Molecular investigation of a new HIV-1 outbreak among people who inject drugs in Greece: evidence for a dense network of HIV-1 transmission

Evangelia Georgia Kostaki ,[1] Evangelia Papadimitriou,[2] Fani Chatzopoulou,[2] Sotirios Roussos,[1] Efrosini Tsirogianni,[3,4] Mina Psichogiou,[5] Ioannis Goulis,[3] Georgios Kalamitsis,[6] Anastasia Maria Kefala,[7] Lemonia Skoura,[2] Theofilos Chrysanthidis,[8] Symeon Metallidis,[8] Chrysa Tsiara,[9] Dimitra Paraskeva,[9] Gkikas Magiorkinis,[1] Apostolos Beloukas ,[7] Angelos Hatzakis,[1,10] Vana Sypsa,[1] Dimitrios Chatzidimitriou,[2] Dimitrios Paraskevis[1]

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For numbered affiliations see end of article.

Correspondence to
Prof Dimitrios Paraskevis; dparask@med.uoa.gr

DC and DP contributed equally.

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ABSTRACT

Objectives An HIV-1 outbreak was identified among people who inject drugs (PWID) in Thessaloniki, Greece, during 2019–2021. We aimed to investigate the characteristics of this outbreak by means of molecular epidemiology.

Methods We analysed 57 sequences from PWID sampled in Thessaloniki during 2019–2023. Phylogenetic trees were inferred using all subtype A sequences from PWID sampled since 1999 in Greece and reference sequences (n=4824). Phylodynamic analysis was performed using the Bayesian birth-death skyline serial model.

Results Most of the 57 study sequences belonged to sub-subtypes A6 (49, 86%) and A1 (4, 7%). Phylogenetic analysis revealed that two (50%) A1 sequences clustered together and 47 (95.9%) A6 sequences fell within three PWID-specific phylogenetic clusters. The 99.6% and 77.9% of pairwise genetic distances within the largest and second largest PWID clusters were lower than 0.015 substitutions/site. Using a more stringent threshold (0.0015 substitutions/site), we identified five networks of sequences from PWID infected within 1 year. The effective reproduction number (R_e) started to increase at the beginning of 2019 and remained high almost until the end of 2021. The estimated time from HIV-1 infection to diagnosis showed an increasing trend during 2020–2023 (p<0.001).

Conclusions The regional clustering of the PWID sequences and their low genetic divergence confirm its local spreading and the recent nature of the outbreak. Using a stringent genetic distance threshold, we showed that HIV-1 transmission occurred among large groups of PWID. The time of epidemic growth coincided with the time of the initial identification, and HIV-1 transmission continued at high rates until 2021.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The study aimed to estimate the characteristics of a new HIV-1 outbreak among people who inject drugs (PWID) identified during an intervention for PWID in 2019–2021 in Thessaloniki, Greece, using molecular epidemiology.

WHAT THIS STUDY ADDS

⇒ The analysis showed high levels of regional clustering of the sub-subtype A6 and A1 PWID sequences, with a high percentage of pairwise genetic distances within the A6 PWID clusters being lower than 0.015 substitutions/site, indicating a close epidemiological link between people infected within the clusters.

⇒ The analysis also identified that the exponential growth of the epidemic (Re>1) occurred at the beginning of 2019 and lasted until the end of 2021, and the time interval between HIV-1 infection and diagnosis showed an increasing trend over time; both highlighting the recent nature of the outbreak.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study is one of the few that combined different molecular epidemiology methods and through a detailed analysis showed high levels of transmission networking among PWID at the early stage of the outbreak.

⇒ It demonstrated that the transmission rate has decreased since 2022 due to intervention and harm reduction programme implementation, highlighting the added value of implementing molecular epidemiology methods for outbreak investigation.

