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ORIGINAL RESEARCH

Absence of Integrase Inhibitor-Associated Resistance Among Antiretroviral Therapy-Naïve HIV-1-Infected Adults in Guangdong Province, China, in 2018

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Background: Antiretroviral therapy (ART) containing an integrase strand transfer inhibitor (INSTI) plus two nucleoside reverse-transcriptase inhibitors has been recommended as a first-line regimen for ART-naïve HIV-1-infected patients in the latest Chinese Guidelines for Diagnosis and Treatment of HIV/AIDS.

Objective: To determine the prevalence of INSTI-related mutations among ART-naïve HIV-1-infected adults in Guangdong, China, in 2018.

Methods: The entire *integrase* gene was amplified from blood plasma. Demographic and epidemiological information was collected. INSTI mutations and antiretroviral susceptibility were interpreted using the Stanford University HIV Drug Resistance Database HIVdb program.

Results: Of 927 samples, 827 integrase sequences were successfully obtained. Among them, no major resistance mutations to INSTIs were identified, and four accessory mutations, including T97A (0.12%, 1/827), A128T (0.24%, 2/827), E157Q (0.85%, 7/827), and G163R (0.24%, 2/827), were found in twelve individuals. Two patient samples contained the G163R mutation conferring low-level resistance to elvitegravir and raltegravir.

Conclusion: The overall prevalence of INSTI mutations remains low. Drug resistance mutation testing for the detection of INSTI drug resistance mutations in HIV treatment-naïve patients should be considered due to the circulation of polymorphisms contributing to INSTI resistance and the expected increasing use of this class of drugs.

Keywords: HIV-1, integrase inhibitor, drug resistance mutation, Guangdong

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Introduction

Human immunodeficiency virus-1 (HIV-1) remains a global public health problem of unprecedented proportions. Antiretroviral therapy (ART) is a good choice for the prevention and treatment of HIV infection and has reduced the mortality and morbidity associated with HIV infection.[1] However, the long-term success of ART is now threatened by HIV drug resistance.[1,2] The World Health Organization (WHO) recommends monitoring and periodic surveillance for the appearance of HIV resistance to all antiretroviral treatment programmes.[3]

HIV-1 integrase plays an important role in HIV-1 replication by catalysing two distinct reactions, 3′-end processing and strand transfer.[4] Integrase strand transfer inhibitors (INSTIs) have become an important part of ART because INSTIs have

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high efficiency, good tolerability, low toxicity, and reduced concern about CYP3A4-associated drug–drug interactions for most patients.[5]

INSTI-containing regimens are recommended as first-line treatment in the latest Chinese Guidelines for Diagnosis and Treatment of HIV/AIDS after the efficacy of INSTIs was demonstrated in randomized clinical trials.[6–9] In Guangdong, the following regimens which include INSTIs are recommended as first-line regimens for approximately 2 years: two nucleotide reverse transcriptase inhibitors backbone with raltegravir (RAL) or Dolutegravir (DTG). Although INSTIs have a higher genetic barrier to the development of HIV-1 resistance,[9–11] INSTI inevitably shows drug resistance similar to other anti-HIV drugs.[12] Some data show that rare major INSTI mutations were found among primary infected HIV-1 patients in Europe, Venezuela, Canada, sub-Saharan Africa, and the US.[13–17] In China, few data are available on the prevalence of HIV-1 INSTI resistance among ART-naïve patients: no major INSTI drug resistance mutations and rare accessory INSTI drug resistance mutations (0.52%, 2/385) were found among 385 ART-naïve individuals in Beijing; a total of 10 (1.95%) major INSTI drug resistance mutations and 8 (1.56%) accessory INSTI drug resistance mutations were found among 513 ART-naïve individuals in Yunnan.[18,19] Given that INSTIs are currently used in HIV-1-infected individuals in Guangdong, a developed province on the southern coast of China, it is essential to obtain the resistance status to this class of antiretrovirals, but there is no information about mutations related to INSTIs among HIV-1-infected individuals in Guangdong. Here, we characterized HIV-1 INSTI-associated resistance among ART-naïve HIV-1-infected adults in Guangdong Province, China.

