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Combining molecular transmission network analysis and spatial epidemiology to reveal HIV-1 transmission pattern among the older people in Nanjing, China

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Abstract

Background In China, the problem of HIV infection among the older people has become increasingly prominent. This study aimed to analyze the pattern and influencing factors of HIV transmission based on a genomic and spatial epidemiological analysis among this population.

Methods A total of 432 older people who were aged ≥ 50 years, newly diagnosed with HIV-1 between January 2018 and December 2021 and without a history of ART were enrolled. HIV-1 *pol* gene sequence was obtained by viral RNA extraction and nested PCR. The molecular transmission network was constructed using HIV-TRACE and the spatial distribution analyses were performed in ArcGIS. The multivariate logistic regression analysis was performed to analyze the factors associated with clustering.

Results A total of 382 sequences were successfully sequenced, of which CRF07_BC (52.3%), CRF01_AE (32.5%), and CRF08_BC (6.8%) were the main HIV-1 strains. A total of 176 sequences entered the molecular network, with a clustering rate of 46.1%. Impressively, the clustering rate among older people infected through commercial heterosexual contact was as high as 61.7% and three female sex workers (FSWs) were observed in the network. The individuals who were aged ≥ 60 years and transmitted the virus by commercial heterosexual contact had a higher clustering rate, while those who were retirees or engaged other occupations and with higher education degree were less likely to cluster. There was a positive spatial correlation of clustering rate (Global Moran $I = 0.206$, $P < 0.001$) at the town level and the highly aggregated regions were mainly distributed in rural area. We determined three large clusters which mainly spread in the intra-region of certain towns in rural areas. Notably, 54.5% of cases in large clusters were transmitted through commercial heterosexual contact.

Conclusions Our joint analysis of molecular and spatial epidemiology effectively revealed the spatial aggregation of HIV transmission and highlighted that towns of high aggregation were mainly located in rural area. Also, we found vital role of commercial heterosexual contact in HIV transmission among older people. Therefore, health resources

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should be directed towards highly aggregated rural areas and prevention strategy should take critical persons as entry points.

Keywords HIV/AIDS, Older people, Molecular network, Transmission cluster, Spatial analysis

Introduction

In the era of antiviral therapy (ART), HIV is no longer a life-limiting infection and AIDS has become a manageable chronic disease. The population of older people with HIV (normally defined as those aged 50 years or older) is increasing [1, 2], and the number of older people with HIV globally increased from 5.4 million in 2015 to 8.1 million in 2020 [1]. In recent years, the number of newly reported older people with HIV who were aged ≥ 50 years in China has also been increasing [3], and the problem of HIV infection among older people has become a prominent challenge in the HIV prevention and control. The older people still have sex needs to a certain extent, but due to their low level of education, poor knowledge about HIV, and low rate of condoms use, they are prone to HIV infection. Meanwhile, they have poor awareness of active detection, resulting in late diagnosis. A meta-analysis pointed out that the HIV infection rate of older people in China was 2.1% [4], which was much higher than that of the general population in China (0.09%) [5]. Hence, controlling HIV infection among this population is of great significance to curb the epidemic of HIV in China.

Using the similarity of viral sequences, HIV-1 molecular transmission network can be constructed to analyze transmission patterns, determine potential transmission relationship, and identify active transmission clusters, providing an effective scientific basis for precise intervention [6, 7]. A study used the molecular transmission network and field epidemiology to quantify the local HIV transmission mode and highlight ongoing epidemics, thus developing precise HIV prevention strategies [8]. The spatial distribution characteristics of HIV are closely related to geographical factors, affecting the prevalence and transmission of HIV [9]. However, previous studies have mainly focused on analyses of epidemic trends or infection characteristics only from a temporal perspective, neglecting the spatial information and failing to comprehensively understand the disease and its impact on different regions. Spatial analysis can fully utilize the spatial information in disease data, so it is widely used in describing the spatial distribution characteristics and changing trends of diseases, disease surveillance and so on [10, 11]. A previous study, combining the molecular networks and geographic information, effectively quantified the transmission of HIV-1 between key populations and general heterosexual populations, as well as

between different geographic regions [12]. The HIV-1 molecular transmission networks alone can't capture the spatial characteristics of HIV transmission, so the joint analysis of molecular and spatial epidemiology is conducive to understanding the dynamic changes of HIV-1 subtype and clusters from a spatial perspective, and providing evidences for mapping out regional HIV prevention strategies and optimizing allocation of health resources.

