ORIGINAL RESEARCH

HIV-1 Drug Resistance and Genetic Transmission Networks Among MSM Failing Antiretroviral Therapy in South China 2014–2019

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Background: Guangdong, located in South China, is one of the areas heavily affected by HIV-1 in China. The transmission of HIV-1 among men who have sex with men (MSM) has gradually been increasing in Guangdong.

Objective: To investigate the characteristics of the HIV-1 drug resistance, and genetic transmission networks in MSM with antiretroviral therapy (ART) failure from 2014 to 2019 in Guangdong.

Methods: HIV-1 *pol* gene sequences were amplified. An online subtyping tool was used to determine the genotype, and a maximum likelihood phylogenetic tree was reconstructed to confirm the genotype results. The Stanford University HIV Drug Resistance Database was used to analyse the sequences of drug resistance mutations (DRMs) and drug resistance profiles. A pairwise Tamura-Nei 93 genetic distance-based method was used to analyse the genetic transmission networks.

Results: Of 393 sequences isolated from HIV-infected MSM with ART failure, CRF01_AE (47.3%), CRF07_BC (21.4%) and CRF55_01B (21.4%) were the top three strains. 55.2% individuals harboured NRTI DRMs, whereas 67.4% carried NNRTI DRMs. 96.8% cases harboured mutations resistance to NRTIs or NNRTIs at high-level. The most common DRMs were M184I/V (42.2%), followed by V179D/E (37.9%) and K65R (27.2%). Of the subtype B sequences, no sequence fell into a cluster. Of the CRF01_AE, CRF55_01B, and CRF59_01B sequences, 14.5%, 61.9%, and 33.3% fell into clusters, respectively. Of the CRF07_BC sequences, 39.3% fell into clusters. The majority of MSM in transmission networks were concentrated at age below 35 years old, with multiple links. Moreover, approximately 54.8% of MSM had more than 2 potential transmission partners.

Conclusion: Drug resistance mutations more frequently occurred in NNRTIs among MSM with ART failure in Guangdong Province. Transmission network analysis revealed a complex transmission pattern, and more attention should be given to younger HIV-1-infected MSM with multiple links.

Keywords: HIV-1, drug resistance, genetic transmission networks, MSM

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Introduction

Up to Aug 31, 2018, approximately 850 thousand people living with HIV were reported nationwide in China.[1] The prevalence of HIV-1 infection is still a massive challenge to public health, although highly active antiretroviral therapy (ART) has improved the quality of life of those living with HIV or AIDS and decreased mortality rates.[2] Guangdong, located in South China, is one of the areas in China

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that is most heavily affected by HIV-1. The number of people receiving ART has increased since the nationwide National Free Antiretroviral Treatment Program (NFATP) started in the early 2000s in Guangdong Province. HIV-infected people receiving ART have a lower risk of HIV transmission.[3] However, increasing HIV drug resistance occurs after the expansion of ART and the Treat-all strategy.

Men who have sex with men (MSM) continue to be a major cause of new infections in America, Europe, Oceania, and Asia,[4] including China. The proportion of newly reported HIV/AIDS cases infected via MSM increased continuously, from 0.7% in 2005 to 25.8% in 2014.[5] In Guangdong Province, MSM with HIV increased from 5.0% in 2008 to 11.4% in 2013 of the infected population.[6] The risk factors for HIV transmission in MSM included multiple sex partners, unprotected anal intercourse, low rates of condom use, drug use, and a history of other sexually transmitted diseases.[7] More attention should be paid to MSM population, including the circulating HIV strains.

Here, we focused specifically on the occurrence and characteristics of HIV-1 drug resistance mutations (DRMs) and the extent of changes in drug susceptibility with long-term treatment among ART-experienced MSM adults in Guangdong Province, China, by targeting the polymerase (*pol*) gene. The results will help clarify HIV-1 genetic diversity and the progression of drug resistance among ART-failing MSM in Guangdong Province between 2014 and 2019.

Materials and Methods
Study Population and Data Collection
HIV-infected patients attending NFATP were regularly followed up at sentinel hospitals in 21 prefectures of Guangdong Province. The plasma HIV-1 viral load after 6 months of ART administration was quantified at least once per year at Guangzhou Eighth People's Hospital. Samples with a viral load of ≥1000 copies/mL were subjected to drug resistance genotyping using an in-house RT-PCR method. Patients were eligible for inclusion in this study, including MSM living in Guangdong, followed up from Jan 1st 2014 to Dec 31st 2019, and had a plasma viral load ≥1000 copies/mL. Patients were excluded if they had used ART outside national guidelines or were missing treatment regimen information.

The epidemiological and demographic information of patients was downloaded and recorded from the National Information Surveillance System, including age, occupation, education level, marital status, WHO classification, geographical region, CD4+ T cell count, sampling area and therapeutic regimen.