INTRODUCTION

Injecting drug use remains an important risk factor for HIV acquisition.[1] According to the European Centre for Disease Prevention and Control, 2022 data showed that transmission due to injecting drug use accounted for 4.3% of HIV diagnoses in the European Union and European Economic Area (EU/EEA).[2] Although the proportion of HIV diagnoses reported among people who inject drugs (PWID) in the EU/EEA decreased from 7.7% to 4.7% during 2013–2021, an increase was observed in 2022 (5.9%).[2]

Local HIV outbreaks linked to injecting drug use have been documented in the EU/EEA during the last 15 years.[3–13] Among these, two were reported in Greece.[9–13] Athens, the capital city of Greece, experienced a large HIV-1 outbreak among PWID during 2011–2013,[10 11] which was marked as the largest recent HIV outbreak in PWID in Europe and North America.[14] Molecular epidemiology studies revealed that most transmissions in PWID occurred within four phylogenetic clusters classified as sub-subtype A1, subtype B, CRF14_BG and CRF35_AD.[10 11] HIV-1 incidence decreased rapidly from 7.8 new infections/100 person-years in 2012 to 1.7 in 2013,[12] and a significant reduction in the number of HIV-1 infections was documented in all four clusters after 2013 due to the intervention and harm reduction programmes that took place in Athens for the control of the outbreak ('ARISTOTLE').[15] However, recent studies revealed ongoing HIV-1 transmission among PWID during 2014–2020.[16 17] Furthermore, a new phylogenetic cluster in PWID classified as sub-subtype A6 was identified for those diagnosed during 2016–2019.[17]

During an intervention for PWID (community-based programme 'ALEXANDROS') in 2019–2021 in Thessaloniki, the second largest city in Greece, a new HIV-1 outbreak among PWID was identified.[13] Among PWID recruited, only 4.8% of those injecting in the past 12 months had received syringes in that period. The HIV prevalence was estimated at 7.0% (95% CI 5.6% to 8.7%) with an increasing trend over 2019–2021, and the HIV incidence was estimated at 7.0 new infections/100 person-years (95% CI 4.8 to 10.2). Furthermore, homelessness and syringe sharing were associated with an increased risk of HIV acquisition.[13]

Our aim was to estimate the characteristics of this new HIV-1 outbreak identified among PWID in Thessaloniki, using molecular epidemiology. Specifically, we aimed to investigate the patterns of viral spread among PWID, to identify any phylogenetic clusters or genetic distance networks among them, to estimate the time to the most recent common ancestor (t_{MRCA}) of the PWID clusters, the effective reproduction number (R_e) over time and the time interval from HIV-1 infection to diagnosis.

MATERIALS AND METHODS

Study sample

We analysed 57 sequences from PWID available in the *pol* gene (PR/RT) sampled in Thessaloniki during 2019–2023 (2019: two, 2020: 13, 2021: 11, 2022: 22, 2023: nine). The data were derived from the National AIDS Reference Centre of Northern Greece in Thessaloniki. Injecting drug use was self-reported by individuals before their testing for HIV, and this information was recorded in the context of the national HIV-1 surveillance system. Alternatively, this information was provided by their attending physician at the corresponding Infectious Diseases Clinic.

HIV-1 subtyping, phylogenetic and genetic distances analyses

Study sequences were subtyped using online automated HIV-1 subtyping tools (COMET,[18] REGA, http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/).

Phylogenetic analysis was performed to investigate the patterns of viral spread among PWID in Thessaloniki by using an approximately maximum likelihood method. Given that the vast majority (n=53, 93%) of the study sequences was found to belong to subtype A, a phylogenetic tree was inferred using all the available subtype A sequences from PWID sampled in Greece (ie, 108 sequences sampled during 1999–2023 in Athens and 93 sequences sampled during 2000–2023 in Thessaloniki) and a high number of reference sequences (n=4623). Also, the pairwise genetic distances were estimated within each one of the two larger sub-subtype A6 PWID clusters identified by phylogenetic analysis, and two different thresholds of 0.015 and 0.0015 substitutions per site were used for the distances. The details of the phylogenetic and genetic distances analyses are provided in online supplemental information.

