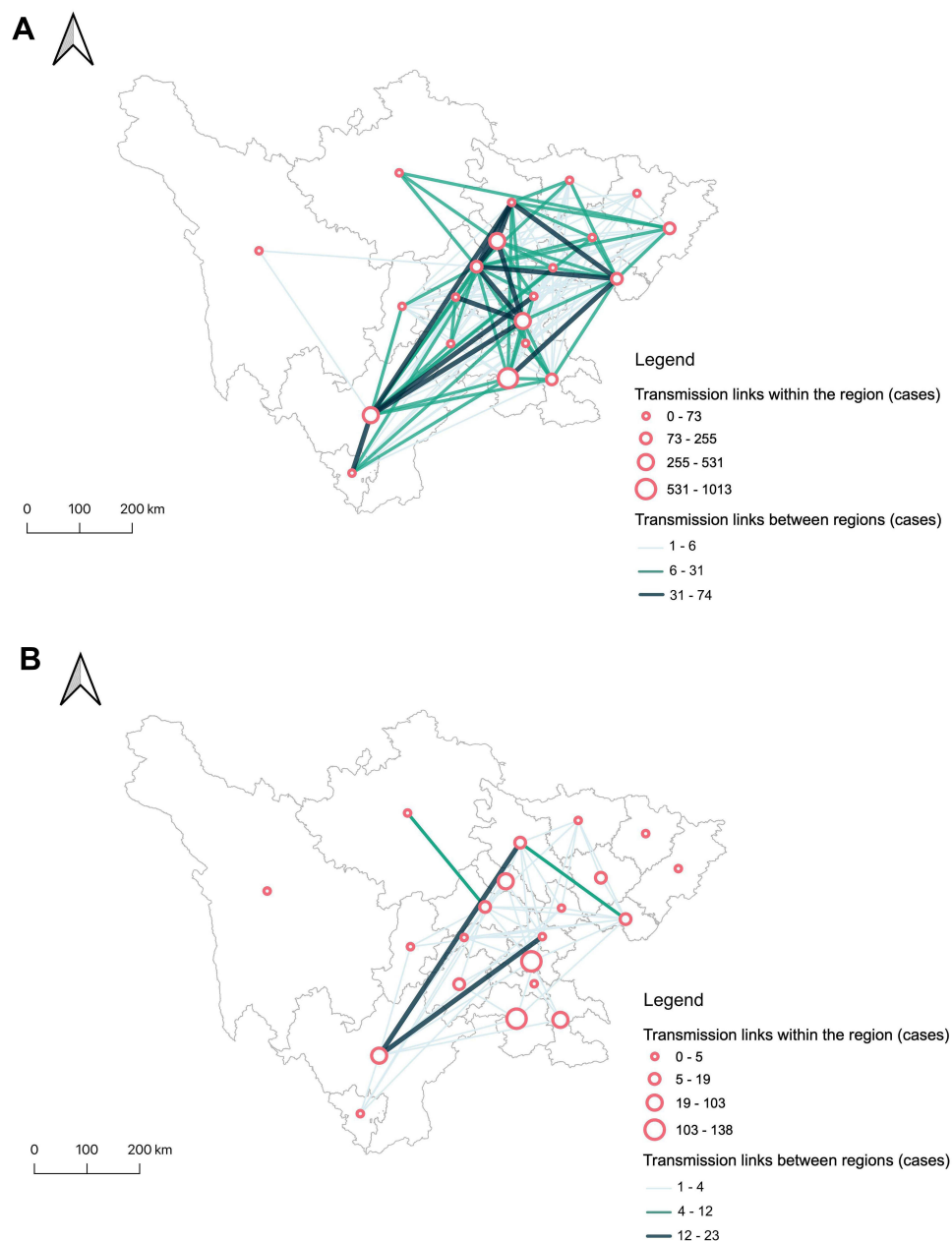
Abbreviations: CI, confidence interval; Hetero, heterosexual transmission; MSM, men who have sex with men; IDUs, injection drug users; MCT, mother to child transmission.

mainly intracity transmission, but cross-regional transmission should not be ignored (Figure 4B).

