HIV-1 gp41 genetic diversity and enfuvirtide resistance-associated mutations among enfuvirtide-naïve patients in southern China

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Research article

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Abstract

Background: Human immunodeficiency virus (HIV) increasing molecular diversity and emergence of drug resistant mutants remain a major concern in Southern China, enfuvirtide (ENF/T-20) is the first entry inhibitor used in patients failing highly active antiretroviral therapy (HAART). However, data on HIV-1 gp41 genetic diversity and primary ENF resistance-associated mutations among treatment-naïve patients in southern China is limited. The objective was to identify molecular diversity and ENF resistance patterns of HIV-1 in southern China, using envelop (env) gp41 sequences and bioinformatics tools, which may help optimize ART.

Methods: From December 2018 to January 2019, 439 blood plasma samples from ENF-naïve HIV-1 patients were collected from Shenzhen, Wuhan and Chongqing, of which, 396 HIV env regions were sequenced and subtyped, and were performed the analysis of drug resistance-associated mutations (DRMs).

Results: The main subtypes were circulating recombinant form (CRF) 01_AE (30.6%) and CRF07_BC (48.7%), CRF55_01B had been the fourth subtype in our work, many rare CRFs were observed. Notably, CRF02_AG and CRF_BF strains typically found in Africa and US respectively were identified amongst Chinese HIV-1 patients. Known DRMs were detected in 27.5% (109/396) of ENF treatment-naïve patients. One major DRM (L44M), many secondary DRMs including (N126K, E137K, S138A) and lots of polymorphisms were found in the study, which have been proved to elevate resistance to ENF.

Conclusion: HIV-1 molecular diversity among people in southern China observed in the work indicates that HIV-1 variability is becoming increasingly complex in the south of China. A diverse set of primary DRMs discovered in this study described the serious threat to ART, which remind us the urgent need of timely surveillance of HIV-1 viral diversity and drug resistance in China.

Introduction

Human immunodeficiency virus type one (HIV-1) presents high genetic diversity, and is suppressed by antiretroviral therapy (ART), but may acquire many drug resistance-associated mutations (DRMs). HIV-1 genetic studies have revealed four groups (M, N, O and P). The M group of HIV-1 is classified into nine phylogenetic subtypes (A, B, C, D, F, G, H, J and K) and at least 98 circulating recombinant forms (CRFs) [1, 2]. Viral diversity analysis has significant implication for investigating the global origin and evolution of HIV-1 isolates [3], and future vaccine development [4]. Molecular epidemiological studies demonstrated that CRF07_BC, CRF01_AE and CRF08_BC were the dominant subtypes among the population of southern China, with identification of drug-resistant strains and novel unique recombinant forms (URFs) [5–7].

In 2003, Chinese government made it possible to have free access to ART [8], which not included enfuvirtide (ENF). ENF was the first HIV fusion inhibitor based on the amino acid sequence of the heptad repeat-2 (HR2) in the glycoprotein 41 (gp41) of HIV-1 envelope (env) protein, which blocks HIV-1 entry and
restrict HIV-1 replication, used in combination with other antiretroviral drugs as an alternative to the ordinary ART [9, 10]. HR1 region substitutions in HIV-1 env gp41, including A30V, L33V, L34M, G36D/S, I37V, V38A, Q39H/R, Q40H, N42T/D, N43D, L44M, L45M, R46M, L54M and et al, have been proved to be associated with ENF resistance in vitro and vivo [11–16]. The major ENF resistance-associated mutations were identified in amino acid position 36–45 of HR1 domain. Other secondary mutations including N126K, E137K, S138A, N140I also were demonstrated to reduce ENF susceptibility [17–20]. ENF resistance-associated mutations have been reported in patients who have accepted highly active antiretroviral therapy (HAART), but less data about the ENF resistance associated mutations among ENF-naïve patients. Hence, before ENF is approved for routine ART by authority in China, the information about ENF primary drug resistance is required.

In this study, we investigated HIV-1 gp41 genetic diversity and the prevalence of antiretroviral drug resistance associated with ENF among treatment-naïve population from southern China.

**Methods**

**Study samples**

From December 2018 to January 2019, 439 blood plasma samples from ENF treatment-naïve HIV-1 population were collected from the Third People’s Hospital of Shenzhen (228 samples), Wuhan Jinyintan Hospital (44 samples) and Chongqing Centers for Disease Control and Prevention (CDC) (167 samples), and quantitated by HIV-1 nucleic acid detection kit (Zhuhai Livzon Diagnostics Inc., Zhuhai, China). Among these samples, 396 HIV env regions were successfully sequenced.