Nucleic Acid Extraction, Amplification, and Sequencing
Viral RNA was extracted from 140 μL of plasma using a QIAamp Viral RNA Mini Kit (Qiagen, Germany). The amplification of the HIV-1 pol gene fragments (HXB2 2253–3318, covering the full-length protease and the first 240 amino acids of reverse transcriptase codons) was performed using one-step reverse transcription PCR (RT-PCR) by using the PrimeScript one-step RT-PCR Kit Ver. 2 (Takara, China), followed by nested PCR using *Ex Taq* Hot Start Version DNA Polymerase (Takara, China). The amplified PCR products were electrophoresed on a 1.0% agarose gel with the DL2000 DNA marker (Takara, China). The positive products were purified using the Agarose Gel DNA Extraction Kit (Takara, China), according to the manufacturer's protocol and sent to Tianyi Huiyuan Genomics Company for Sanger's sequencing.

Sequence Assembly and Phylogenetic Analyses
The obtained sequences were assembled and cleaned by DNA sequence analysis software Sequencher V5.4.6 [Gene codes, US.], and the alignments were performed by BioEdit V7.2.

To determine the genotype, the sequences were submitted to the online subtyping tool COMET developed by the Luxembourg Institute of Health (https://comet.lih.lu/).[8] Then, phylogenetic analysis was conducted to confirm the genotype results.

The alignments, including all the obtained sequences, were merged with HIV-1 subtyping references retrieved from Los Alamos HIV Sequence Database using BioEdit software. Then, the maximum likelihood (ML) tree was reconstructed using PhyML software V3.0[9] based on the GTR substitution model. The approximate likelihood ratio test (aLRT)[10] was used to estimate the branch support. The final trees were visualized using Figtree V1.4.2. The SH-like support aLTR value of 0.9 was selected as the threshold for high reliability of lineages.

Drug Resistance Analyses

The HIVdb program from Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/hivdb/by-sequences/) was used to analyse the sequences for DRMs.[11] DRMs were classified into the PI, NRTI or NNRTI drug classes. Drug resistance was divided into five levels, and sequences with a low-level or greater category of resistance were defined as drug resistance.

HIV-1 Genetic Transmission Network Construction

To avoid biases due to the potential for convergent evolution driven by antiretroviral therapy, 43 codons in PR and RT associated with DRMs were removed according to the major HIV-1 DRMs last updated on Oct 23, 2020.[12]

The pairwise Tamura-Nei 93 (TN93) nucleotide substitution model[13] was used to calculate the genetic distance for the different genotype sequences analysed using the HyPhy software V2.2.4. The genetic transmission network was visualized and analysed using the network software Cytoscape V3.2.1 with a pairwise genetic distance of 0.005–0.015.[14] The genetic distance threshold is defined as the distance that identifies the maximum number of clusters in the transmission networks.

Nucleotide Sequence Data

The nucleotide sequences were submitted to GenBank under the accession numbers MW852088-MW852480.

Statistical Analysis

An Excel database was established, and all statistical analyses were performed using IBM SPSS V25.0. Qualitative statistics were described using frequency, and quantitative statistics were described using the median (IQR). Categorical variables were compared using Fisher's exact tests. Variables for drug resistance with a P-value < 0.05 in the Fisher's exact tests were included in the multivariate logistic regression analysis. For all statistical tests, the level of significance for the evaluation of the two-sided P values was set at 0.05.

Results

Characteristics of the Study Population

Of all the patients who received ART for more than 6 months in Guangzhou Eighth People's Hospital from 2014 to 2019, with once HIV-1 viral load testing per year, a total of 3895 individuals had a plasma viral load of ≥1000 copies/mL. Among the 3895 plasma samples, 3584 HIV pol gene sequences were obtained. A total of 393 sequences were from HIV-infected MSM, accounting for 11.0% (393/3584).

The demographic characteristics of these HIV-infected MSM are shown in Table 1. The participants were aged between 18 and 67 years, with a median age of 32 years,

Table 1 Demographic Characteristics of 393 MSM-Infected Patients with Virologic Failure in ART

Characteristic	
Age (years), n (%)	
15–35	246(62.6)
36–50	122(31.0)
≥51	25(6.4)
Age (years), median (IQR)	32(18–67)
Geographical region, n (%)	
Pearl River Delta	334(85.0)
Eastern	15(3.8)
Western	23(5.9)
Northern	21(5.3)
Marital status, n (%)	
Unmarried	270(68.7)
Married or cohabiting	97(24.9)
Divorced or separated	26(6.6)
Baseline CD4+ T cell count (cells/mm3), n (%)	
≤200	243(61.8)
>200	150(38.2)
Baseline CD4+ T cell count (cells/mm3), median (IQR)	141(1–966)
Years from ACT to ART, median (IQR)	0.10(0.00–8.16)
Years from ART to DRT, median (IQR)	1.42(0.50–8.17)
Initial treatment regimen, n (%)	
ZDV/D4T+3TC+NVP/EFV	74(18.8)
TDF+3TC+EFV/NVP	285(72.5)
ZDV/TDF+3TC+LPV/r	34(8.7)
DRT viral load (log10 copies/mL), median (IQR)	4.37(3.00–6.69)