Molecular clock and phylodynamic analyses

Molecular clock and phylodynamic analyses were conducted on sub-subtype A6 sequences obtained between 2009 and 2023 fell within a large monophyletic cluster in Thessaloniki identified by phylogenetic analysis. This cluster also included three A6 PWID phylogenetic clusters. Molecular clock analysis was used to estimate the t_{MRCA} of the three A6 PWID clusters and phylodynamic analysis to estimate the R_e over time (online supplemental information). Moreover, we estimated the time interval between HIV infection and diagnosis dates for people diagnosed in Thessaloniki whose sequences were found within the large monophyletic cluster in Thessaloniki. Further details about the methods used are provided in online supplemental information.

Estimation of HIV drug resistance

The prevalence of resistance-associated mutations (RAMs) and levels of associated resistance were estimated on PR and RT sequences using the HIVdb program available on the Stanford University HIV drug resistance database assessed on 29 March 2025 (http://hivdb.stanford.edu/).

Statistical analysis

A non-parametric test for trend (Cuzick's test with rank scores) was used to assess whether there was a temporal trend in the interval between HIV-1 infection and diagnosis dates. Statistical analysis was performed using Stata 14.2 (StataCorp, College Station, Texas, USA).[19]

RESULTS

HIV-1 subtyping results

Subtyping analysis of the 57 PWID sequences sampled recently in Thessaloniki (study sample) revealed that most sequences belonged to sub-subtype A6 (n=49, 86%). The rest of the sequences were classified into sub-subtype A1 (n=4, 7.0%), CRF35_AD (n=2, 3.4%) and subtypes B (n=1, 1.8%) and C (n=1, 1.8%).

Patterns of HIV-1 transmission among people who inject drugs in Thessaloniki

We performed phylogenetic analysis using a large set of references to investigate the spread of sub-subtypes A6 and A1 among PWID in Thessaloniki and to estimate the levels of regional transmission. Analysis revealed that two of four (50%) A1 sequences clustered together, and almost all A6 sequences (47 of 49, 95.9%) fell within three phylogenetic clusters (figure 1). The two larger clusters consisted of 14 and 31 PWID sequences, and two and six non-PWID sequences from Thessaloniki, respectively. The smaller cluster included two PWID and one non-PWID sequence from Thessaloniki. These clusters thereafter will be referred to as PWID clusters. The total size of the three PWID clusters, including also sequences sampled in Athens, was three, 17 and 40 sequences. These three groups of recently sampled A6 sequences from PWID were part of a larger monophyletic cluster from Thessaloniki, which also included five PWID sequences

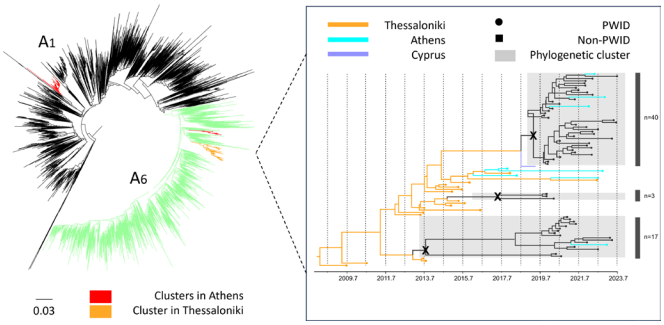

Figure 1 Unrooted phylogenetic tree inferred by using an approximately maximum likelihood method of HIV-1 subtype A sequences from people who inject drugs (PWID) sampled in Athens and Thessaloniki, Greece, and sequences from other geographic regions around the world (a global reference dataset of sequences). Sub-subtype A6 sequences are marked in light green. Clustered sequences from Thessaloniki and Athens are marked in orange and red, respectively. An enlarged view of the dated tree inferred by using the Bayesian birth-death skyline serial model shows the sub-subtype A6 monophyletic cluster consisting of sequences sampled in Thessaloniki, Athens, and Cyprus. Circle shape indicates PWID, and square shape indicates non-PWID. Grey squares indicate the three PWID phylogenetic clusters. X symbol indicates the time to most recent common ancestor (t_{MRCA}) for each one of the three clusters. Tree visualisation and annotation were performed using the FigTree V.1.4 program (http://tree.bio.ed.ac.uk/software/figtree/).

