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Distinct Clusters of HIV-1 CRF01_AE in Zhejiang, China: High-Risk Transmission Cluster 4 Requires Heightened Surveillance

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Background: HIV-1 CRF01_AE is becoming the predominant HIV-1 subtype among patients in China. The distribution and characteristics of transmission clusters of HIV-1 CRF01_AE in Zhejiang, Eastern China remains unclear. This study analyzed the epidemiologic characteristics and transmission clusters of HIV-1 CRF01_AE in Zhejiang.

Methods: Plasma samples obtained from 152 patients of HIV-1 CRF01_AE not undergoing ART were used to amplify HIV-1 *pol* and *env* gene. CRF01_AE drug resistance mutations (DRM) prevalence was analysed using Stanford University's HIV Drug Resistance Database. A phylogenetic tree was constructed using FastTree (version 2.1.11) based on the GTR nucleotide substitution model and visualized using Figtree (version 1.4.4) and The Interactive Tree of Life; the Chinese HIV Gene Sequence Data Platform was used to construct genetic transmission networks.

Results: Majority samples could be grouped into CRF01_AE transmission Clusters 1 (11.2%), 4 (64.5%), and 5 (7.2%). The CD4+ T-cell counts in Cluster 1, 4a, 4b are lower than 5 were 15, 38, 30, and 248 cells/mm^3, respectively ($P < 0.05$). The high X4 tropism rates were 13.2%, 11.8%, 20.0%, and 0.0% in Clusters 1, 4a, 4b, and 5, respectively. DRM rates in Clusters 4a and 4b were 17.6%, and 25.45% respectively ($P < 0.05$), whereas they were 17.6% and 18.2% in Clusters 1 and 5, respectively. In total, 24 transmission genetic networks, comprising 72 sequences and 61 links, were discovered; of them, 61.2%, 11.7%, and 18.2% were from Clusters 4, 1, and 5, respectively ($P < 0.05$).

Conclusion: In Zhejiang, different CRF01_AE clusters displayed unique clinic features. Cluster 4, particularly Cluster 4b, was considered a high-risk transmission cluster. The surveillance of epidemiology of HIV-1 should be enhanced to minimize its transmission.

Keywords: HIV-1 CRF01_AE, drug resistance mutation, transmission cluster

Introduction

Since the discovery of AIDS in 1981, HIV strains have evolved and spread widely.[1] Similarly, the distribution of HIV-1 subtypes has considerably evolved over the years.[2–4] In China, numerous HIV-1 subtypes and circulating recombinant forms (CRFs) have been detected, each with varying effects on disease progression.[5]

HIV-1 CRF01_AE, which originated in Africa,[6] has spread worldwide, primarily through sexual contact, particularly within the men who have sex with men (MSM) community.[7,8] Moreover, CRF01_AE infection may reduce CD4 levels with an accelerated prognosis.[9,10] Since 2007 to 2013 in China, the main HIV-1 subtype in the northwest region is still

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CRF07_BC, while in other regions, a shift from B or CRF08_BC to CRF01_AE has been observed, as reported.[11] In eastern China, the predominant HIV-1 subtypes include CRF01_AE (46.05%) and CRF07_BC (24.93%).[12] From 2014 to 2020, the main newly diagnosed HIV-1 patients in southwestern China were CRF01_AE (56.7%) and CRF08_BC (27.4%).[13] Previous studies also revealed that HIV-1 CRF07_BC and CRF01_AE were the predominant subtype in Zhejiang, China.[14–16] CRF01_AE is becoming the predominant HIV-1 subtype among patients in China.[7,17] Futhermore, CRF01_AE in China can be categorized into seven genetic subgroups, each with unique geographical characteristics and associated risk factors.[18] Recent research indicates that by targeting interventions specific to the diverse epidemic patterns observed within clusters, the overall prevention and control of HIV-1 could be strengthened.[19] However, the characteristics of the CRF01_AE clusters in Zhejiang remain unclear.

Here, we analyzed and assessed the characteristics of the prevalent transmission clusters of CRF01_AE in Zhejiang, China.

Materials and Methods

Study Population

Plasma samples were collected from 152 hIV patients admitted to the First Affiliated Hospital of Zhejiang University in Zhejiang, China, over 2013–2017. All the included patients were aged 18–80 years, were diagnosed or newly diagnosed as subtype of HIV-1 CRF01_AE, and had not yet initiated ART. We also collected relevant demographic characteristics, such as age, sex, marital status, and CD4+ T-cell count.

RNA Extraction, Amplification, and Sequencing

HIV-1 RNA was extracted from the 200 μL plasma samples by using the QIAamp Viral RNA Mini Kit (Tiangen, China). The gene fragments of HIV-1 *pol* (HXB2 2425–3332) and *env* (HBX2 7022–7644) were amplified using nested polymerase chain reaction (PCR). The PCR products were sent to the company for Sanger sequencing.[15] Date sequence data is deposited in National Microbiology Data Center (NMDC) with accession numbers NMDC10018822(https://nmdc.cn/resource/genomics/project/detail/NMDC10018822).

Drug Resistance Mutations (DRMs) Analysis

We identified the major DRMs by submitting the gene sequences to Stanford University's HIV Drug Resistance Database (https://HIVdb.Stanford.edu/HIVdb/by-sequences/). DRMs were classified into the nucleoside reverse transcriptase inhibitors (NRTIs), non- nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs).