Abbreviations: ACT, antibody confirmation test; DRT, drug resistance test; ZDV, Zidovudine; D4T, Stavudine; 3TC, Lamivudine; NVP, Nevirapine; EFV, Efavirenz; TDF, Tenofovir disoproxil fumarate; LPV/r, Ritonavir-boosted lopinavir.

and 62.6% (246/393) of them were 15 to 35 years old. Most of them (334, 85.0%) were distributed in the Pearl River Delta region and unmarried (270, 68.7%). The median (range) baseline CD4$^+$ T cell count was 141 (1–966) cells/mm^3, and 61.8% (243/393) of the participants had a baseline CD4$^+$ T cell count of <200 cells/mm^3. The median (range) HIV-1 viral load (log10 copies/mL) in the drug resistance test was 4.37 (3.00–6.69). The dominant initial treatment regimen was Tenofovir (TDF)+ lamivudine (3TC)+ Efavirenz (EFV)/Nevirapine (NVP) (72.5%, 285/393).

Distribution of Genotypes

The ML phylogenetic analyses based on the *pol* gene regions showed that CRF01_AE was the most common strain circulating among MSM with ART failure in Guangdong Province (47.3%, 186/393), followed by

CRF07_BC (21.4%, 84/393), CRF55_01B (21.4%, 84/393), subtype B (5.1%, 20/393), CRF55_01B (2.3%, 9/393) and others (Figure 1, Table 2).

Drug Resistance Mutations

Of the 393 MSM patients with ART failure, 69.7% (274/393) had at least one DRM. 55.2% patients (217/393) harboured NRTI resistance mutations, whereas 67.4% (265/393) carried NNRTI resistance mutations, with only 0.5% (2/393) PI resistance mutations. Dual-class mutations were present in 53.4% (210/393) of the patients, with 53.2% (209/393) having NRTI plus NNRTI resistance mutations and 0.3% (1/393) having PI plus NNRTI resistance mutations. No triple-class mutations were found.

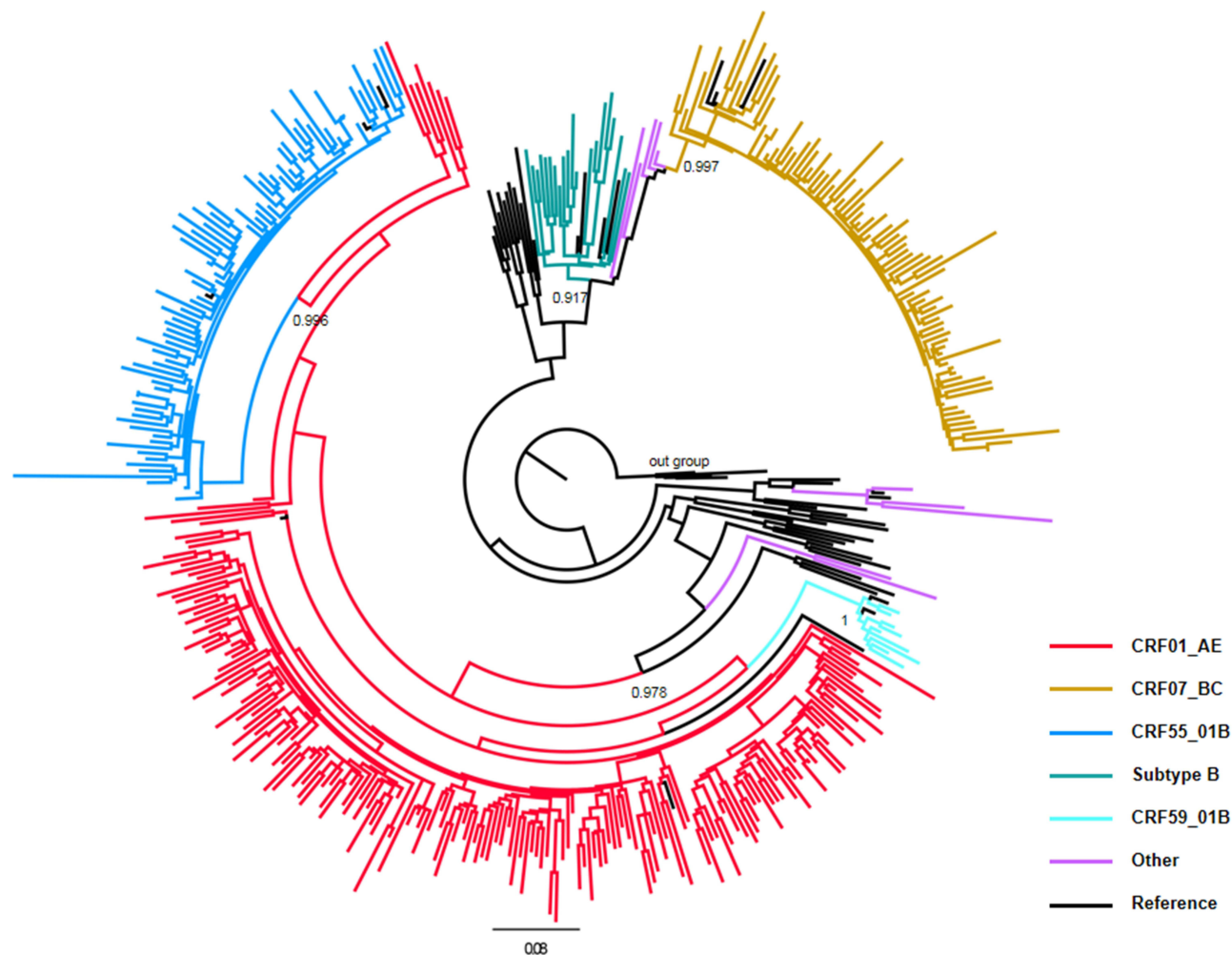One case (0.3%, 1/393) carried PI-related DRMs, K20T, M46I, and K43T. Among the NRTI DRMs, M184I/V