### Analysis Links of HIV DRMs with Subtype in Transmission Network

A total of 2159 nodes were observed in the transmission network, 761 of 2159 DR nodes (35.25%) at least linked one other node, among them 647 nodes (13.36%) were linked to common DRMs (Figure 3D). Table 3 lists all DR-DR links DRMs with five different subtypes among PLWH with virological failure, 12 of 37 DRMs had a significant difference in the subtypes ( $P < 0.001$ ). In our analysis, the DRMs frequency to non-nucleoside reverse transcriptase inhibitors (NNRTIs, 93.20%, 603/647) was much higher than that to nucleoside reverse transcriptase inhibitors (NRTIs, 46.37%, 300/647) and protease inhibitors (PIs, 2.01%, 13/647), and the DRMs of PI had no significant difference in subtypes ( $P > 0.001$ ). Analysis Links of HIV DRMs with subtype in transmission network, the highest rate of drug resistance was observed for subtype B (26.67%, 4/15), followed by CRF08\_BC (18.22%, 92/505), CRF07\_BC (15.49%, 306/1975), CRF85\_BC (12.05%, 108/896) and CRF01\_AE (9.45%, 137/1450). But, these DRMs largely resided in CRF07\_BC (47.30%, 306/647), CRF01\_AE (21.17%, 137/647), CRF85\_BC (16.69%, 108/647) and CRF08\_BC (14.22%, 92/647), there were few individuals of subtype B (0.62%, 4/647).

In 647 DR-DR links, K103N/S/E/Q was the most frequent DRMs, accounting for 45.60% (295/647), followed by M184V/I (27.20%, 176/647), Y181C (17.62%, 114/647), V179D/E (16.38%, 106/647), and K70R/E/T/S/Q/H/G (14.06%, 91/647). In DRMs of CRF07\_BC, 164 links (8.30%) shared a common DRMs of K103N/S/E/Q in the NNRTI coding region and 81 links (4.10%) shared the common site of M184V/I in the NRTI coding region, furthermore, Q58E (0.51%, 10/1975) was the highest mutation probability in the PI coding region, and only detected in CRF07\_BC. The most frequent DRMs in CRF01\_AE was K103N/S/E/Q (3.17%, 46/1450); moreover, M184V/I (2.41%, 35/1450) was quite more. The most common DRMs in CRF08\_BC was V108I (11.09%, 56/505), followed by Y181C (10.30%, 52/505), M184V/I (10.30%, 52/505) and K70R/E/T/S/Q/H/G (10.10%, 51/505), in addition, the mutation frequency of D67N (7.13%, 36/505), T215F/Y/S (8.91%, 45/505), V108I were significantly higher than that of other subtypes. Although there were few kinds of mutation sites of CRF85\_BC, they were mostly concentrated in K103N/S/E/Q (5.58%, 50/896), V179D/E (3.46%, 31/896). It was suggested that the linked sequences with DR tended to have common DRMs and they were interlinked, indicating potential transmitted DR.



**Figure 4** The geographic dimension of HIV-1 Drug-resistance transmission. **(A)** HIV-1 transmission in Sichuan. **(B)** HIV-1 Drug Resistance transmission with virological failure of ART in Sichuan. Circles with different size represents the number of individuals with drug resistance-associated transmission links within the region; the lines with different colors represent the number of individuals with drug resistance-associated transmission links between regions.

## Discussion

We used HIV transmission network analysis to characterize DR transmission links of those infected with ART virological failure. We found the characteristic determining DR phylogenetic clustering and multiple links in the genetic transmission networks. Furthermore, we found that the spatial distribution of DR phylogenetic clustering was mainly clustered in Southern of Sichuan, except for Liangshan. The links within and between the cities, and the strength of the transmission connection are fully

expounded. CRF07\_BC was the most widespread subtype, while CRF85\_BC (66.3%) showed the highest clustering rate. DR-DR links among the transmission network showed that there was a significant correlation between subtypes and common DRMs.

The HIV-1 transmission network is concentrated, four networks contained 831 (38.49%) individuals, with the largest network containing 342 individuals. Among this network, 761 DR subjects were observed, further analysis showed that 373 (49.01%) of individuals had more than 2 DR-DR links,