**RNA extraction, amplification and sequencing**

HIV-1 RNA was extracted from 650 µL plasma using extraction reagent of HIV-1 nucleic acid detection kit (Zhuhai Livzon Diagnostics Inc., Zhuhai, China) according to the manufacturer’s protocol. HIV-1 env gp41 (HXB2: 7648-8365) was amplified with 5×Neoscript RT Premix (RT-PCR) (Zhuhai Biori Biotechnology Co., Ltd, Zhuhai, China). We used the primers env F-1 5’- TRARGGACAATTGGAGAAGTG-3’; env R-1 5’-GTGAGTATCCCTGCCTAAC-3’. The amplification of the env fragment was performed at 50°C for 30 min for reverse transcription and then 95°C for 15 min, followed by 50 cycles at 94°C for 15 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products were purified and sequenced by Invitrogen (Shanghai) trading Co., Ltd using Sanger methods.

**HIV-1 genotype and phylogenetic analysis**

We edited and aligned the sequences using Geneious 9.1.2, compared with gp41 reference sequences. Env sequences were submitted to the Los Alamos HIV BLAST tool for initial HIV-1 subtyping (https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html). All sequences were aligned and compared with HIV-1 reference sequences (Accession numbers were shown in supplementary materials S-Table 1) derived from the Los Alamos HIV sequence database.
and then the nucleotide alignments were subjected to phylogenetic inference for further HIV-1 subtyping through the neighbor-joining method and Kimura 2-parameter model in 1000 bootstrap replicates implemented in the MEGA 7.0.2. For finalization of phylogenetic trees, we used FigTree v1.4.3 (University of Edinburgh, UK) to prepare figures as previously described [21]. Intra-genomic breakpoints and boot-scanning analyses were performed on sequences with possibly unidentified strains through jpHMM program [22]. The sequences did not match established CRFs were identified as URFs. Recombinant HIV-1 Drawing Tool was used to generate the genome maps of URFs.

**Drug resistance mutation analysis**

DRM analyses in *env* regions were performed in the HIVfird program. HIVfird uses HIV-1 HXB2 *env* gp41 protein sequence (nucleotide: 7863-7892, amino acids: 36-45) as a reference sequence to align the user query sequences [23]. The mutation in this region can confer HIV-1 resistance to ENF [24], and polymorphisms are other amino acid differences amongst the gp41 region in the absence of ENF [25].

**Statistical analysis**

Polymorphisms were analyzed using VESPA program. The VESPA program detects signature patterns (atypical amino acid or nucleotide residues) in a set of query sequences relative to a set of background sequences [26]. Chin-square in SPSS 21.0 software was utilized for statistical analyses.

**Results**

**HIV-1 gp41 subtyping analysis**

Among 439 samples, a total of 396 samples were successfully sequenced and included in this study, of which 198, 156, and 42 were collected from Shenzhen, Chongqing, and Wuhan respectively. Gp41 of HIV-1 variant were sequenced and subtyped through jpHMM program and HIV-1 BLAST tool, further confirmed by phylogenetic analysis (Fig. 1). As is shown in Table 1, CRF07_BC (48.7%, 193/396) and CRF01_AE (30.6%, 121/396) were the two main subtypes. A diverse set of genotypes were also identified amongst gp41 regions, including CRF08_BC, CRF55_01B, B, CRF67_01B, CRF_BF, CRF02_AG, CRF52_01B, CRF59_01B, CRF79_0107 and CRF85_BC. Few URFs (0.5%, 2/396) were found in the study (Fig. 2). The distribution of HIV-1 subtypes among the three cities in the study demonstrated that the HIV-1 isolates in Shenzhen were more variable than Chongqing and Wuhan (P < 0.01), and the proportions of CRF07_BC and CRF08_BC in Chongqing were higher than the other two cities (P < 0.01). CRF55_01B outnumbered subtype B to become the fourth HIV-1 subtype.
### Table 1
Gp41 genetic characteristics of HIV-1 infected populations

| Subtypes      | Shenzhen (198) | Chongqing (156) | Wuhan (42) | Total N = 396 |
|---------------|----------------|-----------------|------------|---------------|
| CRF01_AE      | 82 (41.4%)     | 19 (12.2%)      | 20 (47.6%) | 121 (30.6%)   |
| CRF07_BC      | 78 (39.4%)     | 101 (64.7%)     | 14 (33.3%) | 193 (48.7%)   |
| CRF08_BC      | 8 (4.0%)       | 27 (17.3%)      | 2 (4.8%)   | 37 (9.3%)     |
| CRF55_01B     | 16 (8.1%)      | 3 (1.9%)        | 3 (7.1%)   | 22 (5.6%)     |
| B             | 4 (2.0%)       | 4 (2.6%)        | 3 (7.1%)   | 11 (2.8%)     |
| CRF67_01B     | 4 (2.0%)       | -               | -          | 4 (1.0%)      |
| CRF_BF        | 1 (0.5%)       | -               | -          | 1 (0.3%)      |
| CRF02_AG      | 1 (0.5%)       | -               | -          | 1 (0.3%)      |
| CRF52_01B     | -              | 1 (0.6%)        | -          | 1 (0.3%)      |
| CRF59_01B     | 1 (0.5%)       | -               | -          | 1 (0.3%)      |
| CRF79_0107    | 1 (0.5%)       | -               | -          | 1 (0.3%)      |
| CRF85_BC      | -              | 1 (0.6%)        | -          | 1 (0.3%)      |
| URF           | 2 (1.0%)       | -               | -          | 2 (0.5%)      |
Table 2
The characterization of HIV-1 strains with ENF resistance-associated mutations