Figure 1 Distribution characteristics of HIV-1 genotypes among MSM with ART failure in Guangdong Province from 2014 to 2019. The maximum likelihood phylogenetic tree was constructed using PhyML 3.0. The reference sequences, including nine subtypes (A–D, F–H, J and K), CRF01_AE, CRF07_BC, CRF08_BC, CRF55_01B, and CRF59_01B, were downloaded from the Los Alamos HIV Sequence Database (https://www.hiv.lanl.gov/content/index). Different colour lines represent different genotypes. The subtype H sequences were selected as the outgroup. aLTR values higher than 0.9 were used to identify lineages and are indicated at the corresponding nodes of the tree.

Table 2 General Information Accompanied with Drug Resistance Among 393 MSM Patients with ART Failure

Characteristics	HIV Virologic Failure, N=393	Drug Resistance, n (%)	P for Fisher Exact Tests	Multivariate Logistic Regression	
				Adjusted OR(95% CI)	P-value
Age(years)					
15–35	246	160(65.0)	0.656		
36–50	122	78(63.9)			
≥51	25	14(56.0)			
Occupation					
Student	16	8(50.0)	0.085		
Farmer	21	8(38.1)			
Employed	177	118(66.7)			
Unemployed	97	63(65.0)			
Unknown	82	55(67.1)			
Marital status					
Unmarried	270	175(64.8)	0.438		
Married or cohabiting	97	58(59.8)			
Divorced or separated	26	19(73.1)			
WHO classification					
I	58	31(53.5)	0.698		
II	56	36(64.3)			
III	244	157(64.3)			
IV	35	28(80.0)			
Geographical region					
Pearl River Delta	334	213(63.8)	0.734		
Eastern	15	11(73.3)			
Western	23	11(47.8)			
Northern	21	17(81.0)			
Treatment duration(year)					
<1	141	118(83.7)	<0.001	1.000	
1–3	192	113(58.9)		2.562(1.429–4.590)	0.002
>3	60	21(35.0)		7.292(3.436–15.476)	<0.001
Baseline CD4$^+$T cell count (cells/mm^3)					
≤200	243	190(78.2)	<0.001	1.000	
>200	150	62(41.3)		3.896(2.367–6.413)	<0.001

(*Continued*)

Table 2 (Continued).

Characteristics	HIV Virologic Failure, N=393	Drug Resistance, n (%)	P for Fisher Exact Tests	Multivariate Logistic Regression	
				Adjusted OR(95% CI)	P-value
Viral load (log10)					
3~	134	76(56.7)	0.502		
4~	180	118(65.6)			
≥5	79	58(73.4)			
Genotypes					
Subtype B	20	11(55.0)	0.040	1.000	
CRF01_AE	186	131(70.4)		0.667(0.235–1.894)	0.447
CRF07_BC	84	30(35.7)		2.116(0.702–6.376)	0.183
CRF55_01B	84	68(81.0)		0.308(0.097–0.980)	0.046
CRF59_01B	9	4(44.4)		1.977(0.355–11.026)	0.437
Others	10	8(80.0)		0.348(0.047–2.584)	0.302

(42.2%, 166/393) was the most frequent, followed by K65R (27.2%, 107/393). The most common NRTI mutation pattern was M184V+K65R, with a frequency of 15.0% (59/393). The major NNRTI DRMs were V179D/E (37.9%, 149/393), V106I/M (25.7%, 101/393), and K103N/Q (25.2%, 99/393) (Table 3).

Resistance to ART

252 out of 393 MSM (64.1%) were resistant to at least one drug (Table 2). Among them, 244 individuals (96.8%) harboured mutations conveying high-level resistance to NRTIs or NNRTIs. 198 (78.6%) individuals expressed dual resistance to NRTIs and NNRTIs, whereas 13 (5.2%) and 33 (13.1%) ones expressed single resistance to NRTIs and NNRTIs, respectively. Fisher's exact tests showed significant differences in drug resistance rates in terms of different treatment durations, baseline CD4$^+$ T cell counts, and genotypes of HIV-1 (P < 0.05) (Table 2). The patients with a treatment duration of less than one year had the highest drug resistance rate (83.7%, 118/141), compared with the other group; the patients with a baseline CD4$^+$ T cell count ≤200 cells/mm^3 had a higher drug resistance rate (78.2%, 190/243) than the patients with a baseline CD4$^+$ T cell count >200 cells/mm^3; and the CRF55_01B patients had the highest drug resistance rate (81.0%, 68/84) (Table 2).