Phylogenetic Tree Analysis

All 152 gene sequences were aligned and manually adjusted using Bioedit. Next, a phylogenetic tree based on the general time reversible (GTR) nucleotide substitution model was constructed in FastTree (version 2.1.11). Transmission chains were defined as clades with a branch probability of ≥0.80. Finally, the results were visualized using Figtree (version 1.4.4) and The Interactive Tree Of Life (https://itol.embl.de/).

Genetic Transmission Network Analysis

All 152 gene sequences were submitted to the Chinese HIV Gene Sequence Data Platform (https://nmdc.cn/hiv/) for analysing transmission clusters through the use of HIV Trace, with a gene distance threshold of 0.015. Transmission clusters were defined as pairs of sequences with a genetic distance of ≤1.5%.

Viral Tropism Prediction

Viral tropism genotypes were predicted using the Geno2pheno clone algorithm.[20] Sequencing replicates for the position-specific scoring matrices with scores <−6.96 were labelled as R5, whereas those with scores ≥−6.96 were labelled as X4.[21] A high level of accuracy was noted for X4 tropism in CRF01_AE Clusters 4 and 5, specifically in China.[22]

Statistical Analysis

Depending on their distribution, continuous variables are presented as mean ± standard deviation or median (25th and 75th percentiles), whereas categorical variables are reported as numbers (percentages). We used SPSS (version 29.0) to perform the chi-square, independent-samples *t*, and Mann–Whitney *U*-tests. Two-tailed $P < 0.05$ was considered to indicate statistical significance.

Results

Demographic Characteristics of Our Patients

We obtained partial *pol* and *env* sequences for all 152 patients. Of them, most were male (90.1%, 137/152), single or divorced (57.2%, 87/152), middle-aged, and less educated (Table 1).

DRMs

Of all 152 patients, 24 (15.8%) were found to have at least one DRM (Figure 1). Specifically, the rates of DRMs in the NRTIs, NNRTIs and PIs were 8.6%, 9.2%, and 1.3%, respectively (n = 13, 14, and 2, respectively).

The most common NRTI DRM was S68G (3.9%, 6/152), followed by S68SG (1.3%, 2/152). Moreover, the NNRTI DRM with the highest frequency was V179D (3.9%, 6/152), followed by V106I and V179VD (both 1.3%, n = 2/152). Finally, only one PI DRM was observed at M46I (0.7%, 1/152) and an accessory DRM at T74PS (0.7%, 1/152).

Four patients demonstrated DRMs in both NRTIs and NNRTIs, and one demonstrated DRMs in both PIs and NRTIs; however, no patient demonstrated DRMs in all three genes. Finally, one and two patients demonstrated two and three NNRTI DRMs, respectively, and two patients demonstrated two NRTI DRMs.

Distribution of CRF01_AE Transmission Clusters in Zhejiang

To ascertain CRF01_AE cluster distribution in Zhejiang, we next compared 152 sequences from this study (in Zhejiang) with 88 sequences reported in a study[18] that examined the distribution of CRF01_AE clusters in China (Figure 2).

In total, 17 (11.2%), 2 (1.3%), 1 (0.7%), 93 (61.2%), and 11 (7.2%) patients were grouped in Clusters 1, 2, 3, 4, and 5, respectively. Within Cluster 4, 38 (25.0%) and 55 (36.2%) were grouped in Clusters 4a and 4b, respectively. Considering their predominance, we analysed only Clusters 1, 4, and 5 further (Table 2).

Characteristics of DRMs Within Different Clusters

Among Clusters 1, 4, and 5, the data shows there were more males than females in Cluster 1, but compared to other clusters, Cluster 1 had more females (35.3%), as well as in middle-aged and older patients, most of whom were married

Table 1 Demographic Characteristics of Our Patients Living with HIV-1 CRF01_AE Infection

Characteristic	Value
Sex, n (%)	
Male	137 (90.13)
Female	15 (9.87)
Age, n (%)	
≤40 years	108 (71.05)
>40 years	44 (28.95)
Education level, n (%)	
University and higher	48 (31.58)
Middle school	92 (60.53)
Primary school and lower	12 (7.89)
Marital status n (%)	
Married	65 (42.76)
Single or divorced	87 (57.24)
CD4+ T-cell count (cells/mm^3), median, (25th, 75th percentiles)	27 (1, 1106)

