



LETTER

# The Establishment and Spatiotemporal History of A Novel HIV-1 CRF01\_AE Lineage in Shenyang City, Northeastern China in 2002–2019

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Dear Editor,

In China, HIV-1 CRF01\_AE has always been one of the most prevalent subtypes, especially among sexually active population, since it was initially reported in Yunnan and Guangxi Provinces during the early 1990s (Cheng *et al.* 1994; Yu *et al.* 1998; Chen *et al.* 1999). Multiple independent introductions of CRF01\_AE have led to the establishment of at least eight epidemic lineages in China, which have been circulating among different high-risk populations and have variable prevalence and geographic distribution (Li *et al.* 2017). Four main lineages were dominant among heterosexuals/injecting drug users (IDUs) and men who have sex with men (MSM); of these, two homosexual lineages accounted for about 60% cases (Wang *et al.* 2017), in line with that homosexuals are the most high-risk population in China nowadays (Dong *et al.* 2019). Shenyang is located in Northeastern China with > 8 million population and attract migrants from neighboring regions, which might directly or indirectly affect the HIV epidemic in neighboring cities, owing to the frequent population exchange. In Shenyang, multiple HIV subtypes

have co-existed and heterosexuals, IDUs, and those who received blood transfusions were identified as the main risk groups historically (1997–2004) (Han *et al.* 2010). Presently, MSM became the most high-risk group with an HIV prevalence of 10.8% (Zhang *et al.* 2018), and the HIV epidemic was mainly driven by two lineages of CRF01\_AE among this population, accounting for > 70% of all HIV-1 infected individuals in Shenyang. The transmission dynamics of viral lineages would reveal the epidemic characteristics of HIV through time and space and could provide key information for the performed interventions such as targeting the viral sources, sinks, and hubs throughout the viral transmission chains (Vrancken *et al.* 2020a, 2020b).

We collected 3866 HIV-1 CRF01\_AE *pol* sequences (*HXB2*: 2253–3314 nt) from a local HIV-1 drug-resistance database in Shenyang during 2002–2019. Of them, 1631 were from a follow-up cohort in the AIDS Clinic of the First Hospital of China Medical University, which accounted for over half of the HIV-1 infected individuals in Shenyang during 2002–2015; and 2235 were from all newly diagnosed HIV-1 cases in Shenyang during 2016–2019. In order to identify CRF01\_AE epidemic of Shenyang, we reconstructed a phylogenetic maximum likelihood (ML) tree using the 3866 sequences collected in Shenyang and reference sequences (Li *et al.* 2017), and we found that except for three previously reported lineages (Li *et al.* 2017), a novel CRF01\_AE lineage was circulating in Shenyang (Fig. 1A and Table 1). Moreover, the individuals of the novel lineage have significant characteristics for the variables of sex, age, risk group, location, occupation, education and marital status, compared with others (Table 1). This lineage was more prevalent among men born in the 1960s to 1970s who injected drug. Within this novel lineage, more individuals lived in Dadong and Shenhe District, most of whom were unemployed, divorced/widowed and graduated from high school. Next, we downloaded all available HIV-1 CRF01\_AE sequences

