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# Characterization of HIV Transmission in South-East Austria

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**Data Availability Statement:** All viral sequences are available from the Gen Bank database (accession numbers KU574281 - KU574539).

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## Abstract

To gain deeper insight into the epidemiology of HIV-1 transmission in South-East Austria we performed a retrospective analysis of 259 HIV-1 partial *pol* sequences obtained from unique individuals newly diagnosed with HIV infection in South-East Austria from 2008 through 2014. After quality filtering, putative transmission linkages were inferred when two sequences were  $\leq 1.5\%$  genetically different. Multiple linkages were resolved into putative transmission clusters. Further phylogenetic analyses were performed using BEAST v1.8.1. Finally, we investigated putative links between the 259 sequences from South-East Austria and all publicly available HIV polymerase sequences in the Los Alamos National Laboratory HIV sequence database. We found that 45.6% (118/259) of the sampled sequences were genetically linked with at least one other sequence from South-East Austria forming putative transmission clusters. Clustering individuals were more likely to be men who have sex with men (MSM;  $p < 0.001$ ), infected with subtype B ( $p < 0.001$ ) or subtype F ( $p = 0.02$ ). Among clustered males who reported only heterosexual (HSX) sex as an HIV risk, 47% clustered closely with MSM (either as pairs or within larger MSM clusters). One hundred and seven of the 259 sequences (41.3%) from South-East Austria had at least one putative inferred linkage with sequences from a total of 69 other countries. In conclusion, analysis of HIV-1 sequences from newly diagnosed individuals residing in South-East Austria revealed a high degree of national and international clustering mainly within MSM. Interestingly, we found that a high number of heterosexual males clustered within MSM networks, suggesting either linkage between risk groups or misrepresentation of sexual risk behaviors by subjects.

## Introduction

The poor fidelity of the HIV-1 reverse transcriptase leads to significant diversity of the viral population within infected individuals and across populations over time [1]. Viral genetic

study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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sequence information can therefore be used to reconstruct transmission networks as well as the evolutionary history of extant viruses [2,3]. Phylogeography (i.e. reconstruction of the molecular evolutionary history and the spatial diffusion of a virus) can provide clues to the dynamics of viral outbreaks [4]. Integration of molecular, clinical and demographic data offers a unique opportunity to better understand the dynamics of local transmission networks [4–6] and may help to direct intervention and prevention strategies to interrupt ongoing outbreaks, such as the one in Austria.

An estimated 9,000 HIV infected individuals (approximately two-thirds men and one-third women) are currently living in Austria, of which 7–10% live in South-East Austria [7,8]. Although the majority of HIV infected individuals (over 90%) are receiving antiretroviral therapy (ART), the annual number of new HIV diagnoses in Austria has remained stable at about 400–500 new HIV diagnoses per year during the last decade [7]. A major reason for the steady rate of incident diagnoses, despite high ART-coverage, is that despite high numbers of per-capita testing (compared to other European countries), only 20% of individuals are diagnosed with acute or recent HIV infection (defined as seroconversion, with the last negative HIV test not more than 3 years before the first positive test) [7,8]. Among the most important factors associated with late HIV diagnosis (defined as CD4 cell count below 350 at time of HIV diagnosis and/or AIDS within 3 months of HIV diagnosis) are reported heterosexual (HSX) sex as the mode of HIV acquisition and residing in rural areas (i.e. population size of residence below 100,000) [8,9].

To gain deeper insight into HIV transmission, this study aimed to reconstruct the local HIV-1 transmission network in South-East Austria, representing an area of more than 1 million inhabitants, and to investigate putative links with publicly available HIV *pol* sequences from around the world.

## Materials and Methods

### Study Population

The study population included 259 residents of South-East Austria with newly diagnosed HIV-1 infection between 2008 and 2014, who had initial nucleic acid amplification testing (NAT) and/or resistance testing performed at the Institute of Hygiene, Microbiology and Environmental Medicine of the Medical University in Graz. This institute represents the only laboratory performing HIV NAT in this region of more than 1 million inhabitants.