Genetic Transmission Networks of MSM with ART Failure

MSM with subtype B, CRF01_AE, CRF07_BC, CRF55_01B, and CRF59_01B pol sequences were identified and used for genetic transmission network analysis. For different genotypes, transmission networks were constructed with different genetic distance thresholds to identify the maximum number of clusters.

Of the 20 subtype B *pol* sequences, no sequence fell into a cluster at a genetic distance threshold from 0.005 to 0.015. Among 186 CRF01_AE *pol* sequences, 27 (14.5%) sequences fell into clusters at a genetic distance threshold of 0.015, resulting in 8 clusters ranging in size from 2 to 8 (Figure 2A). Of 84 CRF07_BC *pol* sequences, 33 (39.3%) sequences fell into clusters at a genetic distance threshold of 0.013, resulting in 3 clusters ranging in size from 2 to 29 (Figure 2B). Of 84 CRF55_01B *pol* sequences, 52 (61.9%) sequences fell into 3 clusters with sizes of 2 and 48 at a genetic distance of threshold 0.015 (Figure 2C). Of the 9 CRF59_01B *pol* sequences, 3 (33.3%) sequences fell into 1 cluster at a genetic distance threshold of 0.015 (Figure 2D)

Among clusters for CRF01_AE pol sequences, 17 MSM (63.0%) were found to have only one linked individual, and 10 (37.0%) had ≥2 linked individuals. Among

Table 3 The Number and Prevalence (%) of Drug Resistance Mutations Among MSM with ART Failure

DRM	Total	B	CRF01_AE	CRF07_BC	CRF55_01B	CRF59_01B	Others
	N=393	N=20	N=186	N=84	N=84	N=9	N=10
PI							
M46I	1(0.3)	–	–	1(1.2)	–	–	–
K43T	1(0.3)	–	–	1(1.2)	–	–	–
K20T	1(0.3)	–	–	–	1(1.2)	–	–
NRTI							
M184I/V	166(42.2)	8(40.0)	85(45.7)	20(23.8)	45(53.6)	4(44.4)	4(40.0)
K65R	107(27.2)	4(20.0)	57(30.7)	11(13.1)	30(35.7)	1(11.1)	4(40.0)
K70E/Q/R/T	53(13.5)	4(20.0)	25(13.4)	6(7.1)	15(17.9)	1(11.1)	2(20.0)
Y115F	51(13.0)	2(10.0)	33(17.7)	3(3.6)	10(11.9)	–	3(30.0)
D67G/N	41(10.4)	4(20.0)	25(13.4)	7(8.3)	4(4.8)	1(11.1)	–
A62V	22(5.6)	2(10.0)	12(6.5)	–	6(7.1)	1(11.1)	1(10.0)
L74I/V	19(4.8)	–	12(6.5)	3(3.6)	4(4.8)	–	–
K219E/Q	16(4.1)	1(5.0)	7(3.8)	1(1.2)	7(8.3)	–	–
T215F/I/Y	14(3.6)	1(5.0)	6(3.2)	1(1.2)	6(7.1)	–	–
M41L	12(3.1)	–	6(3.2)	2(2.4)	4(4.8)	–	–
V75I/M	12(3.1)	–	9(4.8)	–	3(3.6)	–	–
L210W	2(0.5)	–	–	1(1.2)	1(1.2)	–	–
F77L	1(0.3)	–	1(0.5)	–	–	–	–
T69N	1(0.3)	–	1(0.5)	–	–	–	–
NNRTI							
V179D/E	149(37.9)	5(25.0)	45(24.2)	9(10.7)	84(100.0)	1(11.1)	5(50.0)
V106I/M	101(25.7)	3(15.0)	61(32.8)	12(14.3)	20(23.8)	2(22.2)	3(30.0)
K103N/Q	99(25.2)	6(30.0)	40(21.5)	9(10.7)	39(46.4)	3(33.3)	2(20.0)
G190A/S	64(16.3)	4(20.0)	51(27.4)	4(4.8)	4(4.8)	–	1(10.0)
Y181C	64(16.3)	2(10.0)	34(18.3)	5(6.0)	19(22.6)	1(11.1)	3(30.0)
Y188C/H/L	28(7.1)	2(10.0)	11(5.9)	2(2.4)	12(14.3)	–	1(10.0)
K101E/H/P	26(6.6)	–	20(10.8)	5(6.0)	1(1.2)	–	–
F227L	26(6.6)	–	14(7.5)	9(10.7)	3(3.6)	–	–
H221Y	19(4.8)	1(5.0)	12(6.5)	–	5(6.0)	–	1(10.0)
E138A/G	13(3.)	1(5.0)	2(1.1)	1(1.2)	9(10.7)	–	–
M230L	11(2.8)	–	7(3.8)	–	4(4.8)	–	–
V108I	10(2.5)	–	5(2.7)	–	4(4.8)	–	1(10.0)

(Continued)

Table 3 (Continued).