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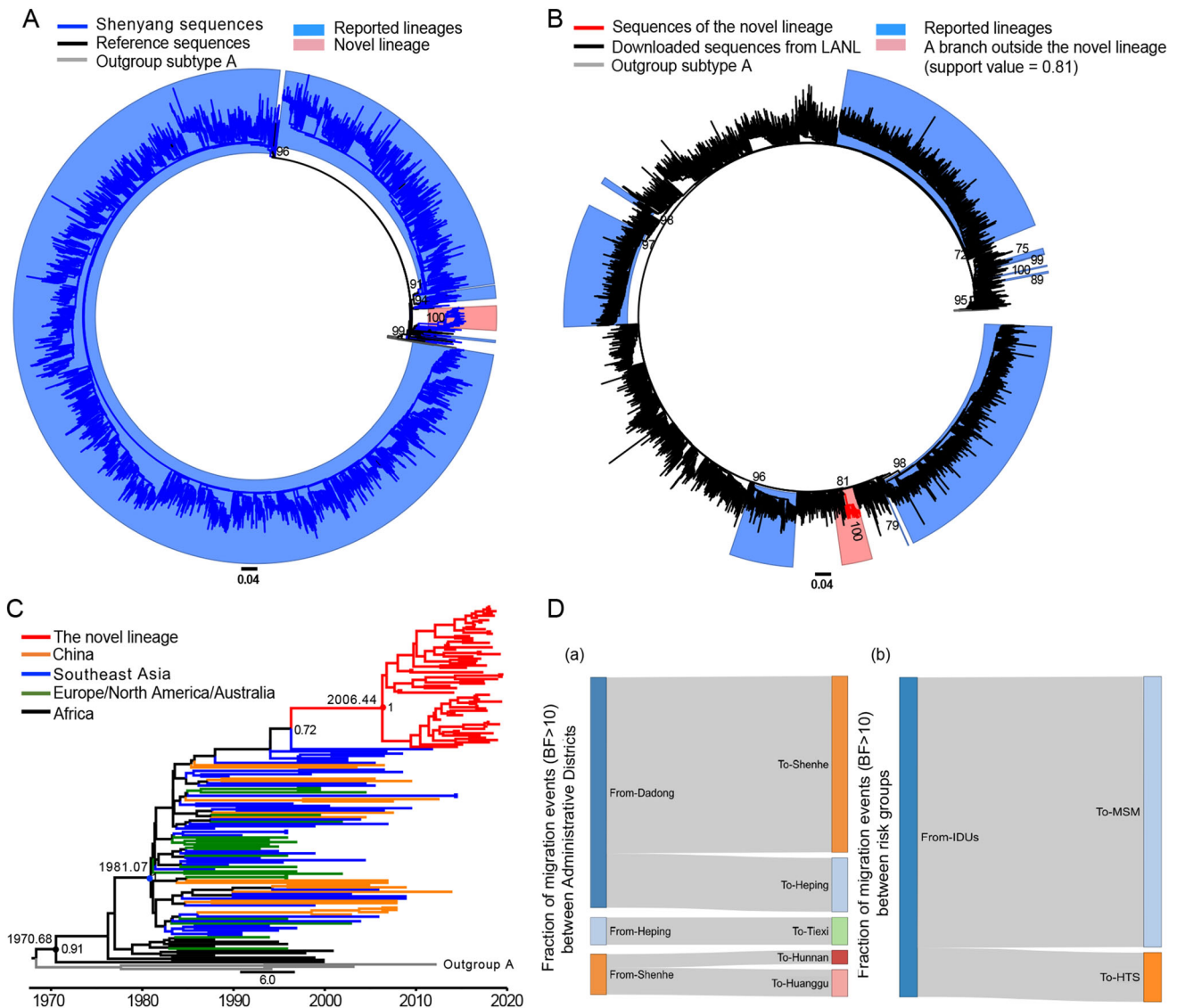
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**Fig.1** The identification and spatiotemporal history of a novel HIV-1 CRF01\_AE lineage in Shenyang. **A** Phylogenetic analysis of all 3866 CRF01\_AE sequences collected in Shenyang during 2002–2019. The blue shadows represent the previously reported HIV-1 CRF01\_AE lineages in China and the red shadow represents the novel lineage in this study (branch support value = 0.7). **B** The phylogenetic analysis of 63 sequences in the novel lineage, and all available CRF01\_AE *pol* sequences downloaded from the Los Alamos HIV database (LANL). The red shadow represents the novel lineage, and the similar sequences to the novel lineage (branch support value = 0.81), which are used in the time-scaled phylogenetic analysis. The both maximum likelihood trees (A and B) are reconstructed under GTR + I + G

model with 1,000 replicates in IQ-Tree v1.6.12. **C** The maximum clade credibility tree under the GTR + I + G substitution model, log normal relaxed molecular clock model, and non-parametric Bayesian SkyGrid mode performed in BEAST v1.10.4. The branch colors depict sampling locations. **D** Migration events of the novel lineage over Administrative Districts (a) and risk groups (b) in Shenyang City. The direction and number of all migration events (BF > 10) are presented here. Left and right sides are the source and destination of migration events, respectively. The fraction of two sides represents the proportion of migration events from sources towards destinations. MSM, men who have sex with men; HTS, heterosexuals; IDUs, injecting drug users

(*HXB2*: 2253–3314 nt; n = 10,736) from 48 countries sampled between 1990 and 2019 in the Los Alamos HIV database (<http://www.HIV.lanl.gov>). The phylogenetic reconstruction revealed that although the novel lineage was unique to any reported lineage (support value = 100) and no one from the 10,736 sequences was within the novel lineage, 40 Asian sequences (21 Thailand, 14 China, two

Vietnam, two Singapore and one Myanmar); three North American sequences; five European sequences; and one Australian were observed within a branch outside this novel lineage (support value = 81) (Fig. 1B).