obtained between 2010 and 2015 and 12 non-PWID sequences from Thessaloniki (figure 1). In addition, a few sequences from Athens (four from PWID and four from non-PWID) and one from Cyprus belonged to this monophyletic cluster. Regarding the sequences from Athens, four of eight fell within the two larger PWID clusters and were sampled during 2022–2023. The single A1 and the three A6 PWID clusters identified in this analysis were distinct from the A1 and A6 PWID clusters previously identified in Athens (figure 1).

HIV-1 genetic distances within the sub-subtype A6 PWID clusters

Pairwise genetic distances were estimated for the sequences within the two larger sub-subtype A6 PWID clusters (separately for each cluster) to identify pairs of sequences with a distance below 0.015 or 0.0015 substitutions/site. These pairs would indicate people with potentially epidemiological links infected within 10 years (distance ≤0.015 substitutions/site) or approximately within 1 year (distance ≤0.0015 substitutions/site).

Analysis revealed that 99.6% (777 of 780) of the pairwise distances within the largest PWID cluster (n=40) and 77.9% (106 of 136) of the corresponding distances within the second largest PWID cluster (n=17) were lower than 0.015 substitutions/site (online supplemental figure 1, online supplemental data). Notably, for both clusters, almost all pairs of sequences had a genetic distance lower than 0.015 substitutions/site (figures 2A and 3A, online supplemental data). Distances were higher than 0.015 substitutions/site only for three pairs of sequences of the largest PWID cluster and for pairs including two sequences of the second largest PWID cluster (figures 2A and 3A, online supplemental data).

Regarding the stringent threshold of 0.0015 substitutions/site, within the largest PWID cluster of 40 sequences, for 17.8% (139 of 780) of the pairs, the genetic distance was below this

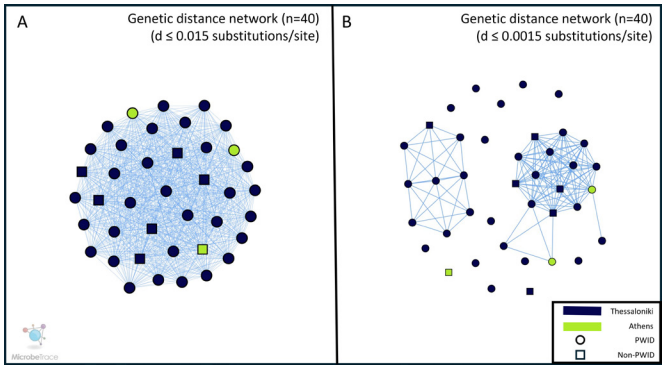

Figure 2 HIV-1 distance network of 40 people infected within the largest sub-subtype A6 phylogenetic cluster of people who inject drugs (PWID) visualised by MicrobeTrace V.0.9.0 web application,[36] with a genetic distance threshold of (A) 0.015 substitutions/site and (B) 0.0015 substitutions/site. The place of diagnosis and mode of HIV acquisition have been mapped to node colour and shape, respectively.

threshold, including two networks of 17 and nine sequences, respectively (figure 2B, online supplemental data). In the larger network (n=17), which included two sequences from PWID sampled in Athens and four non-PWID sequences, 71.3% (97 of 136) of the pairwise genetic distances were lower than 0.0015 substitutions/site. Ten PWID and four non-PWID were the most densely connected within the network, with the pairwise genetic distances being lower than the threshold for at least 13 of the 16 connections per person (figure 2B, online supplemental data). The least connected PWID included one from Athens diagnosed in 2022 and two from Thessaloniki diagnosed in 2022 and 2023 (figure 2B, online supplemental data). In the network of nine sequences, 75% (27 of 36) of the pairwise genetic distances were below 0.0015 substitutions/site, with three sequences from PWID having distances lower than the specified threshold with all other sequences in the network (figure 2B, online supplemental data).