Currently, several analyses highlighted the role of older people in the transmission of HIV-1 in the local context [13, 14]. However, domestic and foreign studies mainly focused on behaviors, attitudes, or factors of HIV infection [15, 16], and reports of spatial analysis combined with transmission network were relatively few for older people. According to the HIV monitoring data in Nanjing, the proportion of older people with HIV in the newly reported cases increased from 10.6% in 2010 to 23.9% in 2023, which suggested it was crucial to analyze HIV transmission characteristics and its impact on different districts of Nanjing among older people. This study utilized the epidemiological data, spatial information, and HIV sequence information of older people with HIV in Nanjing to dissect the spatial distribution characteristics of and detect key persons or critical regions. This could provide reliable information for developing targeted prevention and control strategies, optimal allocation of health resources and precise containment of the local HIV epidemic.

Material and methods

Study participants and data collection

Older people who were aged ≥ 50 years old, newly diagnosed with HIV-1 between January 1, 2018 and November 31, 2022 and without history of ART were enrolled in our study. Before the collection of sample and data, each participant signed a written informed consent. This study was approved by the Ethics Committee of the Nanjing Center for Disease Control and Prevention (Approval No. PJ2020-A001-03). For each participant, 5 ml venous blood was collected, and the plasma was separated and stored in a refrigerator at -80 °C. Meanwhile, epidemiological information was investigated anonymously, including age, gender, marital status, transmission route, number of non-marital heterosexual partners, number of homosexual partners, screening sources, etc.

HIV-1 RNA extraction, amplification and sequencing

HIV-1 RNA was extracted from 200 µl plasma samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the instructions. Reverse transcription and nested PCR amplification of the HIV-1 partial *pol* gene (HXB2: 2253–3313) were performed as previously described [17]. The PCR products were dealt with electrophoresis with 1% agarose gel, and the amplified positive products were purified and sequenced by Sangon Biotechnology Co., Ltd. The sequence database was curated, excluding duplicates, as well as sequences not compliant with quality control [18]. Briefly, we excluded the sequences due to inadequate length, presence of stop codons, bad insertions/deletions and hypermutations. Furthermore, we excluded codon positions associated with drug resistance mutations on the basis of the HIV drug resistance database (<https://hivdb.stanford.edu>), to reduce the potential confounding effects of convergent evolution on sequence analysis [19, 20].

Recombination analysis

Sequencer 4.10.1 (GeneCodes, Ann Arbor, MI) was used for sequence splicing, and BioEdit (version 7.0.9, Informer Technologies Inc.) was used to align with the reference sequences. The reference sequences were downloaded from the LANL HIV database (<https://www.hiv.lanl.gov/content/index>) and contained the major international epidemic strains A–D, F–H, and J–K, as well as the major epidemic recombinant strains in China. The GenBank accession numbers of reference sequences were detailed in additional file 1. Then the maximum likelihood (ML) method and GTR model of the FastTree 2.1 software was used to construct the phylogenetic tree, which was repeatedly constructed 1000 times. A bootstrap value >80% was used to verify the accuracy of subtyping and to determine subtype. The ML phylogenetic tree was imported to FigTree v 1.4.4 for visualization [21]. Intersubtype recombinant sequences were preliminarily analyzed using the Recombination Identification Program (RIP; <https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html>), and Simplot 3.5.1 software was used to analyze the recombination mode in detail.

Construction of HIV molecular transmission network

Pairwise genetic distances were calculated using the Tamura-Nei 93 model, and the HIV-TRACE was used to determine the optimal genetic distance threshold and construct molecular network [22]. At a genetic threshold of 1.10%, the transmission network could identify the most clusters in our study. Cytoscape (version 3.6.1) was used to process and generate the molecular network. According to Technical Guideline

for HIV Transmission Network Monitoring and Intervention released by Chinese Center for Disease Control and Prevention (revision in 2021), a node represents an HIV sequence or a person, an edge represents the potential transmission relationship between two connected nodes, and a link is the number of edges that the node is connected to other nodes, also known as degree. In our study, large transmission cluster was defined as clusters containing more than 10 nodes.

Statistical analysis

SPSS (version 18.0, IBM Corp., Armonk, NY) was used for statistical analysis. Continuous variables with non-normal distribution were reported as median (interquartile range, IQRs), while categorical variables were described by frequency (percentage). The Chi-square test was used to compare difference between groups. The multivariate logistic regression model was conducted to analyze the factors associated with clustering. Variables with a $P < 0.05$ in the Chi-square test were included in the multivariate regression analysis. All the results of statistical significance test were reported as p -values. $P < 0.05$ (two-tailed) was considered statistically significant.