| Code | Source | Subtype | Mutation | Code | Source | Subtype | Mutation |
|------|--------|---------|----------|------|--------|---------|----------|
| 57   | CQ     | 07BC    | S35F     | 10935| CQ     | 08BC    | R46K, S138A|
| 56   | CQ     | 07BC    | S138A    | 10934| CQ     | 07BC    | R46K     |
| 120  | CQ     | 55_01B  | H53R     | 10897| CQ     | 07BC    | R46Q, E49D|
| 11665| CQ     | 08BC    | R46Q     | 10819| CQ     | 08BC    | S35A     |
| 11660| CQ     | 07BC    | R46K     | 10808| CQ     | 07BC    | L33V     |
| 11644| CQ     | 01AE    | E137K    | 10765| CQ     | 07BC    | R46M, E49Q, S138A |
| 11636-2| CQ | 07BC | Q32L, R46Q | 10611| CQ | 08BC | R46Q |
| 11625| CQ     | 08BC    | L33V     | 10579| CQ     | B       | Q32L, L44M|
| 11607| CQ     | 07BC    | A30V     | 10490| CQ     | 07BC    | R46K     |
| 11606| CQ     | 07BC    | R46K     | 10452| CQ     | 07BC    | S138A    |
| 11592| CQ     | 01AE    | E137K    | 10444| CQ     | 07BC    | R46K     |
| 11590| CQ     | 07BC    | R46K     | 10410| CQ     | 07BC    | R46K     |
| 11587| CQ     | 08BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11550| CQ     | 08BC    | R46K     | 10406| CQ     | 07BC    | R46K     |
| 11548| CQ     | 07BC    | R46M     | 10407| CQ     | 07BC    | R46K     |
| 114  | CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11472| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11459| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 114  | CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11472| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11459| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 114  | CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11472| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11459| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 114  | CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11472| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |
| 11459| CQ     | 07BC    | R46K     | 10407| CQ     | 07BC    | R46K     |

SZ: Third People's Hospital of Shenzhen, WH: Wuhan Jinyintan Hospital, CQ: Chongqing CDC.
| Code   | Source | Subtype | Mutation               | Code   | Source | Subtype | Mutation          |
|--------|--------|---------|------------------------|--------|--------|---------|-------------------|
| 11117  | CQ     | 08BC    | L33V, R46K, H53R       | 104−40 | SZ     | 01AE    | Q56K             |
| 11116  | CQ     | 07BC    | R46K                   | S3-4   | SZ     | 08BC    | R46K, S138A       |
| 11100  | CQ     | 07BC    | Q52H                   | S3-29  | SZ     | B       | Q32L, E137K       |
| 11092  | CQ     | 07BC    | R46K                   | S3-23  | SZ     | 01AE    | E137K            |
| 11091  | CQ     | 07BC    | E137K, S138A           | S3-20  | SZ     | 07BC    | E49Q             |
| 11089  | CQ     | 07BC    | R46Q                   | S3-2   | SZ     | BF1     | E49D             |
| 11086  | CQ     | 07BC    | R46K                   | S32    | SZ     | 01AE    | L34M, S35T        |
| 11080  | CQ     | 07BC    | R46K, H53R             | S2-9   | SZ     | 02AG    | R46K, Q56R, E137K |
| 11069  | CQ     | 07BC    | R46K                   | S2-43  | SZ     | 01AE    | E137K            |
| 11067  | CQ     | B       | H53Q                   | S2-39  | SZ     | 07BC    | S138A            |
| 11034  | CQ     | B       | R46M, E137K            | S2-29  | SZ     | 07BC    | R46K, S138A       |
| 11012  | CQ     | 08BC    | R46K                   | S2-28  | SZ     | 08BC    | S35F             |
| 10939  | CQ     | 07BC    | R46K                   | S2-19  | SZ     | 01AE    | E137K            |
| 10938  | CQ     | 07BC    | R46K                   | S2-13  | SZ     | 07BC    | R46K             |
| 10936  | CQ     | 07BC    | S35F                   | S20    | SZ     | 01AE    | S35A, H53R        |
| 3–4    | SZ     | 59_01B  | E137K                  | S16    | SZ     | 07BC    | S35F             |
| 2–8    | SZ     | B       | E137K                  | S15    | SZ     | 01AE    | Q52H             |
| 2–7    | SZ     | 67_01B  | Q52H                   | 452    | WH     | 07BC    | H53N             |
| 24     | SZ     | 07BC    | R46K                   | 3497   | WH     | B       | E137K            |
| 2–12   | SZ     | 07BC    | R46K, H53R             | 3493   | WH     | 01AE    | Q52H, H53R        |
| 2      | SZ     | 01AE    | Q52H                   | 3437   | WH     | 01AE    | Q39H             |
| 18     | SZ     | 07BC    | S138A                  | 3387   | WH     | 01AE    | E137K            |
| 104−42 | SZ     | 07BC    | R46K                   | 3382   | WH     | 07BC    | R46K             |
| 104−41 | SZ     | 01AE    | R46K                   | 3381   | WH     | B       | R46Q             |