**Table 3** Links of HIV DRMs with Subtype in Transmission Network

DRMs	B (n=15)		07BC (n=1975)		01AE (n=1450)		08BC (n=505)		85BC (n=896)		$\chi^2$	P
Total	4	26.67%	306	15.49%	137	9.45%	92	18.22%	108	12.05%	40.83	<0.001
PI	0	0.00%	11	0.56%	1	0.07%	1	0.20%	0	0.00%	10.84	0.028
M46I/L	0	0.00%	0	0.00%	1	0.07%	1	0.20%	0	0.00%	4.46	0.347
I54V	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
V82A	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
K43T	0	0.00%	2	0.10%	0	0.00%	0	0.00%	0	0.00%	1.38	0.847
L10F/I	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
L24I	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
T74P	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
Q58E	0	0.00%	10	0.51%	0	0.00%	0	0.00%	0	0.00%	14.54	0.006
NRTI	3	20.00%	169	8.56%	56	3.86%	63	12.48%	9	1.00%	113.24	<0.001
A62V	0	0.00%	1	0.05%	2	0.14%	0	0.00%	0	0.00%	2.28	0.686
D67N	0	0.00%	22	1.11%	15	1.03%	36	7.13%	1	0.11%	122.01	<0.001
E44D/A	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
K65R/E	1	6.67%	12	0.61%	20	1.38%	11	2.18%	2	0.22%	23.63	<0.001
K70R/E/T/S/Q/H/G	1	6.67%	17	0.86%	20	1.38%	51	10.10%	2	0.22%	213.25	<0.001
K219E/Q/R	1	6.67%	20	1.01%	18	1.24%	45	8.91%	0	0.00%	178.59	<0.001
L74I/V/F	1	6.67%	4	0.20%	4	0.28%	1	0.20%	0	0.00%	32.56	<0.001
L210W	0	0.00%	10	0.51%	3	0.21%	0	0.00%	0	0.00%	8.19	0.085
M41L	0	0.00%	13	0.66%	3	0.21%	0	0.00%	1	0.11%	9.49	0.05
M184V/I	2	13.33%	81	4.10%	35	2.41%	52	10.30%	6	0.67%	97.89	<0.001
T215F/Y/S	0	0.00%	6	0.30%	7	0.48%	45	8.91%	1	0.11%	277.76	<0.001
V75I/M	0	0.00%	5	0.25%	3	0.21%	0	0.00%	0	0.00%	3.42	0.49
Y115F/Y	0	0.00%	3	0.15%	3	0.21%	0	0.00%	1	0.11%	1.22	0.875
NNRTI	4	26.67%	273	13.82%	134	9.24%	89	17.62%	103	11.50%	33.03	<0.001
A98G/A	0	0.00%	3	0.15%	4	0.28%	0	0.00%	1	0.11%	2.11	0.715
E138A/Q/G/K	0	0.00%	7	0.35%	2	0.14%	1	0.20%	0	0.00%	4.31	0.365
F227L/C	0	0.00%	5	0.25%	3	0.21%	0	0.00%	2	0.22%	1.30	0.862
G190A/S/C/Q	1	6.67%	14	0.71%	20	1.38%	10	1.98%	2	0.22%	19.55	0.001
H221Y	0	0.00%	8	0.41%	2	0.14%	7	1.39%	3	0.33%	14.49	0.006
K101E/Q/H/P/A	1	6.67%	13	0.66%	10	0.69%	2	0.40%	1	0.11%	14.36	0.006
K103N/S/E/Q	1	6.67%	164	8.30%	46	3.17%	34	6.73%	50	5.58%	39.26	<0.001
K238T/N	0	0.00%	0	0.00%	1	0.07%	0	0.00%	0	0.00%	2.34	0.674
L100I/S/V/F	0	0.00%	1	0.05%	1	0.07%	0	0.00%	0	0.00%	0.90	0.925
M230L/I	0	0.00%	2	0.10%	0	0.00%	1	0.20%	0	0.00%	3.47	0.483
P225H	1	6.67%	0	0.00%	2	0.14%	2	0.40%	1	0.11%	57.38	<0.001
V106M/V/A	2	13.33%	24	1.22%	18	1.24%	2	0.40%	10	1.12%	22.17	<0.001
V108I	0	0.00%	6	0.30%	14	0.97%	56	11.09%	0	0.00%	334.58	<0.001
V179D/E	0	0.00%	44	2.23%	22	1.25%	9	1.78%	31	3.46%	10.55	0.032
Y181C	1	6.67%	45	2.28%	11	0.76%	52	10.30%	5	0.56%	168.44	<0.001
Y188L/C	0	0.00%	4	0.20%	6	0.41%	0	0.00%	1	0.11%	3.99	0.407

**Notes:** PI: PI resistance in both connected sequences; NRTI: NRTI resistance in both connected sequences; NNRTI: NNRTI resistance in both connected sequences; each drug resistance site stands for the drug resistance site in both connected sequences; n: the total number of sides after the subtype sequence constructs the molecular network.