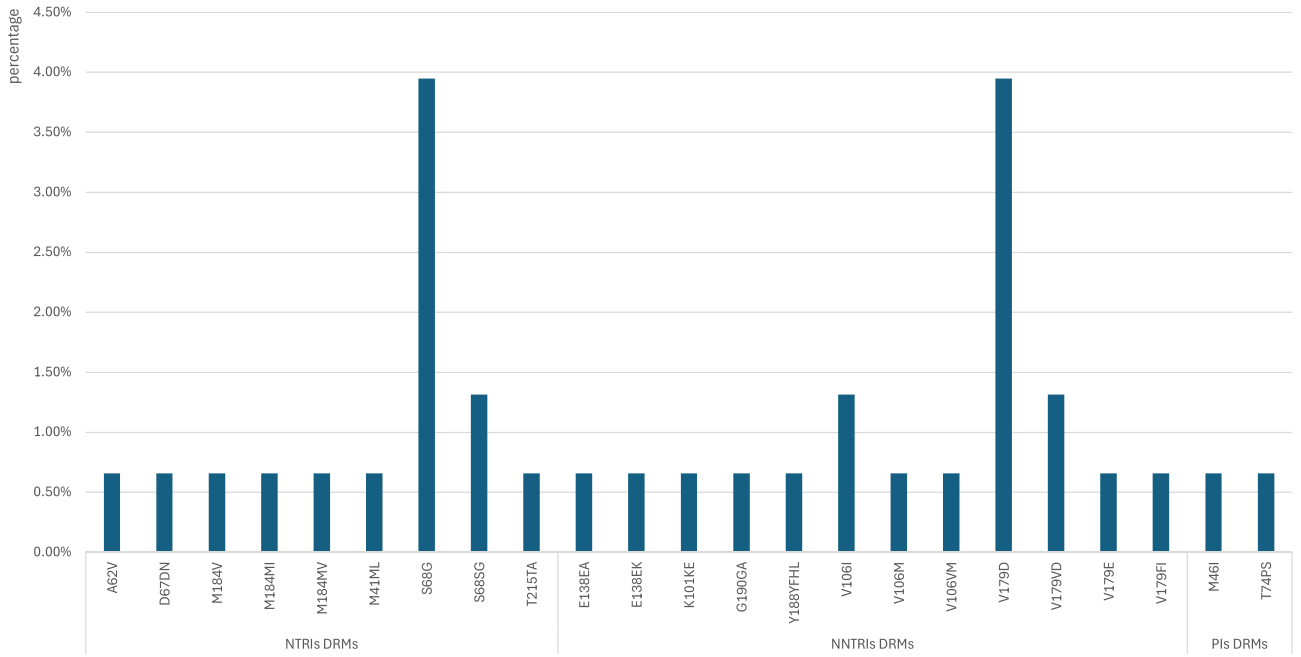

Figure 1 Percentages of CRF01_AE DRMs.

(all P < 0.05). Cluster 4, potentially representing the MSM cluster, could be further subdivided into Clusters 4a from northern China and Cluster 4b from eastern and southern China. Both Clusters 4a and 4b primarily consisted of patients who were single or divorced [65.8% (25/38) and 69.1% (38/55), respectively], were aged <41 years [84.2% (32/38) and 78.2% (43/55), respectively], and had middle school education [57.9% (22/38) and 60% (33/55), respectively]. Finally, patients in Cluster 5 demonstrated the highest CD4+ T-cell counts (P < 0.05); however, their other characteristics were comparable to those in Cluster 4.

DRMs and Viral Tropism Prediction Within Different Clusters

Next, we examined DRMs within each cluster and observed revealed that the rate of DRMs in Clusters 4a and 4b were 7.9% (3/38) and 25.5% (14/55), respectively ($P < 0.05$). Here, S68G and V179D emerged as the most prevalent NRTIs and NNRTIs DRMs.

In contrast, the rates of DRMs in Clusters 1 and 5 were 17.6% (3/17) and 18.2% (2/11), respectively. Notably, Clusters 1, 4a, 4b and 5 harboured S68G, the DRM in NRTIs. Moreover, NNRTI DRMs were predominantly observed in V179D, V179VD, and V106I (Supplementary Figure 1). A highX4 tropism rate of 20.0% (11/55) was observed in Cluster 4b; in contrast, this value was only 13.2% (5/38) and 11.8% (2/17) in Clusters 4a and 1, respectively; however, no high X4 tropism was noted in Cluster 5.

Genetic Transmission Networks

In total, 62 links and 24 genetic transmission networks, including 73 (48.0%) of all 152 patients, were identified (Figure 3). Of the 73 patients, 58.9% shared transmission events with only one patient, whereas 41.1% shared them with at least two other patients. Ten patients demonstrate at least one DRM, with 70.0% carrying an NRTI DRM and 30.0% carrying an NNRTI DRM. The most common DRM was S68G, followed by S68SG. Two patients had multiple DRMs, including S68G+V179D and E138EA+V79FI. Moreover, one patient demonstrated T74PS, a minor PI DRM.

The largest cluster comprised 11 patients, 2 of whom had five links. Two S68G mutations were identified within this cluster, and mutual connections were observed. In this network, the proportion of Cluster 4 (61.2%, 60/98) was significantly higher than that of Cluster 1 (11.7%, 2/17) and Cluster 5 (18.2%, 2/11; $P < 0.05$). Further analysis revealed that 73.7% (28/38) of the sequences belonged to Cluster 4a, whereas 49.1% (27/55) belonged to Cluster 4b (Figure 4).