For the second largest PWID cluster of 17 sequences, the genetic distance was below 0.0015 substitutions/site for 24.3% (33 of 136) of the pairs. Three networks of nine, four and two sequences were identified, with the distance being lower than the threshold for 81%, 50% and 100% of the pairs within each network, respectively (figure 3B, online supplemental data).

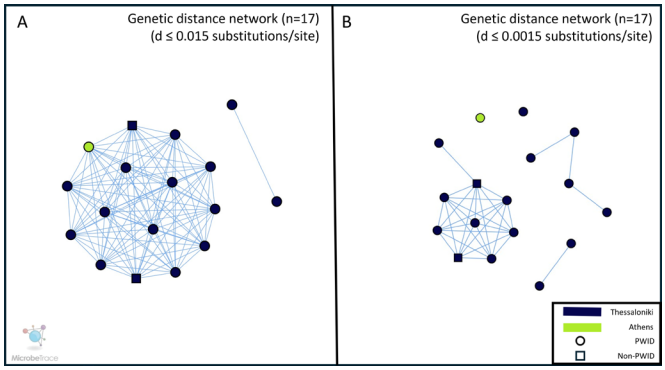

Figure 3 HIV-1 distance network of 17 people infected within the second larger sub-subtype A6 phylogenetic cluster of people who inject drugs (PWID) visualised by MicrobeTrace V.0.9.0 web application,[36] with a genetic distance threshold of (A) 0.015 substitutions/site and (B) 0.0015 substitutions/site. The place of diagnosis and mode of HIV acquisition have been mapped to node colour and shape, respectively.

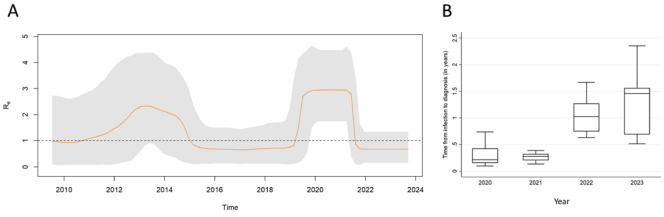

Figure 4 (A) Birth-death skyline plot median estimates (orange line) and 95% highest posterior density intervals (grey shade) for the effective reproduction number (R_e). (B) Time interval between HIV-1 infection and diagnosis dates in years (box plot presentation) over the period 2020–2023.

In the largest network of nine cases, including two sequences from non-PWID, dense connections were detected for eight sequences, with one of them having a genetic distance lower than 0.0015 substitutions/site with all the sequences of the network (figure 3B, online supplemental data). A sequence from a PWID sampled in 2020 was an exception since it had only a single low-distance pair. In the smallest network of four sequences, only one sequence had more than one pair with lower distance than the threshold (figure 3B, online supplemental data).

Temporal characteristics and transmission dynamics of the sub-subtype A6 PWID clusters

Molecular clock analysis showed that the t_{MRCA} of the largest PWID cluster (n=40) was estimated at 4.3 years (median value; 95% highest posterior density (HPD): 3.9–5.0) corresponding to the middle of June 2019 (figure 1). The t_{MRCA} of the second largest PWID cluster (n=17) was estimated at 9.9 years (median value; 95% HPD: 9.6–10.3) corresponding to the beginning of November 2013. For the third PWID cluster (n=3), the t_{MRCA} was estimated at 6.2 years (median value; 95% HPD: 4.3–7.9) corresponding to the middle of July 2017. Moreover, the spatial origin of all three PWID clusters was from Thessaloniki (figure 1). The t_{MRCA} of the large monophyletic cluster from Thessaloniki, including the three sub-subtype A6 PWID clusters and previously sampled sequences, was estimated at 15.3 years (median value; 95% HPD: 14.0–17.0) corresponding to the end of May 2008 (figure 1).