Demographic information and clinical data were retrospectively collected at the Department of Infectious Diseases, Landeskrankenhaus Graz South-West, Austria, where the vast majority of HIV patients were linked to care, and the Department of Medicine, Medical University of Graz, Austria. Data collected included age, sex, HIV risk factor(s), and area of residence for all subjects. All demographic data, except for location of residence, was collected in a de-identified manner and then linked to the unique HIV sequence.

To analyze the geographic network characteristics of viral transmission in the area, the region of South-East Austria under investigation was first divided into five areas (each including at least 15 HIV study participants, in order to reduce the risk of re-identification of participants). These areas included: Graz (i.e. the only urban area within South-East Austria, with about 300,000 inhabitants), the suburban district Graz County (later referred to as “suburban”), and the rural districts of northern Styria, western/southern Styria, and eastern Styria (which included the southern districts of the neighboring state of Burgenland).

### Sequence Analysis and HIV Network Inference

Sequences were generated using the FDA-approved and IVD/CE-labeled TRUGENE HIV-1 Genotyping Kit (Siemens Healthcare Diagnostics, Tarrytown, NY). Analysis was performed

using partial HIV-1 *pol* sequences including the regions encoding reverse transcriptase and protease. All sequences were subtyped using the Subtype Classification using Evolutionary Algorithms (SCUEAL) program [10].

All sequences were made publicly available. The GenBank accession numbers for the *pol* sequences included in this analysis are KU574281—KU574539. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the local ethics committee, Medical University of Graz. All data presented have been de-identified and are therefore not attributable to individual patients. At the participating centers, information of all patients admitted is automatically stored in the electronic hospital database, and written informed consent of participating patients was waived by the local ethics committee.

**Genetic network and clustering analysis.** After removing codons associated with major drug resistance mutations [11], putative transmission clusters were inferred by establishing linkage when two sequences had a Tamura-Nei 93 [12] genetic distance ( $D$ )  $\leq 1.5\%$ . This threshold was selected based on previous work from our group showing that within a mono-infected person, *pol* sequences typically do not diverge more than 1% from baseline over a decade [13], and this threshold is standard in the field [14]. Transmission clusters included all connected nodes such that all nodes (individuals) within a cluster would have a  $D$  of  $< 1.5\%$  from at least one other node in the cluster, but not necessarily within 1.5% of all nodes within the cluster. Demographic information and clinical data analyzed in conjunction with the inferred networks included age, sex, HIV risk factor(s) and area of diagnosis for all subjects.

Transmission clusters inferred by network analysis were confirmed using phylogenetic methods. Subtype specific maximum likelihood trees were inferred using RAxML [15] and included reference sequences from the main subtypes and CRF forms as well as a set of reference sequences from the same subtype. Clusters were confirmed if bootstrap support exceeded 80%.

**Bayesian phylogenetic and phylogeographic analyses.** Phylogenetic analyses were performed by first inferring a time-scaled maximum clade credibility tree using all 259 of the South-East Austrian HIV sequences, performed using a Bayesian Markov Chain Monte Carlo (MCMC) framework as implemented in BEAST v1.8.1 [16] with BEAGLE [17]. We used a discretized gamma distribution (GTR + 4 $\Gamma$ ) to account for among-site rate variation. Time scales of the trees were calibrated with the sampling dates available. We specified an uncorrelated log-normal (UCLN) molecular clock with a gamma distribution prior model that allows rates to vary among the branches of the inferred phylogenies to infer the timescale of HIV evolution for each subject, with a gamma distribution prior on the mean clock rate (shape = 0.001, scale = 1000). A skygrid tree prior was used as a coalescent demographic model with time-aware smoothing [18,19]. Monte Carlo simulations were run for 100 million steps, sub-sampling parameters every 50,000 steps. Convergence of the chains was inspected using Tracer v1.6. Maximum clade credibility trees were obtained with TreeAnnotator v1.8.1 and visualized by using FigTree 1.4 [20].