Spatial autocorrelation analysis

Global Moran's I, a widely used spatial autocorrelation metric in spatial epidemiology, was employed to determine whether there was spatial aggregation. If $I > 0$, it meant there was a positive spatial correlation, showing a clustered distribution; if $I < 0$, it meant that there was a negative spatial correlation, showing a discrete distribution; if $I = 0$, it meant that there was no spatial autocorrelation and the spatial distribution was random. The Anselin local Moran's I statistic was employed to detect spatial clustering patterns, resulting in five categorical values: "high-high" means that a given area with a high clustering rate is surrounded by areas also with a high clustering rate; "low-low" means that an area with a low clustering rate is surrounded by areas also with a low clustering rate; "low-high" means that an area with a low clustering rate is surrounded by areas with a relatively high clustering rate; "high-low" means that an area with a high clustering rate is surrounded by areas with a relatively low clustering rate; a value of zero means that the clustering rates are distributed randomly between regions [23]. A z -test was performed to determine whether each spatial autocorrelation of clustering rate was significantly different from a random distribution. All the spatial descriptions and analyses were performed in ArcGIS (version 10.3).

Results

Characteristics of study population

Our research obtained sample from 432 older people with HIV. After excluding the cases due to failure of HIV-1 RNA extraction and amplification (29 cases), sequencing failure (12 cases), duplicated cases (3 cases), and sequences not compliant with quality control (6 cases), 382 cases were enrolled for analysis. The median age was 59 (IQR: 54–66) years, ranging from 50 to 89 years. The majority were male (83.2%), 50–59 years old (53.1%), married (71.7%), rural (60.5%), with primary school degree or below (35.9%). Regarding screening source, 57.9% were medical institutions, 24.1% were VCT and 11.6% were sexually transmitted disease (STD) clinic. 36.1% of participants were infected through non-commercial heterosexual contact, followed by homosexual (34.8%) and commercial heterosexual contact (28.0%). The first CD4+T lymphocyte (CD4) count before ART was mainly less than 200 cells/ μ l (40.6%). The first viral load (VL) before ART was mainly 10,000–99999 copies/ml (49.2%) (Table 1). The phylogenetic tree analysis revealed that the most common HIV-1 strains was CRF07_BC (52.3%), CRF01_AE (32.5%), and CRF08_BC (6.8%), accounting for 91.6%, followed by subtype B, CRF55_01B, CRF68_01B, CRF67_01B, CRF119_0107 and unique recombinant forms (URFs) (Fig. 1). The three URFs were one Subtype A1/CRF68_01B/CRF01_AE, one CRF07_BC/CRF57_BC/CRF65_CPX and one Subtype C/CRF08_BC/CRF65_CPX, respectively (Additional file 2).

HIV-1 molecular transmission network analysis

Additional file 3 showed that a threshold of 1.1% was optimal for constructing a molecular transmission network given sensitivity analysis of GD thresholds ranging from 0.25 to 1.5%. A total of 176 sequences were included the network, with a clustering rate of 46.1% (176/382). The network consisted of 44 molecular transmission clusters, 176 nodes, and 1140 edges. The size of molecular clusters ranged from 2 to 33 nodes. The CRF07_BC strain exhibited the most clusters, forming a total of 19 clusters, with a clustering rate of 49.0%, and the median degree value for corresponding nodes was 4 (IQR: 1–14). Of note, a CRF07_BC molecular cluster comprised 33 nodes, making it the largest in the entire network. CRF01_AE strain formed 15 clusters in total, with a clustering rate of 37.9%, and the median degree value for corresponding nodes was 2 (IQR: 1–9). Although only 26 persons were infected with CRF08_BC, 5 clusters were formed, with the highest clustering rate of 69.2% (Table 1, Fig. 2).

Impressively, the clustering rate of older people infected through commercial heterosexual contact was

as high as 61.7% and a total of three FSWs were found to be distributed in three transmission clusters (Fig. 2). The cluster 2 consisted of 1 FSW and 13 older persons, with the median degree of 10.5, and 13 of these persons lived in Longpao Town and the rest one's registered residence was also Longpao Town. The cluster 4 consisted 1 FSW and 8 older persons, with the median degree of 2, and 88.9% (8/9) of these persons lived in Jiangbei New District and the neighboring Luhe District. The other cluster only consisted of 1 FSW and an older person, and both of them lived in Jiangbei New District. Additionally, the pilot in-depth interview conducted in Luhe District found that 4 elderly male clients within the cluster 2 had engaged in risky behaviors with the FSW within the same cluster. (Fig. 2). Taken together, these results suggested that commercial heterosexual contact might play an important role in HIV transmission among older people.