Phylodynamic analysis showed that besides the period between 2011 and 2015, the R_e showed a large increase at the beginning of 2019 and remained high ($R_e>1$) almost until the end of 2021 (figure 4A). The estimated value of R_e during 2019–2021 was approximately equal to 3 (median estimate). The increase was followed by a steep decline from the end of 2021 until the end of the study period (September 2023) (figure 4A). Over this period, the R_e remained low (estimated <1), showing that the epidemic was contracting.

Estimated time interval between HIV-1 infection and diagnosis dates

The estimated time interval between HIV-1 infection and diagnosis dates for 55 people (PWID and non-PWID) diagnosed in Thessaloniki and infected within the large monophyletic cluster in Thessaloniki showed an increasing trend during 2020–2023 ($p<0.001$) (figure 4B). The median time from infection to diagnosis was 0.22 years in 2020 (n=19), 0.28 years in 2021 (n=13), 1.03 years in 2022 (n=17) and 1.46 years in 2023 (n=6).

Prevalence of resistance-associated mutations

The prevalence of RAMs in the PR/RT region was 14.0% (8/57) for non-nucleoside reverse transcriptase inhibitors (NNRTIs), 8.8% (5/57) for nucleotide reverse transcriptase inhibitors (N(t)RTIs) and 14.0% (8/57) for protease inhibitors (PIs). The prevalence for dual resistance for N(t)RTIs and NNRTIs was 3.5% (2/57), with the overall prevalence being 33.3% (19/57). Specifically, we detected the following RAMs for NNRTIs: E138A in 8.8% (5/57), K103N in 5.3% (3/57) and Y188H in 1.8% (1/57). For N(t)RTIs, we detected S68G in 5.3% (3/57), A62V in 1.8% (1/57) and L210W in 1.8% (1/57). For PIs, M46L was observed in 14.0% (8/57) as a major RAM. For two PWID, we detected dual RAMs: S68G/K103N and S68G/E138A for N(t)RTIs and NNRTIs. The nucleotide sequences in the integrase region were not available for the study population.

DISCUSSION

Our study showed that the vast majority (49 of 53, 92.5%) of subtype A sequences from PWID diagnosed during 2019–2023 in Thessaloniki fell within sub-subtype A6 and A1 PWID-specific clusters. Most of the sequences were obtained from PWID in this region, suggesting that HIV transmission occurred among the members of this group, most probably through injection practices. Pairwise evolutionary distances for sub-subtype A6 sequences that included the majority of PWID sequences revealed that most genetic distances were below 0.015 substitutions/site, indicating a close epidemiological link between people infected within the PWID clusters. Phylodynamic analysis showed that although the t_{MRCA} of the three PWID clusters within the A6 monophyletic cluster from Thessaloniki was inferred approximately in 2019, 2017 and 2013, the exponential growth of the epidemic, as indicated by R_e values >1, occurred at the beginning of 2019 and lasted until the end of 2021. These findings, along with the observation that the time interval between the estimated time of HIV infection and diagnosis has shown an increasing trend over time, provide evidence about the recent nature of the outbreak that ignited in 2019, and lasted until the end of 2021. Phylogenetic analysis indicated that the outbreak originated from locally circulating strains among both PWID and non-PWID in Thessaloniki.