In order to infer the spatiotemporal dynamics of the sampled Austrian HIV epidemic ( $n = 259$  individuals), we performed a phylogeographic analysis in which we categorized individuals into two groups residing in either: 'urban areas' (defined as Graz City and suburban Graz County,  $n = 126$ ) or 'rural areas' (including all the other surrounding rural areas,  $n = 133$ ). After applying these discrete binary geographic traits ('urban' or 'rural') to each taxon, we then evaluated viral migration patterns between these regions using discrete non-reversible diffusion models and a Bayesian stochastic search variable selection (BSSVS) approach [21], as previously described [21].

**Links between the South-East Austrian Epidemic and the Global HIV Epidemic.** Finally, to better understand the intersection between the South-Eastern Austrian HIV Epidemic and

the global HIV epidemic, we applied the network-based approach described above and used the HIV Trace program developed by our colleagues at UCSD to recover all putative links between the 259 sequences from Austria and all publicly available HIV polymerase sequences in the Los Alamos National Laboratory HIV sequence database ( $n = 148,598$  sequences) [22].

## Statistical analysis

Statistical analysis was performed using SPSS, version 22 (SPSS, Inc., Chicago, IL, USA). Demographic data are displayed as absolute numbers plus percentages, medians plus inter-quartile ranges (IQR) or means plus 95% confidence intervals (95%CI), as appropriate. We compared demographic data, HIV risk factors, and HIV subtypes between individuals that clustered within the transmission network to those that did not cluster, by using two tailed Chi-squared or Fisher's exact test for categorical variables, and Mann-Whitney-U test for continuous variables, as appropriate.

## Results

### Study Population

The mean age of the study population at the time of HIV diagnosis was 34 years (range 4–73 years). Eleven individuals (3.8%) were sampled during the acute stage (HIV NAT+/Ab-) of HIV infection. All individuals were ART-naïve at the time the sequences were obtained. Demographic characteristics, risk factors, and HIV subtypes are displayed in [Table 1](#).

The majority of subjects (193/259 [75%]) were male, and 122/193 (63%) of these self-identified as men who have sex with men (MSM), while 54/193 (28%) reported only heterosexual sex as risk factor for HIV acquisition. Injection drug use (IDU) was reported by 17 (9%) males (in 15 IDU was the only risk factor, 2 had also MSM risk). In one individual each, mother to child transmission and hemophilia were reported as risk factors. Of the 54 males reporting heterosexual contact as their only HIV risk, 13 (24%) reported sex with a female from a country with >1% HIV prevalence, 11 (20%) reported origin from a country with >1% HIV prevalence, and 2 (4%) reported sex with a female known to be HIV positive, while 28 (52%) could not specify their risk, other than having heterosexual contacts.

Sixty-six of 259 (25%) subjects diagnosed with HIV were female. The vast majority (61/66) reported heterosexual contact as their only risk factor for HIV acquisition, while two females reported IDU as risk factor and three reported nosocomial transmission. Of the 61 reporting heterosexual contact as risk factor, 24 (39%) reported sex with a male from a country with >1% HIV prevalence, 4 (7%) sex with an IDU male, 2 (3%) sex with a MSM, and 4 (7%) sex with a male known to be HIV positive. The remaining 27 (44%) females could not specify their risk, other than having heterosexual contacts.

Evolution of the relative frequency of the reported risk exposure among HIV individuals from the South-East Austria Cohort between 2008 and 2014 revealed that MSM and heterosexual contact were the two major risk exposures each accounting for 40%-60% of cases each year of the study period.

### Cluster Analysis

Putative transmission linkages were inferred for 45.6% (118/259) of the sequences from South-East Austria ([Fig 1](#)). The likelihood of clustering for an individual did differ by sex ( $p = 0.002$ , males more likely to cluster), race (Caucasians more likely to cluster,  $p = 0.001$ ; Blacks less likely to cluster,  $p = 0.006$ ) but not by area of residence type (urban versus rural; [Table 1](#)). Clustering individuals were more likely to report MSM as their major risk factor ( $p < 0.001$ ) than