**Abbreviations:** NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

indicating the presence of the individuals with transmission risk in the network,<sup>22</sup> 187 (24.57%) subjects had more than 4 links and were considered high-risk spreaders and more likely to spread HIV-1, one individual had 178 links and was

considered a super spreader, which differs from those in other provinces of China.<sup>11</sup> This super spreader is fit with previous studies that individuals with more linkages may have a higher transmission risk because of their high viral

load and high risk behavior (ie, inject drug),<sup>22–25</sup> therefore, these individuals may function as super-spreaders. Most of the individuals linked with the super-spreader were from Liangshan, but they had intercity connections with 14 regions. This finding reveals the existence of a wide spread transmission network of PLWH with virologically failed drug resistance.

Multivariate regression analysis shows among that those who had a history of sexually transmitted diseases, IDUs and CRF85\_BC subtype are more likely to be potential drug-resistant spreaders. PLWH had a history of sexually transmitted diseases, and clustering may be prone to be formed, because of the high-risk behavior features of this population, including multiple sex partners, low rates of condom use.<sup>19,26</sup> Those who inject drug use have more active high-risk behavior, and who reported previous or current injecting drug use were statistically significantly associated with higher rates of antiretroviral resistance,<sup>24</sup> therefore, they have a higher risk of drug resistance transmission. The PLWH of CRF85\_BC due to the short time of formation and transmission in Sichuan, and most of the infected people were concentrated in one area,<sup>27</sup> so, clustering was more likely to occur in the transmission network. This result was similar to that of previous study that different HIV-1 subtypes can affect the presence of drug resistance.<sup>28</sup> In Sichuan, subtype CRF07\_BC and CRF01\_AE subtypes were the main epidemic strains, and studies had shown that the possibility of resistance was higher in CRF01\_AE subtypes,<sup>19</sup> but CRF85\_BC was the subtype with the highest DR phylogenetic clustering rate in our study. This finding reflects the characteristics of HIV-1 DR transmission, which differ from those in other provinces of China,<sup>22,29</sup> and reveals why CRF85\_BC has been widely disseminated in Yibin region recently. Since subtype CRF85\_BC is mainly found in elderly people infected by non-married heterosexual transmission as a previous report,<sup>27</sup> which might be attributed to the hidden high-risk populations (eg, sex workers). Some cities (eg, Luzhou) showed the spread trend except for Yibin; hence, additional molecular epidemiology surveillance is required for CRF85\_BC.

Besides, we find that employed, AIDS-sufferer being involved in clinical stage, ART time >36 years were protective factor for the spread of drug resistance. Compared with farmers, employed patients have higher opportunities for health education than farmers, so the risk of transmission was reduced and further drug resistance was reduced. For AIDS stage patients, the transmission intensity of patients was limited. The patients in this stage may have various

complications, which affected their activities, so the transmission rate of drug resistance is reduced. And that time of ART suggests that ART plays an important role in inhibiting drug-resistant transmission, emphasizing the need for early diagnosis and timely antiretroviral treatment.

High drug-resistant clustering rates of PLWH were distributed in Southern and Northeast Sichuan Province, which reflected the spread of DR in these areas. Previous studies had shown that regions for relatively severe HIV-1 transmission dynamics might be due to human mobility, relatively developed traffic, and strong economic activity,<sup>30,31</sup> notably, regions with high drug-resistant clustering rates of PLWH who virological failure for ART may not be matched for regions with severe epidemics in this study, it may be related to PLWH in southern Sichuan was mainly of age  $\geq 50$ , as ART virological failure usually occurs the elderly people.<sup>14,19</sup> As the worst-hit HIV epidemic region in China,<sup>14</sup> Liangshan had a low drug resistance phylogenetic clustering rate (7.54%), which may be attributed to poor traffic and economic activity. Besides, China's 13th five-year-plan has achieved huge effect for PLWH in Liangshan,<sup>32</sup> to a certain extent, inhibited the spread of HIV, thus affecting the DR phylogenetic clustering rate. In contrast, several of the cities showed higher DR phylogenetic clustering rates with less severe HIV epidemics, such as Neijiang. The result reflects that regions, where the HIV epidemic is not severe in the past, may not be particularly concerned for possible high DR phylogenetic clustering rates, which should arouse attention. Furthermore, previous research proved that spatial clustering can be used to show the geographic transmission of HIV.<sup>33</sup> The high drug resistance phylogenetic clustering rate regions in our study provide a new focus for our geographic intervention.