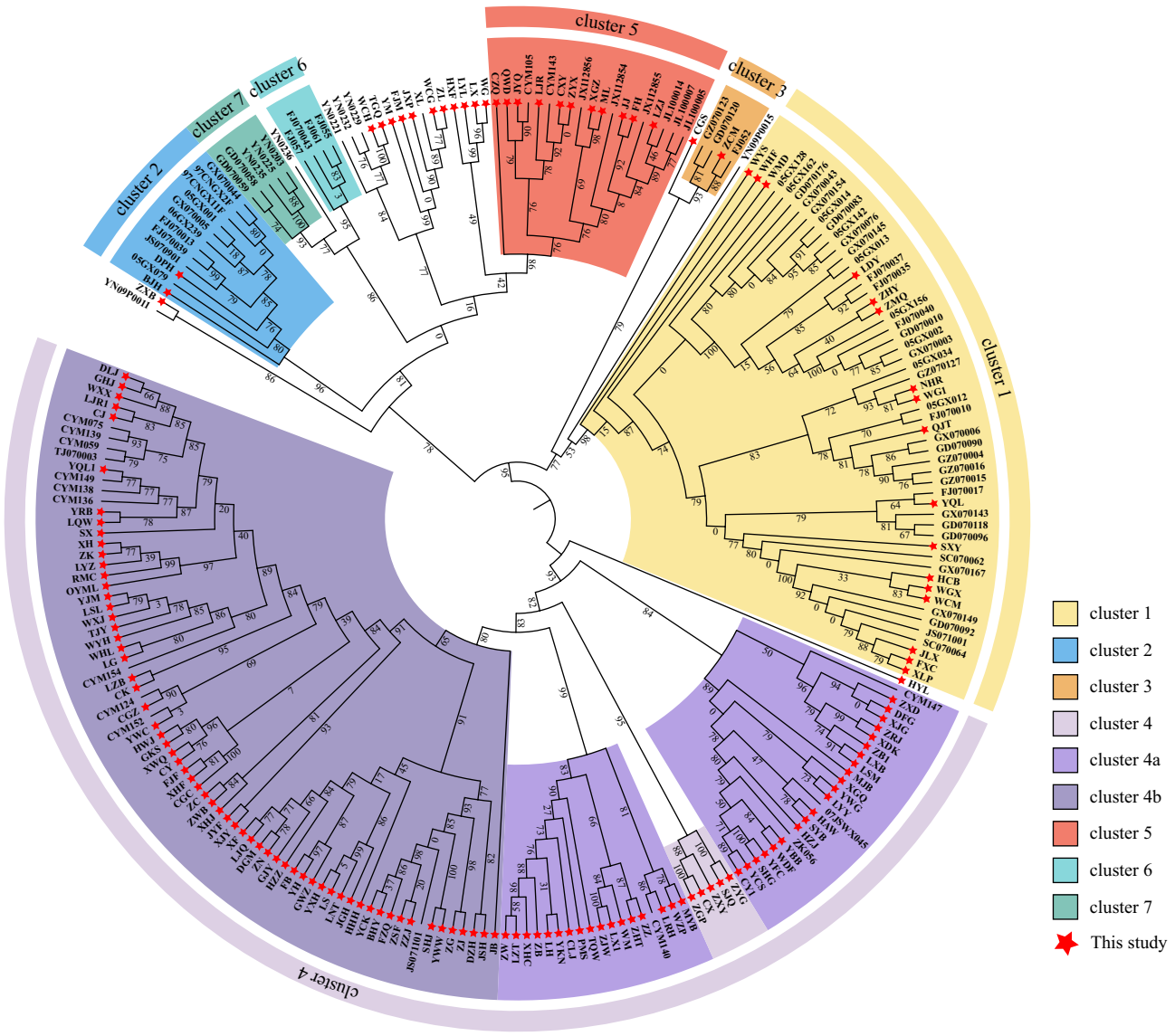

Figure 2 HIV-1 CRF01_AE phylogenetic tree and clusters in Zhejiang, China.

Notably, a comparison between patients in Clusters 4a and 4b within the network revealed that Cluster 4b had moreDRMs ($P < 0.05$) and that Cluster 4a had no DRMs. No differences were noted between the two clusters regarding age, sex, marital status, CD4+ T-cell count, and DRM rate.

Table 2 Demographic Characteristics and DRMs in the HIV-1 CRF01_AE Clusters

Characteristic	Cluster 4a	Cluster 4b	Cluster 1	Cluster 5	*P*
Sex, n (%)					0.001
Male	38 (100.0)	51 (92.7)	11 (64.7)	9 (81.8)	
Female	0 (0.0)	4 (7.3)	6 (35.3)	2 (18.2)	
Age, n (%)					0.003
<41 years	32 (84.2)	43 (78.2)	6 (35.3)	8 (72.7)	
>40 years	6 (15.8)	12 (21.8)	11 (64.7)	3 (27.3)	

(*Continued*)

Table 2 (Continued).

Characteristic	Cluster 4a	Cluster 4b	Cluster 1	Cluster 5	*P*
Marital status, n (%)					0.013
Married	13 (34.2)	17 (30.9)	12 (70.6)	2 (18.2)	
Single or divorced	25 (65.8)	38 (69.1)	5 (29.4)	9 (81.8)	
Education level, n (%)					0.256
University or higher	13 (34.5)	19 (34.5)	2 (11.8)	5 (45.5)	
Middle school	22 (57.9)	33 (60.0)	11 (67.7)	5 (45.5)	
Primary school or lower	3 (7.9)	3 (5.5)	4 (23.5)	1 (9.1)	
CD4+ T-cell count (cells/mm^3), median, (25th, 75th percentiles)	38 (24, 111)	30 (8, 90)	15 (8, 26)	248 (22.5, 508)	0.031
High X4 tropism, n (%)	5(13.2)	11(20.0)	2(11.8)	0(0.0)	0.433
DRMs, n (%)	3(7.9)	14(25.5)	3(17.6)	2(18.2)	0.209

Note: $P < 0.05$ was considered to indicate statistical significance in four clusters.