SZ: Third People's Hospital of Shenzhen, WH: Wuhan Jinyintan Hospital, CQ: Chongqing CDC.
| Code   | Source | Subtype | Mutation     | Code   | Source | Subtype | Mutation     |
|--------|--------|---------|--------------|--------|--------|---------|--------------|
| 104−31 | SZ     | 07BC    | Q56K         | 3370   | WH     | 01AE    | E49K         |
| 104−24 | SZ     | 01AE    | Q56R, E137K  | 3353   | WH     | 07BC    | R46K         |
| 104−22 | SZ     | 07BC    | R46K         | 3350   | WH     | 01AE    | E137K        |
| 104−13 | SZ     | 01AE    | L34M, I62V   | 3247   | WH     | 01AE    | S35A, S138A  |
| 1      | SZ     | 07BC    | S35T         | 165    | WH     | 07BC    | R46K         |
| 39     | SZ     | 55_01B  | H53R         | 115    | WH     | 01AE    | Q56R, E137K  |
| 38     | SZ     | 01AE    | E40Q         |        |        |         |              |

SZ: Third People's Hospital of Shenzhen, WH: Wuhan Jinyintan Hospital, CQ: Chongqing CDC.

ENF resistance-associated mutations and polymorphisms in env gp41 regions

In total, 109 (27.5%, 109/396) HIV-1 isolates had ENF resistance-associated mutations and polymorphisms, of which 22 (20.2%, 22/109) had two different substitutions and 3 HIV-1 strains harbored 3 combined mutations. The prevalence of DRMs and polymorphisms among ENF-untreated individuals from Chongqing, Shenzhen, and Wuhan was 34.0% (53/156), 21.7% (43/198) and 31.0% (13/42), respectively. Only one major ENF resistance-associated mutation (L44M) in amino acid position 36–45 of HR1 domain was seen in the enrolled samples. 8.6% (34/396) HIV-1 strains carried secondary DRMs including N126K (1/396, 0.25%), E137K (18/396, 4.55%), S138A (14/396, 3.54%).

Among the 396 ENF treatment-naive patients, various of polymorphisms were observed in env gp41, R46K/M/Q, E137K and S138A were the major polymorphisms (Fig. 3), R46K/M/Q (19.7%, 38/193) and S138A (5.7%, 11/193) were predominant in subtype CRF07_BC, and E137K (36.4%, 4/11) was a majority in subtype B (P < 0.05), after excluding the subtypes with the number less than ten.

Discussion

To date, this is the first study on the prevalence of ENF resistance-associated polymorphisms and mutations in HIV-1 isolates among treatment-naive patients in southern China. What's more, we investigated the env gp41 diversity of HIV-1 in these patients.

Molecular characterization of the HIV-1 isolates circulating within the HIV-1 patients in southern China revealed the most prevalent subtypes were CRF01_AE and CRF07_BC, while CRF08_BC and subtype B showed the lower prevalence in comparison to the national report in China in 2006 [27]. The prevalence of subtype B mainly transmitted by blood transfusion has significantly decreased, due to the prohibition of
paid blood donation, which is demonstrated by the low percentage of subtype B in the present study. The absorption of HIV-1 patients from Guizhou, Yunnan and Sichuan provinces in this study may account for the observed low proportion of CRF08_BC, since CRF08_BC predominates in these areas [27]. The geographic subtype distribution (Numbers of samples ≥ 10) suggested that CRF07_BC and CRF01_AE were dominant subtypes in three areas, which was consistent with previous reports among high-risk populations [28–30]. Notably, high prevalence of CRF55_01B was observed in our study, compared with the previous reports [27, 29, 31]. CRF55_01B firstly identified in Shenyang composed of subtype B and CRF01_AE, with 4 intra-genomic breakpoints in the polymerase (pol) gene [32]. Evidence showed that CRF55_01B originated and outbroke from men who have sex with men (MSM) in Shenzhen [33], and CRF55_01B have spread throughout China, along with population migration [34]. Furthermore, several rare CRFs including CRF52_01B, CRF59_1B, CRF67_01B, CRF79_0107 and CRF85 were isolated from HIV-1 patients, describing that HIV-1 recombination between different genotypes is becoming increasingly complex in China.