Materials and Methods

Study Subjects and Sample Collection

Guangdong Province has established a system of sentinel hospitals in which patients wishing to receive HIV treatment are cared for by highly trained Infectious Disease specialists. The Guangzhou Eighth People's Hospital was the leader of all these sentinel hospitals. The enrolment of the subjects included 927 ART-naïve patients who were over 16 years old and who had initiated their therapy at the eleven sentinel tertiary care hospital of Guangdong from January to December 2018, randomly. Five millilitres of blood with EDTA were drawn prior to ART, and the samples were centrifuged immediately, aliquoted, and stored at −80°C. The demographic and epidemiological information of the patients, including risk group, sex, age, ethnicity, and marital status, was collected. The baseline plasma viral load plus $CD4^+$ T cell count were quantified and drug resistance was monitoring at Guangzhou Eighth People's Hospital. Patients were previously genotyped based on partial *pol* regions (HXB2 2253–3318) and were excluded if they had ever used antiretroviral drugs.

RNA Extraction and RT-PCR Amplification

HIV-1 RNA was isolated from 200 μL plasma samples using the magnetic-bead-based Viral RNA Extraction Kit (Daan, China) according to the manufacturer's instructions. The amplification of the entire *Integrase* gene (864 bp, HXB2 4230–5093) was performed using one-step reverse transcription PCR (RT-PCR) using the PrimeScript One Step RT-PCR Kit (Taraka, China), followed by nested PCR using PrimeSTAR HS DNA Polymerase (Taraka, China). The outer and inner primers were synthesized as previously described.[20]

The cycling conditions for cDNA synthesis and the first round of PCR amplification were as follows: 45 minutes at 50°C (cDNA synthesis) and initial denaturation for 3 minutes at 94°C, 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension at 72°C for 5 minutes. The cycling conditions for the second round of PCR amplification were as follows: initial denaturation for 3 minutes at 94°C, 40 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 70 seconds, followed by a final extension at 72°C for 5 minutes. Negative controls were utilized to detect possible contamination.

The amplified PCR products were electrophoresed on a 1% agarose gel with negative and positive controls and a DNA size marker (Takara, China). After gel electrophoresis, the positive products were purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany) according to the manufacturer's protocol and sequenced by a genomics company (Tianyi Huiyuan, China). The primers used for Sanger dideoxy sequencing were the inner primers used for the second round of PCR amplification.

Drug Resistance Testing and Interpretation

The obtained sequences were assembled and edited using Sequencher DNA sequence analysis softwareV5.4.6. All

sequences were manually edited and then submitted to the Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/) to identify INSTI mutations. Sequences with a low-level category of resistance or greater were defined as having Integrase drug resistance.

Results

A total of 927 HIV-1 patients were tested using the protocol described above, and 100 of them were excluded for cannot obtain the positive PCR products, so the *integrase* region in 827 patients was successfully amplified, purified and sequenced. The demographic characteristics of these patients (n=827) are shown in Table 1. The participants were aged between 17 and 79 years, with a median age of 35 years. Of these patients, 86.22% (713/827) were male, with a median age of 34 years (ranging from 17 to 79), and 13.78% (114/827) were female, with a median age of 43 years (ranging from 17 to 69). Most of them (91.17%, 754/827) were diagnosed in the last two years (2017–2018). The dominant transmission route was men who have sex with men (MSM; 49.46%, 409/827), followed by heterosexual contact (HET; 39.66%, 328/827). The median (range) HIV-1 viral load was 520,801 (1035–6765,100) IU/mL. The median (range) $CD4^+$ T cell count was 234 (1–1425) cells/mm^3, and 42.32% (350/827) of the participants had a $CD4^+$ T cell count of <200 cells/mm^3. Based on partial pol sequences, HIV-1 genotypes were identified: CRF01_AE (37.97%), CRF07_BC (32.41%), CRF55_01B (12.33%), B (3.39%), CRF59_01B (2.66%), CRF08_BC (2.18%), Other (0.97%), and URF (8.10%).