We also used Bayesian molecular clock and phylogeographic approaches to reconstruct its epidemic history over time and space and identify transmission hotspots to guide

**Table 1** The demographic characteristics of CRF01\_AE-infected individuals among all newly diagnosed cases during 2016–2019

|                         |                    | Total (n = 2235) | Novel lineage (n = 48) | P-value |
|-------------------------|--------------------|------------------|------------------------|---------|
| Sex                     | Male               | 2101             | 38 (79.17%)            | < 0.001 |
|                         | Female             | 132              | 9 (18.75%)             |         |
|                         | Unknown            | 2                | 1 (2.08%)              |         |
| Birth period            | < 1960             | 167              | 1 (2.08%)              | < 0.001 |
|                         | 1960s              | 323              | 20 (41.67%)            |         |
|                         | 1970s              | 333              | 21 (43.75%)            |         |
|                         | ≥ 1980             | 1410             | 5 (10.42%)             |         |
|                         | Unknown            | 2                | 1 (2.08%)              |         |
| Risk group <sup>a</sup> | MSM                | 1839             | 11 (22.92%)            | < 0.001 |
|                         | HTS                | 320              | 2 (4.17%)              |         |
|                         | IDUs               | 41               | 34 (70.83%)            |         |
|                         | Unknown/other      | 35               | 1 (2.08%)              |         |
| Location                | Dadong             | 298              | 16 (33.33%)            | < 0.001 |
|                         | Shenhe             | 187              | 14 (29.17%)            |         |
|                         | Heping             | 285              | 4 (8.33%)              |         |
|                         | Tiexi              | 295              | 4 (8.33%)              |         |
|                         | Huanggu            | 320              | 4 (8.33%)              |         |
|                         | Yuhong             | 231              | 2 (4.17%)              |         |
|                         | Hunnan             | 188              | 1 (2.08%)              |         |
|                         | Unknown/other      | 431              | 3 (6.25%)              |         |
| Occupation              | Officer            | 204              | 1 (2.08%)              | < 0.001 |
|                         | Unemployed         | 1395             | 43 (89.58%)            |         |
|                         | Famer              | 57               | 1 (2.08%)              |         |
|                         | Unknown/other      | 579              | 3 (6.25%)              |         |
| Education               | Primary school     | 100              | 1 (2.08%)              | < 0.001 |
|                         | Junior high school | 572              | 26 (54.17%)            |         |
|                         | Senior high school | 475              | 17 (35.42%)            |         |
|                         | College            | 1056             | 3 (6.25%)              |         |
|                         | Unknown/other      | 32               | 1 (2.08%)              |         |
| Marital status          | Single             | 1360             | 14 (29.17%)            | < 0.001 |
|                         | Married            | 465              | 11 (22.92%)            |         |
|                         | Divorced/widowed   | 404              | 22 (45.83%)            |         |
|                         | Unknown/other      | 6                | 1 (2.08%)              |         |

a: MSM, men who have sex with men; HTS, heterosexuals; IDUs, injecting drug users.

targeting interventions. After verification of temporal signals in TempEst, the time-scale phylogenetic tree, reconstructed using 63 sequences from Shenyang, 56 sequences close to the novel lineage, and 42 reference sequences (An *et al.* 2020), revealed that the median time to most recent common ancestor (tMRCA) of the novel lineage was 2006.44, with a narrow time range corresponding to the