**Table 1. Baseline Demographic and Viral Characteristics.**

	South-East Austria 2008–2014	Individuals clustering within the network	Non-clustering individuals	<i>P</i> value
N (%)	259 (100%)	118 (46%)	141 (54%)	-
<b>Age (years)</b>				
Mean (range)	34 (4–73)	33 (17–65)	34 (4–73)	<i>n.s.</i>
Young (<24)	20.5% (n = 53)	20.3% (n = 24)	20.6% (n = 29)	<i>n.s.</i>
Older (>50)	10.4% (n = 27)	8.5% (n = 10)	12.1% (n = 17)	<i>n.s.</i>
<b>Sex</b>				
Male	74.5% (n = 193)	83.9% (n = 99)	66.7% (n = 94)	<b>0.002</b>
Female	25.5% (n = 66)	16.1% (n = 19)	33.3% (n = 47)	<b>0.002</b>
<b>Race</b>				
Caucasian	85% (n = 221)	93% (n = 110)	79% (n = 111)	<b>0.001</b>
Black	13% (n = 34)	7% (n = 8)	18% (n = 26)	<b>0.006</b>
Asian	2% (n = 4)	0	3% (n = 4)	<i>n.s.</i>
<b>Risk Factor</b>				
MSM	47.1% (n = 122)	59.3% (n = 70)	36.9% (n = 52)	<b>p &lt; 0.001</b>
HSX	44.4% (n = 115)	31.4% (n = 37)	55.3% (n = 78)	<b>p &lt; 0.001</b>
IDU†	6.6% (n = 17)	9.3% (n = 11)	4.3% (n = 6)	<i>n.s.</i>
Others/unknown	1.9% (n = 5)	0% (n = 0)	3.5% (n = 5)	<i>n.s.</i>
<b>HIV Subtype</b>				
B	66% (n = 171)	76.3% (n = 90)	57.4% (n = 81)	<b>p = 0.003</b>
CRF01_AE	12.4% (n = 32)	9.3% (n = 11)	14.9% (n = 21)	<i>n.s.</i>
CRF02_AG	6.9% (n = 18)	3.4% (n = 4)	9.9% (n = 14)	<b>p = 0.049</b>
F	5.8% (n = 15)	9.3% (n = 11)	2.8% (n = 4)	<b>p = 0.033</b>
C	5.0% (n = 13)	0% (n = 0)	9.2% (n = 13)	<b>p &lt; 0.001</b>
A	1.5% (n = 4)	0% (n = 0)	2.8% (n = 4)	<i>n.s.</i>
Others §	2.3% (n = 6)	1.7% (n = 2)	2.8% (n = 4)	<i>n.s.</i>
<b>Residency</b>				
Urban	48.6% (n = 126)	49.2% (n = 58)	48.2% (n = 68)	<i>n.s.</i>

†: Category 'IDU' also includes men who are both MSM and IDU.

§: Includes G, H, J and K subtypes.