Significantly, CRF_BF and CRF02_AG strains typically found in South American countries and Africa respectively, were identified among Chinese HIV-1 patients [35–38]. To our knowledge, CRF_BF was firstly reported in China. More importantly, the two uncommon strains were both found in Shenzhen, which was an international metropolis with progressively international travel and immigration. Therefore, the identification of imported CRF_BF and CRF02_AG in Chinese indicates the newly emerging migration modes in the global HIV-1 pandemic. Furthermore, Few URFs were available in our study, HIV recombination can be presented in different regions (gag, pol and env) between different subtypes, therefore, more gene regions should be included in the further study for comprehensive and accurate HIV-1 classification.

In this study, we evaluated the distributions of env gp41 polymorphisms and ENF resistance-associated mutations among HIV-1 strains from 396 ENF-naïve HIV-1 infected population in Shenzhen, Chongqing and Wuhan in the period 2018–2019. In total, 27.5% HIV-1 isolates had primary ENF resistance-associated mutations and polymorphisms, higher than a previous study in Hong Kong (20.8%), China [39]. Only one major ENF resistance-associated mutation (amino acid positions: 36–45), L44M, was discovered in the study, which has associated with 1.8 fold resistance to enfuvirtide in vitro [40]. In China, ENF has been applied as salvage therapy for patients failing ordinary ART since 2015, but not been included in the Free AIDS Antiretroviral Therapy Manual [8], the low usage of ENF in China may contribute to the observed low prevalence of major ENF resistance mutations in our study (0.25%, 1/396), compared to the reports in Brazil (3.8%, 3/80) [19], Spain (3.0%, 6/200) [41] and US (16.7%, 2/12) [42] among ENF-untreated patients. However, several mutations (N126K, E137K, S138A) in the HR2 region presented in the study can lead to increased viral fusion activity and reduced susceptibility to ENF [43]. Notably, these mutations in HR2 domain may dramatically reduce ENF susceptibility about 100-1000-fold, in combination with substitutions in HR1 domain (amino acid positions: 36–45) [43, 44]. In addition, a lot of minor mutations and polymorphisms (A30V, Q32L, L33V, L34M, S35A/F/T, Q39H, R46K/M/Q, E49D/K/Q, Q52H, H53N/Q/R, Q56K/R, I62V) associated with potential resistance ENF were discovered in our study have reported in previous studies [16, 25, 45, 46].
The subtype distribution of three dominant mutations indicated R46K/M/Q and S138A were predominant in subtype CRF07_BC, while E137K was mainly found in subtype B. The prevalence of resistance-associated mutations and polymorphisms may have relationship with HIV-1 subtypes. However, DRM determinations were analyzed by HIVrd Program, which is based on subtype B and has biased the results for non-B strains [23], further phenotypic resistance analysis should be performed to confirm the susceptibility to ENF among non-B subtypes.

**Conclusion**

In summary, our study demonstrated increasing HIV-1 genetic diversity and high prevalence of primary ENF resistance-associated mutations and polymorphisms in the south Chinese population. The identification of imported CRF02_AG and CRF_BF strains in Chinese individuals, especially the first emergence of US-derived CRF_BF isolate, suggest the newly emerging migration modes of the global HIV-1 pandemic. Additionally, though few major DRM was observed, a large amount of secondary and minor DRMs were isolated from ENF treatment-naïve patients, which can be a serious threat to ART after the failure of the first-line ART. The complex HIV-1 evolution and high prevalence of DRMs to ENF remind us the urgent need for continued molecular surveillance to monitor the HIV-1 diversity and primary DRMs in Chinese population.

**Abbreviations**

HIV: human immunodeficiency virus; ENF: enfuvirtide; HAART: highly active antiretroviral therapy; env: envelope; DRMs: drug resistance-associated mutations; CRFs: circulating recombinant forms; URFs: unique recombinant forms; HR: heptad repeat; pol: polymerase

**Declarations**

**Data Availability**

The data used to support this study are available from the corresponding author upon request.

**Ethics approval and consent to participate**

The study was approved by the institutional review boards in Third People’s Hospital of Shenzhen, Wuhan Jinyintan Hospital and Chongqing CDC respectively. The methods in the study were in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects participating in this research.

**Consent for publication**

Not applicable.

**Conflicts of Interest**
The authors declare that they have no conflicts of interest.

Authors’ contribution

LC and JZ contributed equally to this study. LW, JZ and LC and designed the study. JZ, LC and FG conducted the laboratory tests. JZ, HJ, LZ and XJ collected and analyzed data and prepared the manuscript. LW and LC edited and reviewed the manuscript. All Authors critically reviewed and revised the manuscript drafts, approved the final version of the manuscript and take responsibility for the integrity of the data and accuracy of data analysis.