DRM	Total	B	CRF01_AE	CRF07_BC	CRF55_01B	CRF59_01B	Others
	N=393	N=20	N=186	N=84	N=84	N=9	N=10
P225H	8(2.0)	2(10.0)	4(2.2)	–	2(2.4)	–	–
L100I	7(1.8)	2(10.0)	I(0.5)	–	3(3.6)	–	I(10.0)
A98G	5(1.3)	–	3(1.6)	–	2(2.4)	–	–
K238T	3(0.8)	–	I(0.5)	–	2(2.4)	–	–
L234I	2(0.5)	–	2(1.1)	–	–	–	–

Note: The major mutations were denoted by bolding.

clusters for CRF07_BC pol sequences, 16 MSM (48.5%) were found to have only one linked individual, and 17 (51.5%) had ≥2 linked individuals. Among the clusters for CRF55_01B pol sequences, 17 MSM (32.7%) were found to have only one linked individual, and 35 (67.3%) had ≥2 linked individuals. Of all 15 transmission networks, 69 (60.0%, 69/115) sequences from patients aged 15 to 35 years old were dispersed among 73.3% (11/15) of transmission networks, and 59 (51.3%, 59/115) sequences from patients with drug resistance were dispersed among 86.7% (13/15) of transmission networks (Figure 2).

There were no significant differences between genetic linkages in terms of age, occupation, marital status, WHO classification, geographical region, treatment duration, baseline CD4^{+} T cell count, viral load (log10) in ART failure, or individuals who showed or did not show drug resistance after ART failure (Table 4).

Discussion

HIV epidemics in Guangdong have expanded beyond injection drug users and grown into sexual populations, including MSM and heterosexual contact, since 2013.[15] The occurrence of HIV-1 acquired drug resistance becomes a critical clinical and public health issue and will lead to ART failure. This study analysed the prevalence of resistant variants and linking-associated factors for genetic transmission networks of MSM with ART failure in Guangdong, providing implications for the management of ART.

The results show that CRF01_AE (47.3%) and CRF07_BC (21.4%) were the dominant circulating HIV-1 strains in MSM with ART failure in Guangdong. A recent nationwide survey in China revealed that CRF01_AE and CRF07_BC accounted for more than 80% of HIV-1 infections among MSM in nine major

Chinese cities,[16] which was consistent with our results. CRF55_01B is the CRF01_AE/B recombinant strain originating from MSM in Shenzhen, Guangdong, and was first reported in 2013.[17,18] In the present study, CRF55_01B was the third most dominant circulating genotype, accounting for 21.4%, higher than the 8.5% in Guangdong in 2013,[19] 9.2% in Shenzhen in 2006–2013,[18] and 10.7% in Guangzhou in 2018.[20]

The overall prevalence of drug resistance was 66.7% in MSM with ART failure in Guangdong, which was similar to that in Yunnan (66.27% in MSM with ART failure from 2014 to 2019)[21] and relatively higher than those in other areas of China, such as Sichuan (50.7% in MSM with ART failure from 2011 to 2017)[22] and Hunan (41.3% in MSM with ART failure from 2012 to 2017).[23]