95% highest posterior density (HPD) interval of 2003.56–2008.07. The long branch away from the node of the novel lineage was also observed in the maximum clade credibility (MCC) tree (Fig. 1C), similar to the ML tree (Fig. 1A and 1B). Additionally, the novel lineage had been further divided into two sub-lineages. The tMRCAs of HIV-1 CRF01\_AE from Africa and Thailand were

estimated to be 1970.68 and 1981.07, respectively, of which the 95% HPD were 1960.67–1982.97 and 1979.58–1985.8, respectively (Fig. 1C), consistent with previous studies (Feng *et al.* 2013; Han *et al.* 2013). The estimated evolutionary rate based on *pol* sequences (*HXB2*: 2253–3314 nt) was  $1.36 (1.121.59) \times 10^{-3}$  substitutions/site/year. Finally, we estimated the well-supported direction and number of migration events based on the BF > 10 calculated under Bayesian stochastic search variable selection (BSSVS) using phylogeographic inference (Fig. 1D). Dadong District was the initial and dominant source (77.27%), from which the virus had spread to Heping and Shenhe District. Shenhe and Heping were the secondary centers, of which Shenhe was also the main destination (59.09%), suggesting it may be a potential hub of the viral spread. Between risk groups, the virus had been transmitted by IDUs to MSM, who were the main risk population of HIV-1 infection in Shenyang, accounting for 84.62% of all migration event.

Preliminary phylogenetic analyses (Fig. 1A and 1B) revealed that the novel lineage was independent of other previously reported CRF01\_AE lineages (Li *et al.* 2017), and no sequence was clustered within this novel lineage. Furthermore, the tMRCA of the novel lineages was 2006 (Fig. 1C), far later than other known lineages that typically appeared during the 1980s and 1990s in China (Feng *et al.* 2013). Notably, there were more Southeast Asian sequences located outside the lineage (Fig. 1C), suggesting that the ancestral strain of the lineage was introduced to Shenyang directly or indirectly from other Southeast Asian countries (Chen *et al.* 2020; Hemelaar *et al.* 2020) and the novel lineage has most likely sustained only by local transmission since its entrance to Shenyang, and yet not spread elsewhere. However, among newly diagnosed cases in Shenyang between 2016 and 2018, recent infections could be found in this lineage (Zhao *et al.* 2021), according to the results of HIV-1 limiting antigen avidity enzyme immunoassay (LAG-Avidity EIA) (Xia *et al.* 2020); this predicted that the novel lineage was spreading and required urgent interventions to prevent its expansion.

The viral migration history (Fig. 1D) revealed that IDUs living in Dadong District were viral sources and the strains have been circulating in some main districts of Shenyang (Dadong, Shenhe, Heping, Huanggu, Tiexi and Hunnan). As a large part of the transmissions from Dadong and toward Shenhe District, interventions focusing on the two areas would likely reduce viral spreading on a large scale. Moreover, Shenyang, as the capital of Liaoning Province and the political, economic, and cultural center of Northeast China, attracts more labor migration, rural–urban migration, and circular population migration; this could directly or indirectly affect the HIV epidemic of neighboring areas.

Additionally, identifying the individuals who are more likely to be at the highest risk of being infected or infecting others will be directly actionable in HIV prevention. From the demographic information of the patients of the novel lineage (Table 1), more elders (> 50 years) and IDUs were observed, most of who were living in Dadong and Shenhe District, coinciding with the Bayesian inferences (Fig. 1D). Owing to the needle exchange programs, from 2000, that freely provided needles to IDUs, both the HIV incidence and prevalence among IDUs have rapidly decreased (Liu *et al.* 2007). Now MSM have become the dominant risk group for HIV infection. Obviously, the number of MSM was followed by IDUs among all newly diagnosed cases in Shenyang during 2016–2019 (Table 1). Importantly, in this novel lineage, the strains have been transmitted from IDUs to MSM (Fig. 1D), indicating that this lineage has the potential to further expand via homosexual contact. Other demographic characteristics related to occupation, marital status and education would also provide more information to identify the intervention targets (Table 1). Further, more individuals who have marriage history were observed within lineage, inferring that they as the higher risk transmitter would have more chances to transmit virus to their partners via heterosexual contact.

Taken together, the interventions targeting geographical hotspots and high-risk groups would have direct benefits on reducing risks of HIV transmission and infection. Here, a focus on IDUs and MSM in Dadong and Shenhe District, such as enhancing health education and increasing testing frequency, will likely have the maximum effect in controlling the epidemic of this novel lineage locally and elsewhere. Such continued molecular monitoring can uncover more active viral transmission chains to ensure rapid response to new situations of the HIV epidemic.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare no competing interest.

**Animal and Human Rights Statement** This study was approved by the Medical Ethics Committee of the First Hospital of China Medical University and informed consent was obtained (ethics number 2019–153-2 and 2019–150-2).

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