Discussion

The current results clarified the characteristics of transmission clusters in HIV-1 CRF01_AE in Zhejiang, China.

HIV patients from different regions have been noted to display varied DRMs.[23] In Ethiopia, K103N and K219Q were the predominant NNRTI and NRTI DRMs among pretreatment drug–resistant HIV patients over 2003–2018.[24] In China, K103N was the most prevalent NNRTI DRM among HIV patients in 2017, followed by V179D and E138A.[25] In the present study, the most frequent NNRTI DRM was V179D (3.9%, 6/152), followed by V179VD (1.3%, 2/152) and V106I (1.3%, 2/152). The most commonly observed NRTI DRM was S68G (3.9%,6/152). S68G, particularly prevalent among CRF01_AE strains, has been noted to be the most frequent natural polymorphism, typically followed by K65R; it can partially offset the replication impairment linked to K65R, another NRTI DRM.[26,27] Nevertheless, in the current

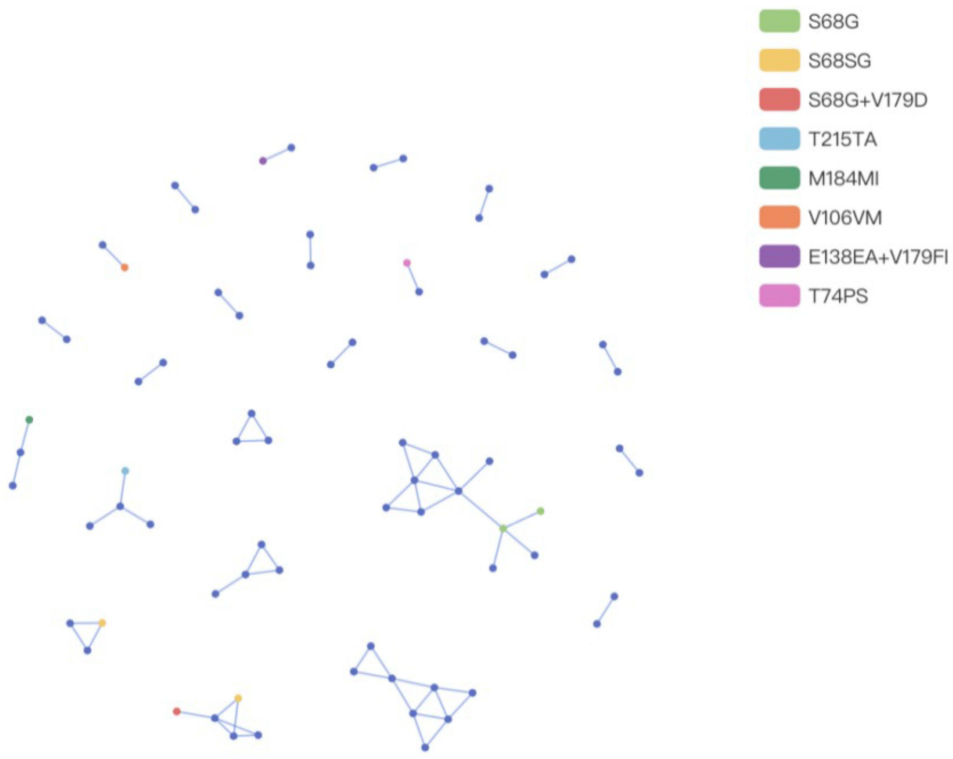

Figure 3 HIV-1 CRF01_AE transmission clusters in Zhejiang, China. Transmission clusters were identified using the Chinese HIV Gene Sequence Data Platform (https://nmdc.cn/hiv/). DRMs are colour coded.

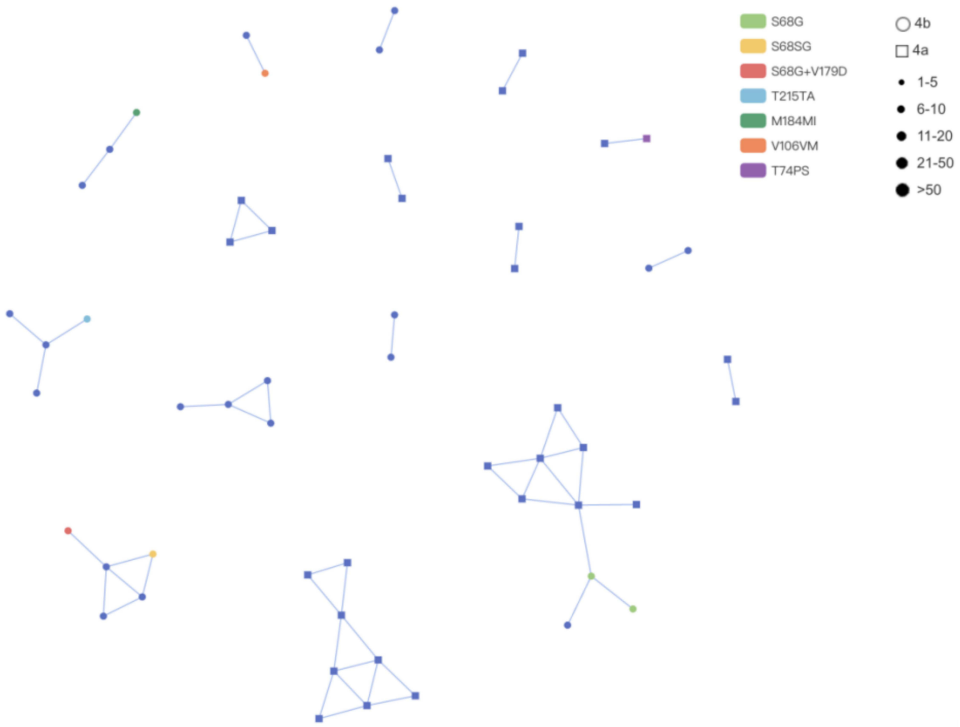

Figure 4 Transmission Clusters 4a and 4b. DRMs are colour coded; squares and circles represent Clusters 4a and 4b, respectively.