The finding that all sequences from PWID in Thessaloniki were classified as sub-subtypes A1 and A6 poses an additional concern. Previous studies have shown that subtype A1/A6 was independently associated with increased risk for virological failure to cabotegravir+rilpivirine long-acting therapy[20 21] and thus limiting the potential therapeutic options for this population, which has been identified as a priority population for treatment including the potential for benefits of long-acting ART.[22] Furthermore, the detection of RAMs for NNRTIs in 14% of PWID with E138A identified in 8.8% of PWID further increases the risk for virological failure in rilpivirine long-acting ART regimens. The combination of subtype A1/A6 and the presence of NNRTI RAMs highlight the need for resistance testing before treatment initiation.

The substantial increase in the time from infection to diagnosis observed in 2022 and 2023, as compared with 2020 and 2021, may be attributed to the discontinuation of the 'ALEXANDROS' programme. This community-based seek-test-treat intervention was implemented in multiple rounds of respondent-driven sampling and reached 1100 PWID.[13] Two-thirds of the HIV cases identified through this programme (52/77, 67.5%) were new diagnoses.[13] Based on unpublished data, 'ALEXANDROS' has reached a significant portion of the PWID population in Thessaloniki.

Almost a decade ago, a large HIV-1 outbreak was identified in Athens starting in 2011 and lasting until 2013.[9–12] Similar to the current situation, the majority of sequences fell within four PWID phylogenetic clusters, and the exponential growth of the clusters lasted approximately 12–20 months until the start of intervention programmes targeting this population.[10 11 15] Moreover, in the Athenian outbreak, the time interval between HIV infection and diagnosis showed a similar increasing trend suggesting that the proportion of PWID with recent infection is decreasing over time and a larger proportion of newly diagnosed cases have been infected in the previous year(s).[17] Both outbreaks occurred in a setting with low coverage of needle and syringe programmes and low HIV testing levels in this population.[9 11 13] In Thessaloniki, as shown by the 'ALEXANDROS' programme, the risk for HIV acquisition was the highest for homeless PWID, a situation similar to Athens as well as outbreaks in other settings.[12–14] Although HIV transmission continues to occur in Athens in the form of a 'slow burn' outbreak,[17] the outbreak in Thessaloniki originated from locally circulating strains.

During the last 10 years, several HIV outbreaks have been identified in Europe including Bucharest (Romania),[3 4] Athens (Greece),[9–12] Glasgow (Scotland),[6 7] Luxembourg,[8] Dublin (Ireland)[5] and Thessaloniki (Greece),[13] and also in North America and Israel.[14 23–25] In all cases, molecular epidemiology studies showed that sequences from PWID were closely related, suggesting that transmission occurred among the members of this population.[4 7 8 10 11 15] Several studies have used genetic distance methods to identify clusters of epidemiologically linked people, specifically applying a genetic distance threshold of 0.015–0.020 substitutions/site, for the region used routinely for resistance testing (PR/RT).[7 26–29] This method has been implemented in several studies for detecting clusters and real-time monitoring of HIV transmission hot spots and enhanced public health intervention.[7 26–28 30 31] In our case of a recently identified outbreak, using the threshold of 0.015 substitutions/site for the genetic distance, we found that the vast majority of sequence pairs within the largest PWID cluster were below this threshold. Similarly, in the second largest PWID cluster, only pairwise genetic distances between different sublineages with an earlier t_{MRCA} than the starting time of the outbreak in 2019, were above this threshold.

Using a stricter threshold,[32] we identified two and three networks within the two major sub-subtype A6 PWID clusters in Thessaloniki. Notably, the majority (71%–81%) of the pairwise genetic distances were below 0.0015 substitutions/site within the three largest networks, showing low genetic divergence. Given that the selected genetic distance threshold corresponds to recently infected people, the identification of these networks is a proxy of the number of PWID infected over a short period, probably as a result of injecting practices. Five networks were identified consisting of 17, nine, nine, four and two sequences, showing that HIV transmission occurred among large groups of PWID. Moreover, in the larger networks, a high proportion of PWID showed numerous potential links with the other members, suggesting high connectivity. The large number of PWID within the networks and the dense connectivity among the networks' members suggest that HIV transmission occurred effectively among injector groups. These findings explain the high HIV transmissibility, as shown by the elevated R_e that was estimated to be approximately equal to 3. The networks provide a proxy for the characteristics of the groups where HIV transmission occurred from a common source over a short time and provide evidence about the high-risk behaviours that ignited the outbreak in Thessaloniki. Moreover, the networks included a few PWID from Athens and some non-PWID who have probably been infected sexually or whose injecting history was not accurately reported.