Factors associated with clustering

Univariate analyses showed that there were significant differences between different age groups, education degrees, current addresses, occupations, transmission routes, screening sources, numbers of non-marital heterosexual partners, numbers of homosexual partners, and subtypes ($P < 0.05$). Multivariate logistic regression analysis revealed that compared with homosexual contact, commercial heterosexual contact (OR = 2.295, 95% CI 1.260–4.183) was more likely to cluster within the network. Compared with the older people aged between 50 and 59, those aged 60–69 years (OR = 2.029, 95% CI 1.127–3.656) and those aged ≥ 70 years (OR = 3.467, 95% CI 1.607–7.482) were more likely to cluster. Moreover, compared with the older people who were with primary school degree or below, those with a college degree or above (OR = 0.356, 95% CI 0.130–0.974) were less likely to cluster. Additionally, compared with farmers, retirees (OR = 0.361, 95% CI 0.180–0.725) or other occupations (OR = 0.498, 95% CI 0.267–0.928) were less likely to cluster (Table 1). Further comparative analysis of older people between the rural area and urban area showed that farmer (39.0% vs 6.0%, $\chi^2 = 54.760$, $P < 0.001$), primary school degree or below (49.8% vs 14.1%, $\chi^2 = 70.024$, $P < 0.001$), and commercial heterosexual contact (36.5% vs 14.8%, $\chi^2 = 52.502$, $P < 0.001$) accounted for a significantly higher proportion in rural area.

Analysis of large transmission clusters

We further analyzed the features of three large transmission clusters consisting of more than 10 nodes, including two CRF07_BC clusters and one CRF01_AE cluster (Fig. 2). These clusters were composed of 33, 14, and 13 cases, respectively, accounting for 34.1% (60/176) of all the clustered cases. The large molecular clusters

Table 1 Analysis of factors associated with clustering in molecular network of older people with HIV in Nanjing

Variables	Total (%)	Clustering (%)	Chi-square Test		Multivariate analysis	
			χ^2	P value	aOR(95% CI)	P value
Gender						
Male	318 (83.2)	140 (44.0)	3.205	0.073		
Female	64 (16.8)	36 (56.3)				
Age group (yrs)						
50~59	203 (53.1)	72 (35.5)	22.299	<0.001	1.000	
60~69	124 (32.5)	67 (54.0)			2.029(1.127 – 3.656)	0.018
≥ 70	55 (14.4)	37 (67.3)			3.467(1.607 – 7.482)	0.002
Marital status						
Single	26 (6.8)	15 (57.7)	2.123	0.346		
Married	274 (71.7)	127 (46.4)				
Divorced/widowed	82 (21.5)	34 (41.5)				
Current address						
Urban area	151 (39.5)	49 (32.5)	18.652	<0.001		
Rural area	231 (60.5)	127 (55.0)				
Occupation						
Farmers	100 (26.2)	68 (68.0)	26.267	<0.001	1.000	
Retiree	102 (26.7)	40 (39.2)			0.361(0.180 – 0.725)	0.004
Others	180 (47.1)	68 (37.8)			0.498(0.267 – 0.928)	0.028
Education degree						
Primary school degree or below	137 (35.9)	83 (60.6)	30.099	<0.001	1.000	
Junior	113 (29.6)	55 (48.7)			1.133(0.628 – 2.046)	0.678
Senior	94 (24.6)	31 (33.0)			0.668(0.347 – 1.283)	0.226
College or above	38 (9.9)	7 (18.4)			0.356(0.130 – 0.974)	0.044
Transmission route						
Homosexual	133 (34.8)	40 (30.1)	23.495	<0.001	1	
Non-commercial heterosexual	138 (36.1)	70 (50.7)			1.502(0.854 – 2.639)	0.158
Commercial heterosexual	107 (28.0)	66 (61.7)			2.295(1.260 – 4.183)	0.007
Others	4 (1.0)	0 (0)			-	0.999
Screening source						
VCT	92 (24.1)	37 (40.2)	6.666	0.083		
STD clinic	44 (11.6)	18 (40.9)				
Medical institution	221 (57.9)	104 (47.1)				
Others	25 (6.5)	17 (68.0)				
Number of non-marital heterosexual partners						
0	182 (47.6)	71 (39.0)	12.744	0.002		
1–2	144 (37.7)	68 (47.2)				
≥ 3	56 (14.7)	37 (66.1)				
Number of homosexual partners						
0	249 (65.2)	136 (54.6)	21.034	<0.001		
1–2	62 (16.2)	19 (30.6)				
≥ 3	71 (18.6)	21 (29.6)				
The first CD4+T cells (cells/ μ l)						
< 200	155 (40.6)	63 (40.6)	3.479	0.176		
200–499	134 (35.1)	69 (51.5)				
≥ 500	93 (24.3)	44 (47.3)				
The first viral load before ART (copies/ml)						
< 10,000	55 (14.4)	18 (32.7)	5.110	0.078		
10,000~99,999	188 (49.2)	94 (50.0)				