study, patients with S68G did not exhibit K65R. As such, further investigation on the relationship between S68G and K65R is warranted.

Previous study determined the strain lineage of HIV-1 CRF01_AE in China and noted the unique characteristics of each cluster.[18] In the present study, we noted that most CRF01_AE strains in Zhejiang could be placed in Clusters 1, 4, and 5—with each strain exhibiting unique traits.

Cluster 4, as the largest CRF01_AE strain in Zhejiang, originated from the northern MSM population and was transmitted to Zhejiang, strongly indicating the high prevalence of MSM transmission of CRF01_AE strains in Zhejiang. The patients in Cluster 4 were young or middle-aged men, most of whom were single and therefore may have had multiple sexual partners,[28] which could make HIV transmission risky.[5] Moreover, Studies have indicated that high X4 tropism is correlated with low CD4+ T-cell counts[29] and that genetic clusters exhibiting the X4 phenotype reduce ART efficacy.[22] The high X4 tropism rates (13.2% and 20.0%, respectively) and the low CD4+ T-cell counts (median 38 and 30, respectively) in Clusters 4a and 4b are similar to previous studies, suggesting that patients in Cluster 4a and 4b may experience a more challenging immune recovery process. Additionally, DRM rates were more variable and higher in Cluster 4b than in Cluster 4a. And the predominant DRM was V179D in Cluster 4b, which can lead to decreased susceptibility to efavirenz and nevirapine.[30] As such, the use of these drugs in ART for patients in Cluster 4b should be reevaluated. In summary, Cluster 4, particularly Cluster 4b, may be a high-risk cluster in Zhejiang, with young men being the most affected population. Patients in Cluster 4 may require immune reconstitution over an extended period and be relatively vulnerable to severe opportunistic infections. Careful consideration of the timing of ART and detection of opportunistic infections may improve the quality of life of these patients.

Cluster 5 was noted to share similarities with Cluster 4 in terms of its characteristics; however, it was associated with considerably higher CD4+ T-cell counts but without any X4 tropism. This result corroborates the findings of previous studies, indicating that Cluster 5 is stable.[29]

Cluster 1 may have been transmitted to Zhejiang via heterosexual individuals and injection drug users in southern China. In our study, we propose that middle-aged and older married individuals, particularly women, should be considered the key target group for Cluster 1. Furthermore, equal attention should be paid to marital harmony and well-

being among this population. As reported, the likelihood of CD4+ T-cell count recovery and immune reconstruction is relatively low among Cluster 1 patients.[31] Our result showed that the median CD4+ T-cell count in Cluster 1 was 15, which means that Cluster 1 in Zhejiang may be at a greater risk of virological failure. More frequent and longer-term monitoring strategies are necessary to improve the immune status of Cluster 1.

In the present study, 48.0% (73/152) of individuals constituted the genetic transmission networks. It appears that there is a higher transmission risk of HIV-1 CRF_01AE in Zhejiang than in the southwest border region of China (32.2%) or in Guangdong, China (25.8%).[32,33] Due to the extremely high presence of individuals from Cluster 4 in the genetic transmission networks, it is possible that Cluster 4 is a high-risk transmission cluster in Zhejiang. Cluster 4b showed a higher frequency of DRMs than Cluster 4a, suggesting a higher likelihood of drug resistance transmission. We also found a relatively high prevalence of CRF01_AE pretreatment drug resistance at 11.8% (Cluster 4 at 13.3%), in contrast to the 4.65% prevalence in China in 2017 and the 6.73% prevalence in the nearby city of Nanjing from.2019−2021[25,34] Furthermore, we noticed a likelihood of sharing DRM (S68G) between them. We believe that the spread of CRF01_AE resistance in Zhejiang should not be underestimated, especially when considering the impact of the widely present Cluster 4.Thus, to minimize the spread of Cluster 4 hIV-1 CRF01_AE strains and potentially prevent their evolution, drug resistance testing and dynamic transmission monitoring of these strains, particularly Cluster 4b strains, should be prioritized.

This study has a few limitations. First, our HIV patient sample was small and enrolled from a single centre; this may have led to sampling errors and limited the generalizability of our findings size. Consequently, further comprehensive analysis of the characteristics of patients with HIV-1 CRF01_AE in Zhejiang as a whole is warranted. Secondly, as a comprehensive hospital in Zhejiang Province, our patients mainly included newly diagnosed HIV-1 patients and those with advanced HIV; thus, the higher proportion of DRMs among them could have been due to impairments in their immune function and infection status.Thirdly, according to our observation, a majority of hospitalized patients at our institution are male, potentially introducing a gender bias in the data. Further inquiry is warranted to elucidate the underlying factors contributing to this observation.