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References

1. Hemelaar J: The origin and diversity of the HIV-1 pandemic. Trends in molecular medicine 2012, 18(3):182-192.

2. HIV Circulating Recombinant Forms (CRFs) [https://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html]

3. Ou CY, Ciesielski CA, Myers G, Bandea CI, Luo CC, Korber BT, Mullins JI, Schochetman G, Berkelman RL, Economou AN et al: Molecular epidemiology of HIV transmission in a dental practice. Science 1992, 256(5060):1165-1171.

4. Cohen KW, Frahm N: Current views on the potential for development of a HIV vaccine. Expert opinion on biological therapy 2017, 17(3):295-303.

5. Chen S, Cai W, He J, Vidal N, Lai C, Guo W, He H, Chen X, Fu L, Peeters M et al: Molecular epidemiology of human immunodeficiency virus type 1 in Guangdong province of southern China. PloS one 2012, 7(11):e48747.

6. Zhou PP, Yu G, Kuang YQ, Huang XH, Li Y, Fu X, Lin P, Yan J, He X: Rapid and complicated HIV genotype expansion among high-risk groups in Guangdong Province, China. BMC infectious diseases 2019, 19(1):185.

7. Zeng P, Liu Y, He M, Wang J, Keating S, Mao W, Huang M, Ma H, He W, Bi X: The infection staging and profile of genotypic distribution and drug resistance mutation among the human immunodeficiency
virus-1 infected blood donors from five Chinese blood centers, 2012-2014. PloS one 2017, 12(6):e0179328.

8. National Center for AIDS/STD Control and Prevention CC: Free AIDS Antiretroviral Therapy Manual in China. Beijing, China: People's Medical Publishing House; 2016.

9. Cardoso LP, Stefani MM: High level of multidrug resistance mutations in HIV type 1 pol gene and resistance-associated mutations to enfuvirtide (T-20) among antiretroviral-experienced patients from central Brazil. AIDS research and human retroviruses 2009, 25(10):943-950.

10. Derdeyn CA, Decker JM, Sfakianos JN, Zhang Z, O’Brien WA, Ratner L, Shaw GM, Hunter E: Sensitivity of human immunodeficiency virus type 1 to fusion inhibitors targeted to the gp41 first heptad repeat involves distinct regions of gp41 and is consistently modulated by gp120 interactions with the coreceptor. Journal of virology 2001, 75(18):8605-8614.

11. Xu L, Hue S, Taylor S, Ratcliffe D, Workman JA, Jackson S, Cane PA, Pillay D: Minimal variation in T-20 binding domain of different HIV-1 subtypes from antiretroviral-naive and -experienced patients. AIDS (London, England) 2002, 16(12):1684-1686.

12. Su Y, Chong H, Xiong S, Qiao Y, Qiu Z, He Y: Genetic Pathway of HIV-1 Resistance to Novel Fusion Inhibitors Targeting the Gp41 Pocket. Journal of virology 2015, 89(24):12467-12479.

13. Ray N, Harrison JE, Blackburn LA, Martin JN, Deeks SG, Doms RW: Clinical resistance to enfuvirtide does not affect susceptibility of human immunodeficiency virus type 1 to other classes of entry inhibitors. Journal of virology 2007, 81(7):3240-3250.

14. Ramakrishnan R, Mehta R, Sundaravaradan V, Davis T, Ahmad N: Characterization of HIV-1 envelope gp41 genetic diversity and functional domains following perinatal transmission. Retrovirology 2006, 3:42.

15. Melby T, Sista P, DeMasi R, Kirkland T, Roberts N, Salgo M, Heilek-Snyder G, Cammack N, Matthews TJ, Greenberg ML: Characterization of envelope glycoprotein gp41 genotype and phenotypic susceptibility to enfuvirtide at baseline and on treatment in the phase III clinical trials TORO-1 and TORO-2. AIDS research and human retroviruses 2006, 22(5):375-385.

16. Leung PH, Chen JH, Wong KH, Chan KC, Lam HY, Cheng VC, Yuen KY, Yam WC: High prevalence of primary Enfuvirtide (ENF) resistance-associated mutations in HIV-1-infected patients in Hong Kong. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2010, 47(3):273-275.

17. Xu L, Pozniak A, Wildfire A, Stanfield-Oakley SA, Mosier SM, Ratcliffe D, Workman J, Joall A, Myers R, Smit E et al: Emergence and evolution of enfuvirtide resistance following long-term therapy involves heptad repeat 2 mutations within gp41. Antimicrobial agents and chemotherapy 2005, 49(3):1113-1119.