Of 827 successfully amplified and sequenced integrase sequences, no individual with major INSTI mutations was identified. Twelve individuals had INSTI accessory mutations. The overall prevalence of INSTI drug resistance mutations was 1.45% (12/827). The mutations were T97A (0.12%, 1/827), A128T (0.24%, 2/827), E157Q (0.85%, 7/827), and G163R (0.24%, 2/827). No sample with dual or triple INSTI drug resistance mutations was found in the study.

The characteristics of the twelve individuals harbouring INSTI mutations are shown in Table 2. Unequal distributions of mutations were found in the transmission routes, geographical region and genotype. The rates of INSTI mutations associated with the transmission routes were 5.71%, 1.83%, and 0.98% for IDU, HET, and MSM,

Table 1 Demographic and Viral Characteristics of 827 Study Population

Characteristics	Individuals (%)	Cases Related to INI Mutations (%)	
		Cases	%
Patient, no.	827	12	1.45
Gender, no. (%)			
Male	713(86.22)	10	1.40
Female	114(13.78)	2	1.75
Route of infection, no. (%)			
Heterosexual	328(39.66)	6	1.83
MSM	409(49.46)	4	0.98
IDU	35(4.23)	2	5.71
Blood	5(0.60)	0	0.00
SexIDU	9(1.09)	0	0.00
Others	43(5.20)	0	0.00
Geographical region, no. (%)			
Pear River Delta	689(83.31)	11	1.60
Eastern	39(4.72)	0	0.00
Western	48(5.80)	1	2.08
Northern	51(6.17)	0	0.00
HIV-1 subtype based on PR/RT sequences, no. (%)			
CRF01_AE	314(37.97)	1	0.32
CRF07_BC	268(32.41)	6	2.24
CRF55_01B	102(12.33)	0	0.00
B	28(3.39)	2	7.14
CRF59_01B	22(2.66)	1	4.55
CRF08_BC	18(2.18)	1	5.56
Other (C, G, CRF67_01B, CRF68_01B)	8(0.97)	0	0.00
URF	67(8.10)	1	1.49
HIV-1 viral loads (Log 10, IU/mL), no. (%)			
<4	197(23.82)	3	1.52
4~5	141(17.05)	2	1.42
>5	489(59.13)	5	1.02
$CD4^+$ T cell count (cells/mm^3), no. (%)			
<200	350(42.32)	3	0.86
200–350	296(35.79)	5	1.69
351–500	115(13.91)	2	1.74
>500	66(7.98)	0	0.00

Note: Others including unknown and not clear.
Abbreviations: INI, integrase inhibitor; MSM, men who have sex with men; IDU, injection drug user; URF, unique recombination form.

Table 2 Characteristics of Patients Harboring HIV-1 Integrase Inhibitor Mutations

Sample ID	Geographical Region	Gender	Age	Transmission Route	Genotype Based on PR/RT Sequences	INI Resistance Mutations	
						Major	Accessory
01032	Pear River Delta	Male	35	MSM	B	–	E157Q
02063	Pear River Delta	Male	65	HTS	CRF_07BC	–	G163R
02072	Pear River Delta	Male	44	HTS	CRF_07BC	–	E157Q
03012	Pear River Delta	Male	40	IDU	CRF_08BC	–	E157Q
03028	Pear River Delta	Male	45	HTS	CRF_5901B	–	E157Q
03059	Pear River Delta	Male	44	HTS	B	–	E157Q
08060	Pear River Delta	Male	28	MSM	CRF_01AE	–	E157Q
08062	Pear River Delta	Male	29	MSM	URF	–	T97A
00067	Western	Female	58	HTS	CRF_07BC	–	E157Q
00169	Pear River Delta	Male	32	MSM	CRF_07BC	–	G163R
00292	Pear River Delta	Female	66	HTS	CRF_07BC	–	A128T
00346	Pear River Delta	Male	22	IDU	CRF_07BC	–	A128T

Abbreviations: INI, integrase inhibitor; MSM, man who have sex with man; IDU, injection drug user; URF, unique recombination form.

respectively. The rates of INSTI mutations associated with the geographical region were 2.08% and 1.60% for the Western and Pear River Deltas, respectively. No INSTI mutations were detected in eastern and northern Guangdong. The rate of INSTI mutations among Subtype B was the highest (7.14%, 2/28), followed by CRF08_BC (5.56%, 1/18), CRF59_01B (4.55%, 1/22), CRF07_BC (2.24%, 6/268), URF (1.49%, 1/67), and CRF01_AE (0.32%, 1/314).