Conclusion

Our study revealed the presence of HIV-1 CRF01_AE Clusters 1, 4, and 5 in Zhejiang, China, with each cluster demonstrating distinct characteristics. Cluster 4 was noted to be the most significant in terms of both DRMs and transmission risks. To minimize the spread of HIV strains, effective targeted drug resistance testing and ongoing transmission dynamics surveillance are essential.

Abbreviations

AIDS, Acquired immunodeficiency syndrome; HIV, Human immunodeficiency virus; CRFs, Circulating recombinant forms; MSM, Men who have sex with men; DRM, Drug resistance mutations; NNRTI, Non-nucleoside reverse-transcriptase inhibitor; NRTI, Nucleoside reverse-transcriptase inhibitor; PI, Protease inhibitor; INSTI, Integrase strand transfer inhibitor.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study protocol was approved by the ethical committee of the First Affiliated Hospital, Zhejiang University School of Medicine (No. IIT2022052B-R1) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants in this study provided written informed consent prior to the collection of serum samples.

Consent for Publication

All the authors declare their consent for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All the authors declare that they have no competing interests in this work.

References

1. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. *Curr Opin HIV AIDS*. 2019;14(3):153. doi:10.1097/COH.0000000000000534
2. Xiao P, Zhou Y, Lu J, et al. HIV-1 genotype diversity and distribution characteristics among heterosexually transmitted population in Jiangsu province, China. *Virol J*. 2019;16(1):51. doi:10.1186/s12985-019-1162-4
3. Ye M, Chen X, Duo L, et al. Identification of two novel HIV-1 circulating recombinant forms of CRF111_01C and CRF116_0108 in southwestern Yunnan, China. *Virulence*. 2022;13(1):19–29. doi:10.1080/21505594.2021.2010399
4. Lan Y, He X, Li L, et al. Complicated genotypes circulating among treatment naïve HIV-1 patients in Guangzhou, China. *Infect Genet Evol*. 2021;87:104673. doi:10.1016/j.meegid.2020.104673
5. Pang X, Liang S, Tang K, et al. Disparity of HIV-1 Pretreatment Drug Resistance in Men Who Have Sex With Men and the Heterosexual Population in Guangxi, China. *Open Forum Infect Dise*. 2023;10(2):ofad016. doi:10.1093/ofid/ofad016
6. Lau KA, Wong JJL. Current Trends of HIV Recombination Worldwide. *Infect Dis Rep*. 2013;5(Suppl 1):e4. doi:10.4081/idr.2013.s1.e4
7. Li Y, Han Y, Xie J, et al. CRF01_AE subtype is associated with X4 tropism and fast HIV progression in Chinese patients infected through sexual transmission. *AIDS*. 2014;28(4):521. doi:10.1097/QAD.0000000000000125
8. Li W, Chu J, Wei F, et al. Molecular characteristic of HIV-1 CRF01_AE in Nanjing from 2015 to 2017. *Infect Genet Evol*. 2019;75:104038. doi:10.1016/j.meegid.2019.104038
9. Ng OT, Lin L, Laeyendecker O, et al. Increased Rate of CD4+ T-Cell Decline and Faster Time to Antiretroviral Therapy in HIV-1 Subtype CRF01_AE Infected Seroconverters in Singapore. *PLoS One*. 2011;6(1):e15738. doi:10.1128/JVI.77.24.13376-13388.2003
10. Chu M, Zhang W, Zhang X, et al. HIV-1 CRF01_AE strain is associated with faster HIV/AIDS progression in Jiangsu Province, China. *Sci Rep*. 2017;7(1):1570. doi:10.1038/s41598-017-01858-2
11. Li X, Li W, Zhong P, et al. Nationwide Trends in Molecular Epidemiology of HIV-1 in China. *AIDS Res Hum Retroviruses*. 2016;32(9):851–859. doi:10.1089/aid.2016.0029
12. Xiao P, Li J, Fu G, Zhou Y, Huan X, Yang H. Geographic Distribution and Temporal Trends of HIV-1 Subtypes through Heterosexual Transmission in China: a Systematic Review and Meta-Analysis. *Int J Environ Res Public Health*. 2017;14(7):830. doi:10.3390/ijerph14070830
13. Xu X, Luo L, Song C, et al. Survey of pretreatment HIV drug resistance and the genetic transmission networks among HIV-positive individuals in southwestern China, 2014–2020. *BMC Infect Dis*. 2021;21(1):1153. doi:10.1186/s12879-021-06847-5
14. Zhang J, Xu K, Jiang J, et al. Combining molecular network analysis and field epidemiology to quantify local HIV transmission and highlight ongoing epidemics. *Inter J Infect Dis*. 2023;128:187–193. doi:10.1016/j.ijid.2022.12.033
15. Zhang J, Guo Z, Yang J, et al. Genetic diversity of HIV-1 and transmitted drug resistance among newly diagnosed individuals with HIV infection in Hangzhou, China. *J med virol*. 2015;87(10):1668–1676. doi:10.1002/jmv.24223
16. Xu Y, Peng X, Peng X, et al. Characterization of HIV-1 subtypes and transmitted drug resistance among treatment-naive HIV-infected individuals in Zhejiang, China, 2014-2017. *Arch Virol*. 2018;163(8):2233–2237. doi:10.1007/s00705-018-3839-1
17. Liu X, Wang D, Hu J, et al. Changes in HIV-1 Subtypes/Sub-Subtypes, and Transmitted Drug Resistance Among ART-Naïve HIV-Infected Individuals — China, 2004–2022. *China CDC Wkly*. 2023;5(30):664–671. doi:10.46234/ccdcw2023.129
18. Feng Y, He X, Hsi JH, et al. The rapidly expanding CRF01_AE epidemic in China is driven by multiple lineages of HIV-1 viruses introduced in the 1990s. *AIDS*. 2013;27(11):1793. doi:10.1097/QAD.0b013e328360db2d
19. An M, Zheng C, Li H, et al. Independent epidemic patterns of HIV-1 CRF01_AE lineages driven by mobile population in Shenzhen, an immigrant city of China. *Virus Evolution*. 2021;7(2):veab094. doi:10.1093/ve/veab094