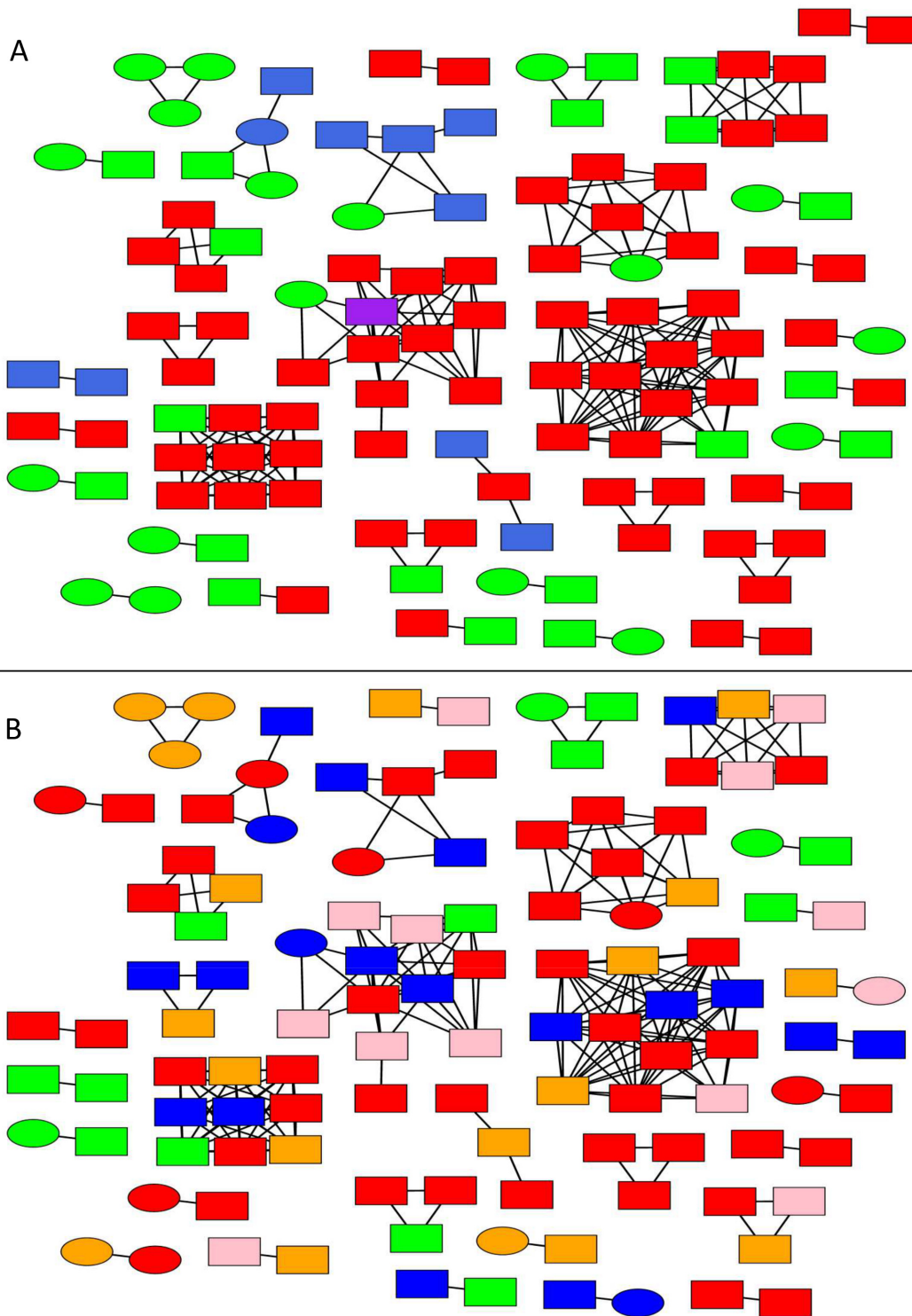
Abbreviations: CRF, circulating recombinant forms; HSX, heterosexual orientation; IDU, injection drug use; MSM, men who have sex with men; n.a., data not available; n.s., not significant.

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the individuals who did not belong to any detected cluster in this dataset. A higher prevalence of subtype B ( $p < 0.001$ ) and F ( $p = 0.033$ ) was found in clustering individuals (Table 1, Fig 2a). HIV subtypes by risk behavior are displayed in Fig 2b. We found a significantly higher proportion of MSM among individuals infected by subtype B ( $p < 0.001$ ) and a higher proportion of heterosexuals ( $p < 0.001$ ) and IDU ( $p = 0.002$ ) among non-B individuals.