18. Cunyat F, Marfil S, Garcia E, Svischer V, Perez-Alvarez N, Curriu M, Perno CF, Clotet B, Blanco J, Cabrera C: The HR2 polymorphism N140I in the HIV-1 gp41 combined with the HR1 V38A mutation is associated with a less cytopathic phenotype. Retrovirology 2012, 9:15.
19. Teixeira C, de Sa-Filho D, Alkmim W, Janini LM, Diaz RS, Komninakis S: Short communication: high polymorphism rates in the HR1 and HR2 gp41 and presence of primary resistance-related mutations in HIV type 1 circulating in Brazil: possible impact on enfuvirtide efficacy. AIDS research and human retroviruses 2010, 26(3):307-311.

20. Bai X, Wilson KL, Seederoff JE, Ahrens D, Green J, Davison DK, Jin L, Stanfield-Oakley SA, Mosier SM, Melby TE et al: Impact of the enfuvirtide resistance mutation N43D and the associated baseline polymorphism E137K on peptide sensitivity and six-helix bundle structure. Biochemistry 2008, 47(25):6662-6670.

21. Rodgers MA, Vallari AS, Harris B, Yamaguchi J, Holzmayer V, Forberg K, Berg MG, Kenmence J, Ngansop C, Awazi B et al: Identification of rare HIV-1 Group N, HBV AE, and HTLV-3 strains in rural South Cameroon. Virology 2017, 504:141-151.

22. Anne-Kathrin S, Ming Z, Ingo B, Thomas L, Bette K, Burkhard M, Mario S: jPHMM: Improving the reliability of recombination prediction in HIV-1. Nucleic Acids Research 2009(suppl_2):suppl_2.

23. Barreto Vasconcelos AL, Monteiro-Cunha JP: HIVfird: A Tool for Detection of Resistance to Fusion Inhibitor Drugs in HIV-1 Sequences. AIDS research and human retroviruses 2019.

24. 2019 Update of the Drug Resistance Mutations in HIV-1 [https://www.iasusa.org/wp-content/uploads/2019/07/2019-drug-resistance-mutations-figures.pdf]

25. Carmona R, Pérez-Alvarez L, Mu?oz M, Casado G, Delgado E, Sierra M, Thomson M, Vega Y, Parga EVd, Contreras G: Natural resistance-associated mutations to Enfuvirtide (T20) and polymorphisms in the gp41 region of different HIV-1 genetic forms from T20 naive patients. 32(3):0-253.

26. Korber B, Myers G: Signature pattern analysis: a method for assessing viral sequence relatedness. AIDS research and human retroviruses 1992, 8(9):1549-1560.

27. He X, Xing H, Ruan Y, Hong K, Cheng C, Hu Y, Xin R, Wei J, Feng Y, Hsi JH et al: A comprehensive mapping of HIV-1 genotypes in various risk groups and regions across China based on a nationwide molecular epidemiologic survey. PloS one 2012, 7(10):e47289.

28. Zhao GL, Yu W, Zhang JJ, Chen L, Feng TJ, Wang F, Hong FC, Wang XH, Li Q: [Study on the molecular-epidemiological characteristics of HIV-1 in Shenzhen, 1992-2008]. Zhonghua Liu Xing Bing Xue Za Zhi 2012, 33(1):82-87.

29. Zeng P, Liu Y, He M, Wang J, Keating S, Mao W, Huang M, Ma H, He W, Bi X et al: The infection staging and profile of genotypic distribution and drug resistance mutation among the human immunodeficiency virus-1 infected blood donors from five Chinese blood centers, 2012-2014. PloS one 2017, 12(6):e0179328.

30. Chu XG, Zhan FX, Peng GP, Chen HP, Peng TH, Tang H, Li Y: [Subtype and sequence analysis of the gag genes for HIV-1 strains isolated in Hubei province]. Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology 2012, 26(6):460-463.

31. Su Y, Liu H, Wu J, Zhu L, Wang N: [Distribution of HIV-1 genotypes in China: a systematic review]. Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi 2014, 35(10):1164.
32. Han X, An M, Zhang W, Cai W, Chen X, Takebe Y, Shang H: Genome Sequences of a Novel HIV-1 Circulating Recombinant Form, CRF55_01B, Identified in China. Genome announcements 2013, 1(1).

33. Zhao J, Cai W, Zheng C, Yang Z, Xin R, Li G, Wang X, Chen L, Zhong P, Zhang C: Origin and outbreak of HIV-1 CRF55_01B among MSM in Shenzhen, China. Journal of acquired immune deficiency syndromes (1999) 2014, 66(3):e65-67.

34. Han X, Takebe Y, Zhang W, An M, Zhao B, Hu Q, Xu J, Wu H, Wu J, Lu L et al: A Large-scale Survey of CRF55_01B from Men-Who-Have-Sex-with-Men in China: implying the Evolutionary History and Public Health Impact. Scientific reports 2015, 5:18147.