20. Hwang SS, Boyle TJ, Lyerly HK, Cullen BR. Identification of the Envelope V3 Loop as the Primary Determinant of Cell Tropism in HIV-1. *Science*. 1991;253(5015):71–74. doi:10.1126/science.1905842
21. Jensen MA, Li FS, Wout ABVT, et al. Improved Coreceptor Usage Prediction and GenotypicMonitoring of R5-to-X4 Transition by Motif Analysis of HumanImmunodeficiency Virus Type 1 env V3 LoopSequences. *J Virol*. 2003;77:13376–13388. doi:10.1128/jvi.77.24.13376-13388.2003
22. Ge Z, Feng Y, Li K, et al. CRF01_AE and CRF01_AE Cluster 4 Are Associated With Poor Immune Recovery in Chinese Patients Under Combination Antiretroviral Therapy. *Clinl Infect Dis*. 2021;72(10):1799–1809. doi:10.1093/cid/ciaa380
23. Kirichenko A, Kireev D, Lopatukhin A, et al. Prevalence of HIV-1 drug resistance in Eastern European and Central Asian countries. *PLoS One*. 2022;17(1):e0257731. doi:10.1371/journal.pone.0257731
24. Kiros M, Biset S, Gebremariam B, Yalew GT, Abegaz WE, Geteneh A. Trends in HIV-1 pretreatment drug resistance and HIV-1 variant dynamics among antiretroviral therapy-naive Ethiopians from 2003 to 2018: a pooled sequence analysis. *Virol J*. 2023;20(1):243. doi:10.1186/s12985-023-02205-w
25. Kang RH, Liang SJ, Ma YL, et al. Pretreatment HIV drug resistance in adults initiating antiretroviral therapy in China, 2017. *Infect Diseases Poverty*. 2020;9(1):54. doi:10.1186/s40249-020-00668-5
26. Li S, Ouyang J, Zhao B, et al. The S68G polymorphism is a compensatory mutation associated with the drug resistance mutation K65R in CRF01_AE strains. *BMC Infect Dis*. 2020;20(1):123. doi:10.1186/s12879-020-4836-z
27. Svarovskaia ES, Feng JY, Margot NA, et al. The A62V and S68G Mutations in HIV-1 Reverse Transcriptase Partially Restore the Replication Defect Associated With the K65R Mutation. *J Acquir Immune Defic Syndr*. 2008;48(4):428–436. doi:10.1097/QAI.0b013e31817bbe93
28. Hong H, Shi X, Liu Y, et al. HIV Incidence and Transactional Sex Among Men Who Have Sex With Men in Ningbo, China: prospective Cohort Study Using a WeChat-Based Platform. *JMIR Public Health and Surveil*. 2024;10(1):e52366. doi:10.2196/52366
29. Song H, Ou W, Feng Y, et al. Disparate impact on CD4 T cell count by two distinct HIV-1 phylogenetic clusters from the same clade. *Proc Natl Acad Sci*. 2019;116(1):239–244. doi:10.1073/pnas.1814714116
30. Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Oka S. Combination of V106I and V179D Polymorphic Mutations in Human Immunodeficiency Virus Type 1 Reverse Transcriptase Confers Resistance to Efavirenz and Nevirapine but Not Etravirine. *Antimicrob. Agents Chem*. 2010;54(4):1596–1602. doi:10.1128/aac.01480-09
31. Li K, Chen H, Li J, et al. Immune reconstruction effectiveness of combination antiretroviral therapy for HIV-1 CRF01_AE cluster 1 and 2 infected individuals. *Emerging Microbes Infect*. 2022;11(1):158–167. doi:10.1080/22221751.2021.2017755
32. Lan Y, Li F, Li L, et al. Drug resistance and genetic transmission characteristics of HIV-1 CRF59_01B in infected patients in Guangdong Province, China. *J Antimicrob Chemother*. 2022;77(7):1960–1968. doi:10.1093/jac/dkac132
33. Li D, Chen H, Li H, et al. HIV-1 pretreatment drug resistance and genetic transmission network in the southwest border region of China. *BMC Infect Dis*. 2022;22(1):741. doi:10.1186/s12879-022-07734-3
34. Xu Y, Shi H, Dong X, et al. Transmitted drug resistance and transmission clusters among ART-naïve HIV-1-infected individuals from 2019 to 2021 in Nanjing, China. *Front Public Health*. 2023:11. doi:10.3389/fpubh.2023.1179568