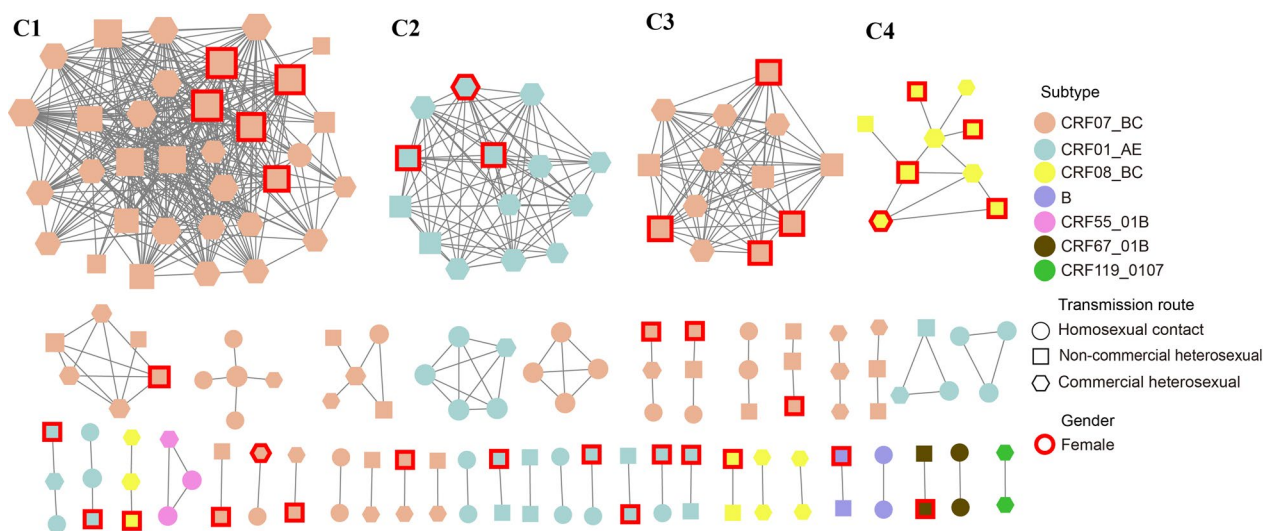


Fig. 2 The HIV-1 molecular transmission network of older people with HIV in Nanjing

proportion of nodes with a degree value ≥ 10 accounted for 84.8% (28/33), 78.6% (11/14) and 92.3% (12/13), respectively (Fig. 2).

Spatial analysis of HIV-1 molecular network and large clusters

The clustering rate and clustered cases in the HIV transmission network for older people varied in geographical distribution. The rural towns of Yongning in Pukou District, Longpao in Luhe District, and Jiangning in Jiangning District, which had more clustered cases (19, 16, 11), also had higher clustering rates (90.5, 100, 68.8%). The standardized clustering rate was calculated according to the age composition of participants in each town, and then the spatial autocorrelation analysis was carried out using the standardized clustering rate. In the geographical space, the global Moran’s I value was 0.206 ($P < 0.001$), indicating that there was a positive spatial correlation of the clustering rate at the town level in Nanjing. Regarding the spatial clustering pattern, the towns of Longpao, Xiongzhou, Changlu, Longchi, Tangquan and Xindian had a significant high-high clustering pattern. These above towns were distributed in Luhe District, Jiangbei New District and Pukou District, all in north of the Yangtze River. The Pancheng Town showed a significant low-high clustering pattern. Also, the towns of Qilin, Xiaolingwei, and Daguanglu showed a significant high-low clustering pattern (Fig. 3).

Further analysis on the spatial distribution characteristics of three large transmission clusters indicated that they were mainly concentrated in rural regions, such as Pukou District (38.3%, 23/60), Luhe District (21.6%, 13/60), Jiangning District (20.0%, 12/60), and Jiangbei

New District (13.3%, 8/60). Cluster 1 (C1) was primarily distributed in Yongning Town in Pukou District and Taishan Town in Jiangbei New District. A few cases were distributed in Xindian Town and Tangquan Town both in Pukou District, and Jiangpu Town and Yanjiang Town both in Jiangbei New District. In addition, two cases spread cross-regionally to Jianye District and Yuhuatai District. All the cases within cluster 2 (C2) lived in Longpao Town, except for one case whose registered residence was Longpao Town and cross-regionally transmitted to the geographically distant Moling Town. Cluster 3 (C3) was mainly concentrated on Jiangning Town and adjacent Guli Town both in Jiangning District, with two cases cross-regional transmitting to Qixia District and the neighboring Yuhuatai District (Fig. 4).