Discussion

Two patient samples contained the G163R mutation, which is polymorphic in subtype F viruses from ARV-naive patients but is otherwise non-polymorphic,[21,22] and confers low-level resistance to elvitegravir (EVG) and RAL. One patient sample contained the T97A mutation, which is a polymorphic INSTI-selected mutation that, depending on subtype, occurs in 1% to 5% of viruses from untreated persons,[23] has minimal effects on INSTI susceptibility but in combination with other major resistance mutations, conferring potential low-level resistance to EVG and RAL. Seven patient samples contained the E157Q mutation, which is a polymorphic mutation that confers potential low-level resistance, and it generally only occurs in combination with other high- or intermediate-level resistance mutations.[24] Two patient samples contained the A128T mutation, which is a relatively nonpolymorphic possibly INSTI-selected mutation that does not appear to reduce INSTI susceptibility.

Updated guidelines containing INSTI regimens have been recommended for approximately 2 years in Guangdong, China. Drug resistance testing is not routinely performed for ART-naïve HIV-1-infected individuals. HIV-1 is characterized by a high degree of natural variability due to its high replication rate and lack of a proofreading mechanism in RNA viral polymerase reverse transcription and genetic recombination.[12,25,26] Small genetic errors or mutations can lead to the development of drug resistance. In the present study, we found that INSTI drug resistance mutations remain uncommon in Guangdong, China. No mutations related to high-level or medial-level resistance to four INSTIs were found. G163R (0.24%, 2/827) confers low-level resistance to EVG and RAL. G163R was also detected in one patient (0.2%, 1/513) in Yunnan Province.[19] Other INSTI accessory mutations found in this study do not reduce susceptibility to INSTIs. The low rates of INSTI drug resistance mutations may be due to natural polymorphisms in the integrase region of HIV-1 virus, and surveillance is also needed for the further use of INSTIs.

In this study, different rates of INSTI mutations among different genotypes were found (Table 2). It has been reported that different genotypes may develop different mutational pathways leading to varying levels of drug resistance. Natural polymorphisms may influence the development of resistance against INSTIs in different HIV-1 subtypes.[27,28] Here, we found that the mutation rate of Subtype B was higher than that of other genotypes, but the total number

and types of drug-resistant sites were limited. Further study will consider grouping the patients according to genotype and following them by using INSTI-containing regimens.

A limitation of this study is that we used Sanger dideoxy sequencing to identify established clinically significant drug resistance mutations. Although Sanger sequencing is the gold standard method in HIV drug resistance testing, it fails to identify drug resistance minority variants that are below 20% of the virus population.[1,29,30] The method probably underestimates the real prevalence of resistance mutations among treatment-naïve patients.

Conclusion

In conclusion, our results demonstrate that drug resistance mutations associated with INSTIs, including T97A, A128T, E157Q, and G163R, were detected among ART-naïve HIV-1-infected adults in Guangdong, China, in 2018. The overall prevalence of INSTI mutations remains low. Drug resistance mutation testing for the detection of INSTI drug resistance mutations in HIV treatment-naïve patients should be considered due to the circulation of polymorphisms contributing to INSTI resistance and the expected increasing use of this class of drugs.

Ethical Approval

This study was approved by the Institutional Review Board of the Guangzhou Eighth People's Hospital (20171491), and written informed consent of the patient was obtained before collecting serum samples. A parent or legal guardian provided informed consent for any patients under the age of 18 years, and that this study was conducted in accordance with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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