M184I/V (42.2%) was the most prevalent NRTI-associated mutation, which might be caused by the frequent use of 3TC in ART-experienced patients.[24] This caused high-level resistance to 3TC and Emtricitabine (FTC) and low-level resistance to Abacavir (ABC) and Didanosine (DDI). In contrast, M184I/V increased susceptibility to Zidovudine (AZT), Stavudine (D4T) and TDF and slowed the emergence of AZT, D4T, and TDF resistance. K65R (27.2%) is the second most common NRTI-associated resistance mutation and has only a relatively low level of reduced drug susceptibility. A high frequency of K65R plus M184V/I (15.0%) was observed in this study, and K65R plus M184 V/I appeared sufficient to abrogate the NRTI activity of a regimen comprising ABC, TDF or D4T.[25] As a consequence, it should be notified that more attention should be reinforced to the possibility of transmitted drug resistance, when pre-exposure prophylaxis (PrEP) was administered in this population. In our study, V179E/D was the most common NNRTI-associated mutation, accounting for 37.9%.
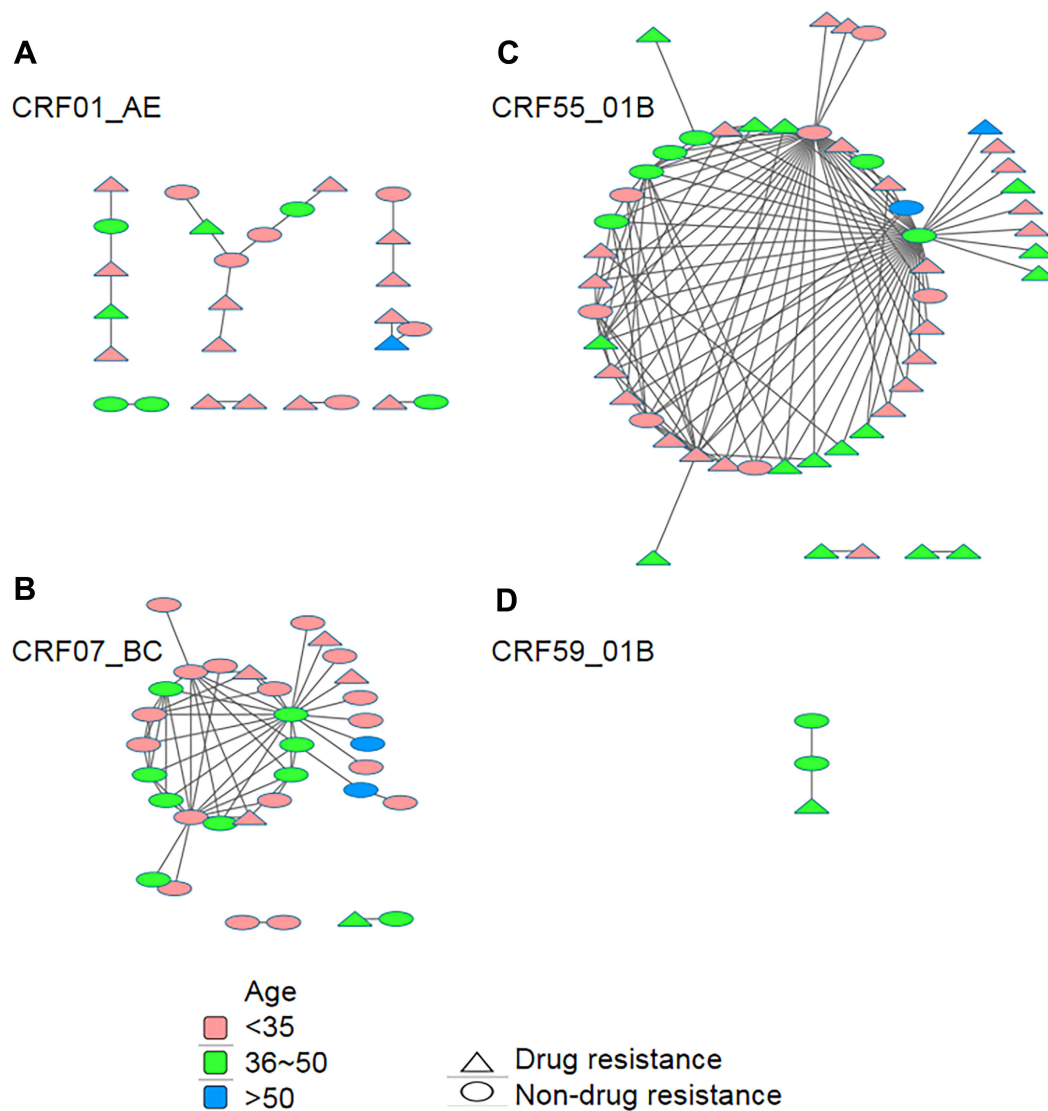
Figure 2 Age- and drug-resistance-associated genetic transmission networks of different genotype sequences. The networks were constructed using Cytoscape with a pairwise genetic distance analysed by using HyPhy software. A total of 27 CRF01_AE sequences (**A**), 33 CRF07_BC sequences (**B**), 52 CRF55_01B sequences (**C**), and 3 CRF59_01B sequences (**D**) were used for genetic transmission network analysis, and the largest cluster had 48 sequences. Different colours and shapes represent sequences from different age groups or those showing drug resistance or not, respectively.

V179E is a nonpolymorphic mutation weakly selected by NVP and EFV.[26] V179D reduces NVP and EFV susceptibility by 2- to 5-fold and reduces ETR and RPV susceptibility by 2- to 3-fold, respectively.[27] V179E/D does not appear to reduce the virological response to a first-line EFV-containing regimen.[15] V106I/M, K103N/Q, G190A/S, and Y181C were the major NNRTI-associated mutations, similar to those in other cities in China and other countries,[23,28–31] and showed broad-spectrum resistance possibly caused by the wide use of NNRTIs.

The parameters examined in this study had no significant effect on drug resistance, leading to treatment failure except treatment duration, baseline CD4+ cell count, and genotype

(P<0.001, <0.001, and 0.040, respectively). Virologic failure without resistance is most often the result of inadequate drug concentrations caused by poor adherence or drug–drug interactions.[32] In the present study, patients with a treatment duration below one year (83.7%) or CD4+ cell count below 200 cells/mm^3 (78.2%) had a higher drug resistance rate than other groups in terms of the corresponding factors. More attention should be paid to differentiate between adherence- and resistance-driven failures, which are not mutually exclusive. In line with the evidence that different frequencies of DRMs among HIV-1 subtypes circulate among drug-naive and treated individuals in China,[33] there were different drug resistance rates among different genotypes in this study (Table 2).