Discussion

In this study, we found that the major strains among the older people with HIV were CRF07_BC and CRF01_AE in Nanjing, which was consistent with the previous reports of the surrounding areas such as Shaoxing City in Zhejiang Province [24] and Pudong New Area in Shanghai [25]. We also found a variety of low epidemic strains: CRF08_BC, B, CRF55_01B, CRF68_01B, CRF67_01B, CRF119_0107 and URFs. CRF119_0107, as a second-generation recombinant strain of CRF01_AE and CRF07_BC, was first reported in the MSM in Nanjing [26]. Here, for the first time, we found that CRF119_0107 had spread from the MSM to older people and even heterosexual individuals. These results indicated that the diversity and complexity of HIV-1 strain among the older people posed a huge challenge for HIV prevention and control.

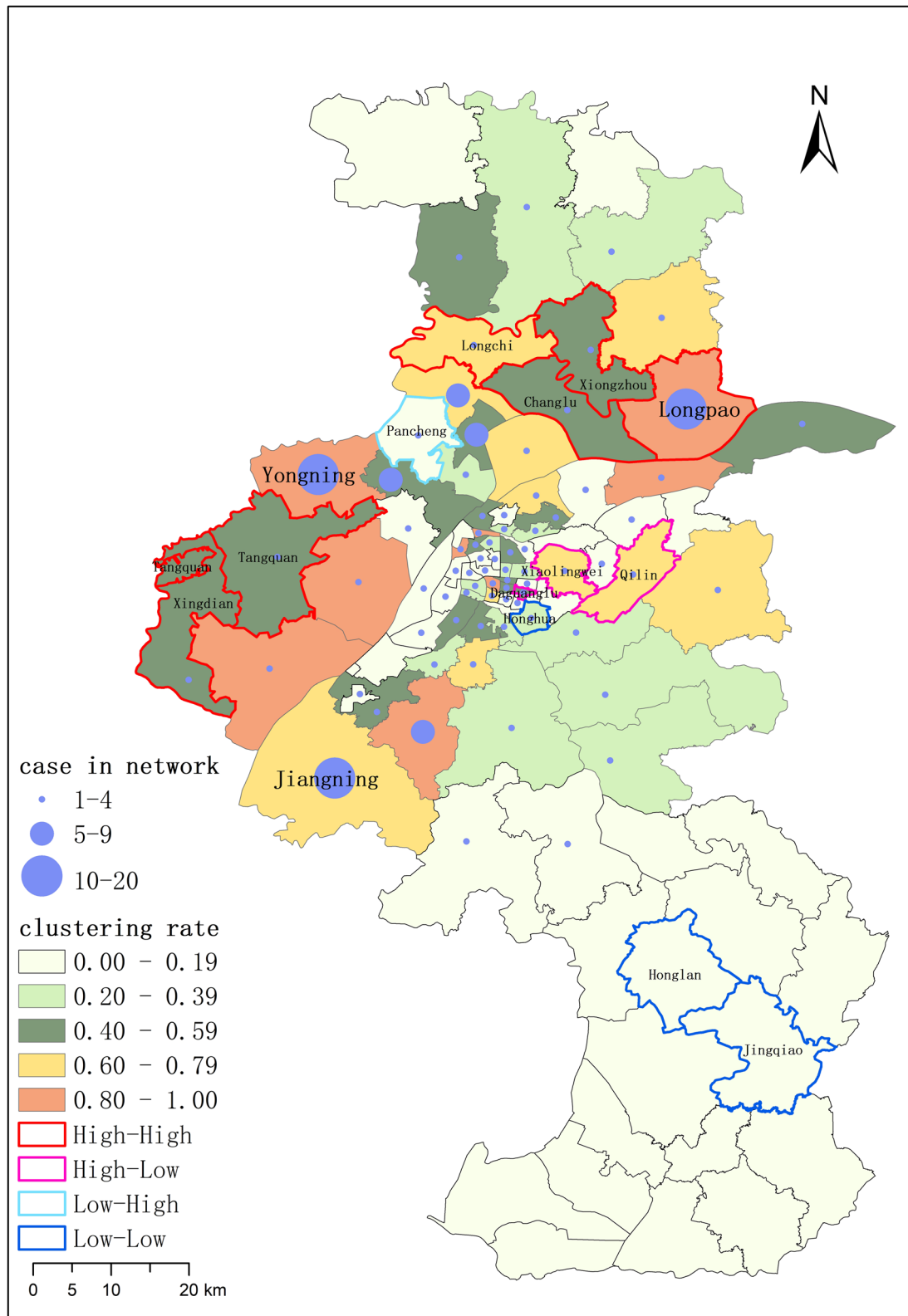


Fig. 3 The spatial distribution graph of molecular transmission network of older people with HIV in Nanjing