Exploring the geographic dimension of HIV-1 DR transmission among PLWH with virological failure, we found Liangshan exhibited the most linkage with the other cities. Interestingly, the major cities of cross-regional HIV-1 transmission with Liangshan are Mianyang and Ziyang, and the geographical distance between Liangshan from the two places is relatively far apart, it may be related to the fact that Ziyang compulsory detoxification center of Sichuan Province guards many IDUs from Liangshan. However, the intensity of transmission of these neighboring cities to Liangshan may not be that strong enough, which indicated a noteworthy long-distance strong interaction. Besides, Yibin, Neijiang, Luzhou showed obvious characteristics of intracity DR-DR links intensity ratio, among these cities with more than

40 DR-DR links, suggesting that there was more likely regional transmission in these areas. Furthermore, among the transmission network, most of DR (85.02%) subjects were linked to other DR sequences, indicating that PLWH with drug resistance were more likely to link with those with drug resistance. Of course, there may be deeper social factors that determine the spatial flow of the virus. As a previous study has indicated, the increase of human exposure and the increase in population mobility lead to the high risk of disease transmission among numerous urban population, cities become incubators that meet all the conditions of the outbreak, and urbanization may lead to the strengthening of disease transmission links between cities in the future.<sup>34</sup>

We analyzed the links of DRMs in the transmission network to further confirm the transmission of DR in PLWH with virological failure. Six hundred and forty-seven of 761 links (85.02%) were linked to common DRMs, suggesting that HIV-1 resistant strains would spread out. The most common linked DRMs found in our study were directed against at NNRTIs, which is consistent with this category drug widely used as standard first-line China's National ART Guidelines,<sup>35,36</sup> the more widespread use of NRTIs and NNRTIs may be the major cause of the higher prevalence of resistance to NNRTI than to PI in Sichuan. In our study, it was found that the drug resistance mutation of DR-DR links in the network significantly differ among the five main subtypes, 12 of 37 drug resistance mutation sites had significant difference ( $P < 0.001$ ), which was different from the results of previous study in other region.<sup>35</sup> The results showed that different subtypes of HIV-1 acquired different drug-resistant mutation sites could occur when exposing them to ART for long term, and these mutation sites may be stably passed; thus, it provides some clues of adaptability of the virus and renewal of the ART regime.

## Limitations

The study has some limitations. First, to compare the drug-resistant links with different subtypes, we selected the threshold value of the highest cumulative cluster number of five subtypes, which may have some deviation from CRF85\_BC, for the subtype is prevalent in Sichuan for a short time. Second, we only used the sequences of Sichuan province, which may not fully reflect the actual situation of drug-resistant transmission, thus, may strengthen regional cooperation especially in Chongqing, which is the province with the most cross-regional HIV-1 transmission of Sichuan; therefore, the result provides

direction, which may strengthen regional cooperation. Third, a potential sampling bias, we could analyze only the individuals with ART virological failure, but those that ART naive newly diagnosed individuals could not be included in the analysis, there may be the possibility of the same drug resistance mutations after ART, therefore, to avoid the interference caused by ART drug-resistant mutation, 62 drug-resistant mutation sites were removed from all sample sequences by comparison reference subtype standard-strain sequences.

## Conclusions

This study shows that transmission network links analysis based on molecular methods can be used to explore the HIV-1 drug-resistant spread influencing factors and transmission patterns of PLWH with ART virological failure. Additionally, we found regions with high drug-resistant transmission of PLWH may not be matched for regions with severe epidemics. Using the network links to infer the patterns of HIV transmission and identify the high-risk populations of drug resistance, the spatial distribution and clustering patterns of the clustering and spatial links of HIV-1 between cities showed the geographic variations in HIV-1 drug-resistant transmission and high-risk areas. Our findings will be helpful in strengthening the evidence-based to drug-resistant transmission targeting interventions.

## Ethics Approval

This study was approved by the local Ethics Committee at Sichuan Center for Disease Control and Prevention, according to the Helsinki II Declaration. All participants in this study provided written informed consent.

## Author Contributions

All authors made a significant contribution to the work reported, CZ and DY designed the study; RK and TF participated in the spatial clustering analysis process; YL, YZ, LL and LY participated in the process of sequences editing and phylogenetic analyses; CZ and DY performed statistical analysis; SL, DY and LS collected sample and the demographic data; CZ, RK and DY participated in the writing process. All authors have agreed on the journal to which the article has been submitted.

## Funding

This study was supported by grants from the Scientific research project of Sichuan Health and Health Commission (20PJ121) and Sichuan Science and Technology program (2020YJ0449).

## Disclosure

The authors declare no conflicts of interest for this work.

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