Table 4 Distributions of HIV-1-Infected MSM with ART Failure Within the Three Major Transmission Networks

Variables	CRF01_AE Sequences				CRF07_BC Sequences				CRF55_01B Sequences		
	Within Transmission Network, n=27(n/N, %)	Total Sequences N=186	P For Fisher Exact Tests		Within Transmission Network, n=33(n/N, %)	Total Sequences N=84	P for Fisher Exact Tests		Within Transmission Network, n= 52(n/N, %)	Total Sequences N=84	P for Fisher Exact Tests
Age(years)											
15–35	19(15.2)	125	0.929		21(41.2)	51	0.750		29(64.4)	45	0.935
36–50	7(13.2)	53			10(40.0)	25			21(60.0)	35	
≥51	1(12.5)	8			2(25.0)	8			2(50.0)	4	
Occupation											
Student	1(25.0)	4	0.482		4(66.7)	6	0.319		3(75.0)	4	0.845
Farmer	2(20.0)	10			2(50.0)	4			2(66.7)	3	
Employed	14(17.5)	80			16(45.7)	35			29(63.0)	46	
Unemployed	4(8.7)	46			6(28.6)	21			12(60.0)	20	
Unknown	6(13.0)	46			5(27.8)	18			6(54.6)	11	
Marital status											
Unmarried	20(14.7)	136	0.802		27(45.0)	60	0.112		31(64.6)	48	0.638
Married or cohabiting	5(12.5)	40			5(22.7)	22			15(53.6)	28	
Divorced or separated	2(20.0)	10			1(50.0)	2			6(75.0)	8	
WHO classification											
I	5(17.2)	29	0.604		4(33.3)	12	0.342		10(90.9)	11	0.157
II	3(17.7)	17			5(27.8)	18			8(53.3)	15	
III	18(15.0)	120			21(42.0)	50			28(56.0)	50	
IV	1(5.0)	20			3(75.0)	4			6(75.0)	8	

	n(%)	N	P	n(%)	N	P	n(%)	N	P
Geographical region									
Pearl River Delta	24(15.1)	159	0.274	30(40.0)	75	0.392	42(59.2)	71	0.476
Eastern	0(0.0)	6		0(0.0)	1		3(75.0)	4	
Western	3(27.3)	11		0(0.0)	3		5(83.3)	6	
Northern	0(0.0)	10		3(60.0)	5		2(66.7)	3	
Treatment duration(year)									
<1	12(16.4)	73	0.311	4(21.1)	19	0.176	22(57.9)	38	0.693
1–3	9(11.0)	82		21(41.2)	51		23(62.2)	37	
>3	6(19.4)	31		8(57.1)	14		7(77.8)	9	
Baseline CD4$^+$T cell count (cells/mm^3)									
≤200	17(12.8)	133	0.356	9(26.5)	34	0.068	31(59.6)	52	0.500
>200	10(18.9)	53		24(48.0)	50		21(65.6)	32	
Viral load (log10)									
3~	3(5.9)	51	0.015	15(33.3)	45	0.287	15(60.0)	25	0.662
4~	21(21.9)	96		16(50.0)	32		24(66.7)	37	
≥5	3(7.7)	39		2(28.6)	7		13(56.6)	22	
Drug resistance									
Yes	16(12.2)	131	0.178	5(16.7)	30	0.002	37(54.4)	68	0.021
No	11(20.0)	55		28(51.9)	54		15(93.8)	16	

The genetic distance cut-off value for constructing the transmission network differs among sequences belonging to different subtypes.[34] In the present study, genetic distances ≤0.013 and ≤0.015 were set as cut-off values for CRF07_BC and CRF01_AE, CRF55_01B, or CRF59_01B, respectively. The results showed that most (70.4% for CRF01_AE, 63.6% for CRF07_BC, and 55.8% for CRF55_01B) of the MSM in transmission networks were concentrated at ages below 35 years old. Moreover, approximately 54.8% (63/115) of MSM had more than 2 potential transmission partners. Individuals with multiple links could have potentially higher transmission risk.[22] Therefore, more attention should be given to younger HIV-1-infected MSM with multiple links.

Conclusions

Our study focused on the genotype diversity, drug resistance and transmission networks among MSM with ART failure in Guangdong Province from 2014 to 2019 and identified CRF01_AE, CRF07_BC and CRF55_01B as the main strains circulating among MSM with ART failure. Drug resistance mutations more frequently occurred in NNRTIs. Transmission network analysis revealed a complex transmission pattern, and more attention should be paid to younger HIV-1-infected MSM with multiple links.

Ethics Approval

This study was approved by the institutional review board of the Guangzhou Eighth People's Hospital. All participants in this study provided written informed consent.

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Disclosure

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