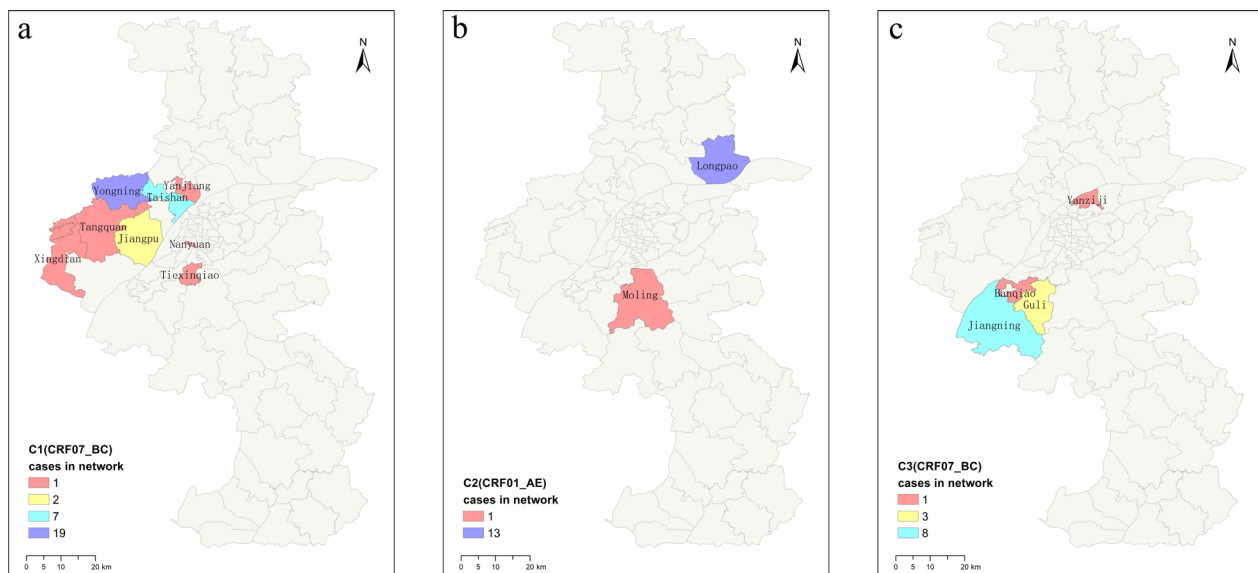


Fig. 4 The spatial distribution graph of three large transmission clusters of older people with HIV in Nanjing. **a** Spatial distribution of cluster 1, **b** Spatial distribution of cluster 2, **c** Spatial distribution of cluster 3

During the five years, three large transmission clusters formed in the network, accounting for over one-third of all the clustered cases. It implied that the overall epidemic trend of HIV-1 among older people was not sporadic, but with a certain aggregation. Furthermore, our study illustrated that the clustering rate among the older people infected through commercial heterosexual contact was more than two times higher than those through homosexual contact. Previous researches have demonstrated that commercial sexual contact acted as a major risk factor for HIV infection among older people [16, 27]. Due to lack of companionship and support from spouses or children, older people often meet psychological and emotional fulfillment through pursuing commercial sexual services [28]. Simultaneously, because of poor awareness of active detection, they have a high rate of late detection [29, 30], which further increases the risk of HIV transmission. In our current study, more than three in five older adults who were infected through commercial heterosexual contact entered the network. Additionally, three FSWs were observed in the network and there was a correlation between the location of these FSWs and the geographical aggregation of corresponding molecular clusters. These findings indicated that commercial sex could play an important role in HIV transmission network, and the older people could also play a bridge role in spread of HIV epidemic from at-risk population to their spouses or the general population.

Our study also revealed that the older the individuals were, the more likely they enter the network, possibly because the older they were, the less inter-regional

mobility they were, the more likely they cause intra-regional transmission of HIV. As for education, older people with a college degree or above were less likely to cluster. Older people with lower education degree usually had weak knowledge of HIV prevention, poor self-protection awareness and low rate of condoms use, so they were prone to engage in risky behaviors, resulting in a higher risk of HIV infection and transmission [28, 31, 32]. In terms of occupation, farmers had a higher risk of clustering, which was consistent with the results reported in Pengzhou City in Sichuan Province [33]. This may be because farmers usually live in rural areas with relatively scarce health resources, and have limited access to health services. Hence, more health service such as publicity on AIDS prevention and treatment, protective skill and HIV testing should be provided to the older farmers [34, 35].