Our study has the limitation that included only HIV-1 sequences from PWID who were linked to care. However, our findings about the high levels of clustering among viral sequences from PWID suggest a common pattern for HIV transmission within this group. Using a larger sample, our results about the networking levels might be different regarding the size of networks of PWID infected with viruses with short pairwise genetic distances. However, our findings that HIV transmission occurred among large groups of PWID were not subject to potential sampling bias. Additionally, a potential limitation of the study was that the results were not reported in real time, but since a community-based programme was already in place, the study was not designed for real-time outbreak investigation. Although our study was not designed for the real-time detection of PWID clusters, we show how phylogenetic and phylodynamic analyses, along with genetic analysis, can be used for detecting clusters of rapid transmission. Several studies have shown that molecular epidemiology can be used for the real-time detection of rapidly expanding clusters and thus for detecting high-risk networks and transmission hotspots.[33–35] This approach has been successfully implemented for the design and implementation of targeted intervention strategies.[33–35] Given the ongoing HIV transmission among PWID, especially across settings with low coverage of harm reduction services, the use of molecular epidemiology can strengthen outbreak detection and public health measures for the control of HIV transmission among the people of this group. Furthermore, in our study, we used only the necessary data, and demographic characteristics were not requested for ethical reasons to minimise the risk of PWID identification.

Our study described in detail the characteristics of the HIV-1 outbreak among PWID in Thessaloniki, providing insights into the characteristics of HIV transmission. To our knowledge, this is one of the few studies that combined different molecular epidemiology methods and, through a detailed analysis of the pairwise genetic distances, showed the high levels of transmission networking among PWID at the early stage of the outbreak. Our findings also demonstrated that the transmission rate, as estimated by the R_e, has decreased since 2022 due to intervention and harm reduction programme implementation, highlighting the added value of implementing molecular epidemiology methods for outbreak investigation.

Author affiliations
[1]Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[2]National AIDS Reference Centre of Northern Greece, Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]4th Department of Internal Medicine, Hippokratio General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece
[4]Greek Organisation Against Drugs (OKANA), Athens, Greece
[5]1st Department of Internal Medicine, Laiko General Hospital, Medical School, National and Kapodistrian University Athens, Athens, Greece
[6]Hellenic Liver Patient Association "Prometheus", Athens, Greece
[7]Department of Biomedical Sciences, University of West Attica, Athens, Greece
[8]Infectious Diseases Division, 1st Department of Internal Medicine, AHEPA University Hospital, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece
[9]National Public Health Organization, Marousi, Greece
[10]Hellenic Scientific Society for the Study of AIDS, Sexually Transmitted Diseases and Emerging Diseases, Athens, Greece

Handling editor Michael Traeger

X Apostolos Beloukas @Beloukas

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Ethics Committees of the Medical School of the Aristotle University of Thessaloniki (protocol number: 2/2024), the Medical School of the National and Kapodistrian University of Athens (protocol numbers: 115/2019 and 1516010195/2015) and the National Public Health Organization (protocol number: 10210/2022). Retrospective and anonymised data were analysed.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available on reasonable request. The dataset corresponds to a dense sampling of people who inject drugs in Thessaloniki. To minimise the risk of identifying individuals living with HIV, sequence data will be made available on reasonable request.

ORCID iDs
Evangelia Georgia Kostaki http://orcid.org/0000-0002-3346-0930
Apostolos Beloukas http://orcid.org/0000-0001-5639-0528

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