35. Carr JK, Avila M, Gomez Carrillo M, Salomon H, Hierholzer J, Watanaveeradej V, Pando MA, Negrete M, Russell KL, Sanchez J et al: Diverse BF recombinants have spread widely since the introduction of HIV-1 into South America. AIDS (London, England) 2001, 15(15):F41-47.

36. Thomson MM, Delgado E, Herrero I, Villahermosa ML, Vazquez-de Parga E, Cuevas MT, Carmona R, Medrano L, Perez-Alvarez L, Cuevas L et al: Diversity of mosaic structures and common ancestry of human immunodeficiency virus type 1 BF intersubtype recombinant viruses from Argentina revealed by analysis of near full-length genome sequences. The Journal of general virology 2002, 83(Pt 1):107-119.

37. Howard TM, Rasheed S: Genomic structure and nucleotide sequence analysis of a new HIV type 1 subtype A strain from Nigeria. AIDS research and human retroviruses 1996, 12(15):1413-1425.

38. Carr JK, Salminen MO, Albert J, Sanders-Buell E, Gotte D, Birx DL, McCutchan FE: Full genome sequences of human immunodeficiency virus type 1 subtypes G and A/G intersubtype recombinants. Virology 1998, 247(1):22-31.

39. Jang DH, Yoon CH, Choi BS, Chung YS, Kim HY, Chi SG, Kim SS: Characterization of Gp41 polymorphisms in the fusion peptide domain and T-20 (Enfuvirtide) resistance-associated regions in Korean HIV-1 isolates. Journal of Korean medical science 2014, 29(3):456-459.

40. Mink M, Mosier SM, Janumpalli S, Davison D, Jin L, Melby T, Sista P, Erickson J, Lambert D, Stanfield-Oakley SA et al: Impact of human immunodeficiency virus type 1 gp41 amino acid substitutions selected during enfuvirtide treatment on gp41 binding and antiviral potency of enfuvirtide in vitro. Journal of virology 2005, 79(19):12447-12454.

41. Carmona R, Perez-Alvarez L, Munoz M, Casado G, Delgado E, Sierra M, Thomson M, Vega Y, Vazquez de Parga E, Contreras G et al: Natural resistance-associated mutations to Enfuvirtide (T20) and polymorphisms in the gp41 region of different HIV-1 genetic forms from T20 naive patients. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2005, 32(3):248-253.

42. Pessoa LS, Valadao AL, Abreu CM, Calazans AR, Martins AN, Azevedo SS, Couto-Fernandez JC, Azevedo MC, Tanuri A: Genotypic analysis of the gp41 HR1 region from HIV-1 isolates from enfuvirtide-treated and untreated patients. Journal of acquired immune deficiency syndromes (1999) 2011, 57 Suppl 3:S197-201.
43. Izumi K, Kawaji K, Miyamoto F, Shimane K, Shimura K, Sakagami Y, Hattori T, Watanabe K, Oishi S, Fujii N et al: Mechanism of resistance to S138A substituted enfuvirtide and its application to peptide design. *The international journal of biochemistry & cell biology* 2013, **45**(4):908-915.

44. Reeves JD, Lee FH, Miamidian JL, Jabara CB, Juntilla MM, Doms RW: Enfuvirtide resistance mutations: impact on human immunodeficiency virus envelope function, entry inhibitor sensitivity, and virus neutralization. *Journal of virology* 2005, **79**(8):4991-4999.

45. Sen S, Tripathy SP, Sahni AK, Gupta RM, Kapila K, Chopra GS, Chimanpure VM, Patil AA, Paranjape RS: Human immunodeficiency virus type 1 gp 41 mutations in proviral DNA among antiretroviral treatment-naive individuals from India. *AIDS research and human retroviruses* 2009, **25**(5):521-523.

46. Smolen-Dzirba J, Rosinska M, Kruszynski P, Bratosiewicz-Wasik J, Wojtyczka R, Janiec J, Szetela B, Beniowski M, Bociaga-Jasik M, Jablonowska E et al: Prevalence of Transmitted Drug-Resistance Mutations and Polymorphisms in HIV-1 Reverse Transcriptase, Protease, and gp41 Sequences Among Recent Seroconverters in Southern Poland. *Medical science monitor : international medical journal of experimental and clinical research* 2017, **23**:682-694.

**Figures**
Figure 1

Phylogenetic tree analysis of env gp-41 sequences among HIV-1 isolates in the southern China. Sequences from HIV-1 infected patients and references are respectively in blank and red in the trees and boxes.

S2-20 (URF)

S3-15 (URF)

S2-9 (CRF02_AG)

S3-2 (CRF_BF)

Figure 2
URFs and two rare recombinant partial genome maps.

Figure 3

Frequency of polymorphisms and mutations detected in HIV-1 gp41 sequences isolated from ENF treatment-naïve patients.

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