Combining molecular and spatial epidemiology analysis, for the first time, our study found that the HIV-1 clustering rate of the older people showed a significant spatial aggregation at the town level. Among the newly reported HIV infection, the clustering rate and the number of clustered cases were higher, indicating that HIV transmission in this area was more active [36, 37]. We found that the areas with a high clustering rate and more clustered cases were mainly concentrated in rural areas, especially in the towns of Yongning, Longpao and Jiangning. This finding illustrated that HIV transmission among older adults was more active in rural areas. Further analysis showed that the older people in rural areas had lower education level and more farmers, which also explained the relatively active HIV transmission in rural areas. Therefore,

it necessary to allocate government input reasonably in prevention for older people and direct health resources toward rural regions of high aggregation. A “County-Township-Village” HIV prevention and control intervention mode could be applied to carrying out HIV publicity based on local cultural characteristics, expanding HIV testing, implementing the whole-process management and treatment mode [38]. This will gradually interrupt the transmission of HIV and reduce the new infection of older people.

We identified three large molecular clusters, in which more than a half were infected through commercial heterosexual contact, further highlighting the important role of commercial heterosexual contact in HIV transmission. Fortunately, the large molecular clusters mainly spread in the intra-region of certain rural towns of Pukou District, Luhe District, Jiangning District and Jiangbei New District. The above four districts were mainly rural area, and three of them except for Jiangning were adjacent and all located in the north of the Yangtze River. Previous studies highlighted that the low-end commercial service places, which mainly served the older males, often appeared in the rural area [16, 39, 40], resulting in continuous spread of HIV in the local area. Therefore, it was essential to conduct in-depth interviews among the clustered cases in the above areas, and find the critical persons accelerating the spread of HIV. Future prevention and treatment strategy such as increasing the frequency of condom use or HIV testing, application of post-exposure prophylaxis (PEP) or pre-exposure prophylaxis (PrEP) for at-risk individuals, and rapid initiation of ART should take clusters or critical persons as entry points. Meanwhile, dynamic surveillance of transmission network should be strengthened, and response to the clusters should be timely adjusted. This will improve prevention and control efficiency under limited resource and contain the expansion of clusters. Besides, there was a warning sign that a very few cases in large molecular clusters had spread to towns in other districts, which implied that cross-regional transmission had started to occur. Therefore, the above-mentioned health sectors may increase information exchange and jointly launch intervention, so as to reduce cross-region HIV transmission in Nanjing [36].

This study showed how molecular transmission network analysis and spatial analyses in combination could be used to disentangle epidemiology of HIV at the local level, to address specific populations and produce useful information in prevention and control efforts. However, there were several limitations in our study. First, this study couldn't obtain the sequences of all older people newly diagnosed with HIV. So our future studies should increase sampling efforts as possible. Only then can we conduct separate analysis for each subtype to further

enhance the validity of the results. Second, we didn't assess transmission dynamics and dynamical change of spatial characteristics over time. Long-term observation studies are needed to gain more information on dynamical HIV transmission patterns. Third, our results only show a representation of the HIV epidemic in Nanjing. Although HIV infections of older people are also increasing in different regions in China, the circumstances may vary from region to region. Therefore, future studies should pay attention to HIV transmission among older people in a larger geographical context because a larger dataset of wider geographical area would offer further insight.

Conclusions

Understanding transmission pattern and geographic distribution of the HIV-1 strain is essential to target limited health resources precisely to the region of most needed and to guide decision-making [41]. This study used interdisciplinary approaches to reveal the spatial aggregation of HIV transmission and highlight that towns of high aggregation are mainly located in rural area. Notably, commercial heterosexual contact played an important role in HIV transmission among older people. Future prevention and treatment strategy for older people will consider highly aggregated towns in rural area and take critical persons as entry points in order to develop precise intervention in the local HIV context. Meanwhile, continuous monitor and rapid response to the network should be strengthened to reduce further HIV transmission among older people.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12985-024-02493-w>.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.

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Author contributions

ZZ and YX conceived and designed the study. YX, TJ, and HS analyzed and wrote the manuscript. MQ provided laboratory supports. XL, SW, XY, RW and JW performed the epidemiology survey and data collection. LJ assisted with data cleaning and paper revision. All authors reviewed the final manuscript.

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Availability of data and materials

All sequences in this study have been deposited in the GenBase in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformatics. The accession numbers can be obtained in additional file 4. All sequences can be found online at <https://ngdc.cncb.ac.cn/genbase>.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of the Nanjing Center for Disease Control and Prevention (Approval No: PJ2020-A001-03). Participants provided written informed consent to participate in this study.

Competing interests

The authors declare no competing interests.

Consent for publication

Not applicable.

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