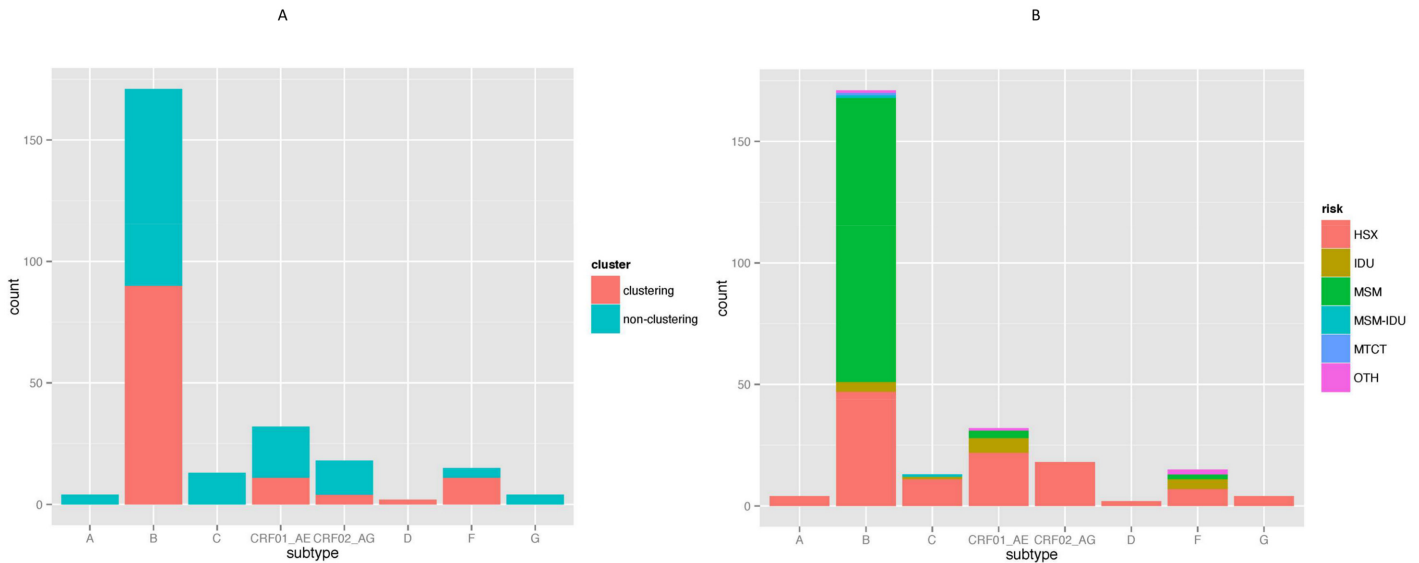
A total of 34 clusters were identified (Fig 1, Table 2). 19 of these clusters (55.9%) were linked pairs, while the remaining 15 clusters (44.1%) had three or more individuals, including two clusters each with 12 individuals. MSM were more likely to be members of larger clusters ( $>2$  individuals) than smaller clusters ( $p = 0.028$ ) (Table 2, Fig 1a). Three of the four transmission clusters that included IDU were non-B clusters, including two subtype F and one CRF01\_AE cluster (Fig 1). Maximum likelihood trees for each subtype were generated to validate the clusters identified by the network inference method above (Fig 3).

Of the clustering males that reported only HSX risk, 47% (9/19) clustered closely with MSM. Of these nine, seven reported that they were certain about heterosexual transmission



**Fig 1. Inferred HIV Transmission Clusters.** HIV-1 transmission clusters (networks) identified in the region of South-East Austria. Sex of the individuals (nodes) is indicated by shape: square (men) and circle (women) **1A**. Nodes are colored by their reported risk factor: red (men who have sex with men [MSM]), green (heterosexual), blue (injection drug use [IDU]) and purple (MSM and IDU), respectively. All edges represent a genetic distance  $\leq 1.5\%$  separating nodes. **1B**. Here, nodes are colored according to the region of residence in red (Graz City), pink (Suburban Graz), green (Eastern Styria), blue (Northern Styria) and orange (Southwestern Styria).

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**Fig 2. HIV Subtype in the South-East Austrian Cohort. 2A.** Comparison between clustering and non-clustering populations by HIV subtype. **2B.** HIV Subtype by Risk factors in the South-East Austrian Cohort. Legend: HSX, heterosexual contact; IDU, injection drug use; MSM, men who have sex with men; MTCT, mother to child transmission; OTH, other.

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without giving further information on risk, while two reported sex with a female from a high prevalence country. Five out of those nine males clustered within the four large MSM clusters. While one of these clusters included four MSM and two HSX males, the remaining three HSX males were the only non-MSM within their respective transmission clusters. The remaining four of nine males reporting HSX contact clustered as pairs ( $n = 3$ ) or triplets ( $n = 1$ ) with MSM (Fig 1a).

Using Bayesian phylogenetic methods, we then inferred the time of most recent common ancestor (tMRCA) for the three largest clusters. These three clusters, which were all subtype B

**Table 2. Characteristics of clusters according to cluster size.**

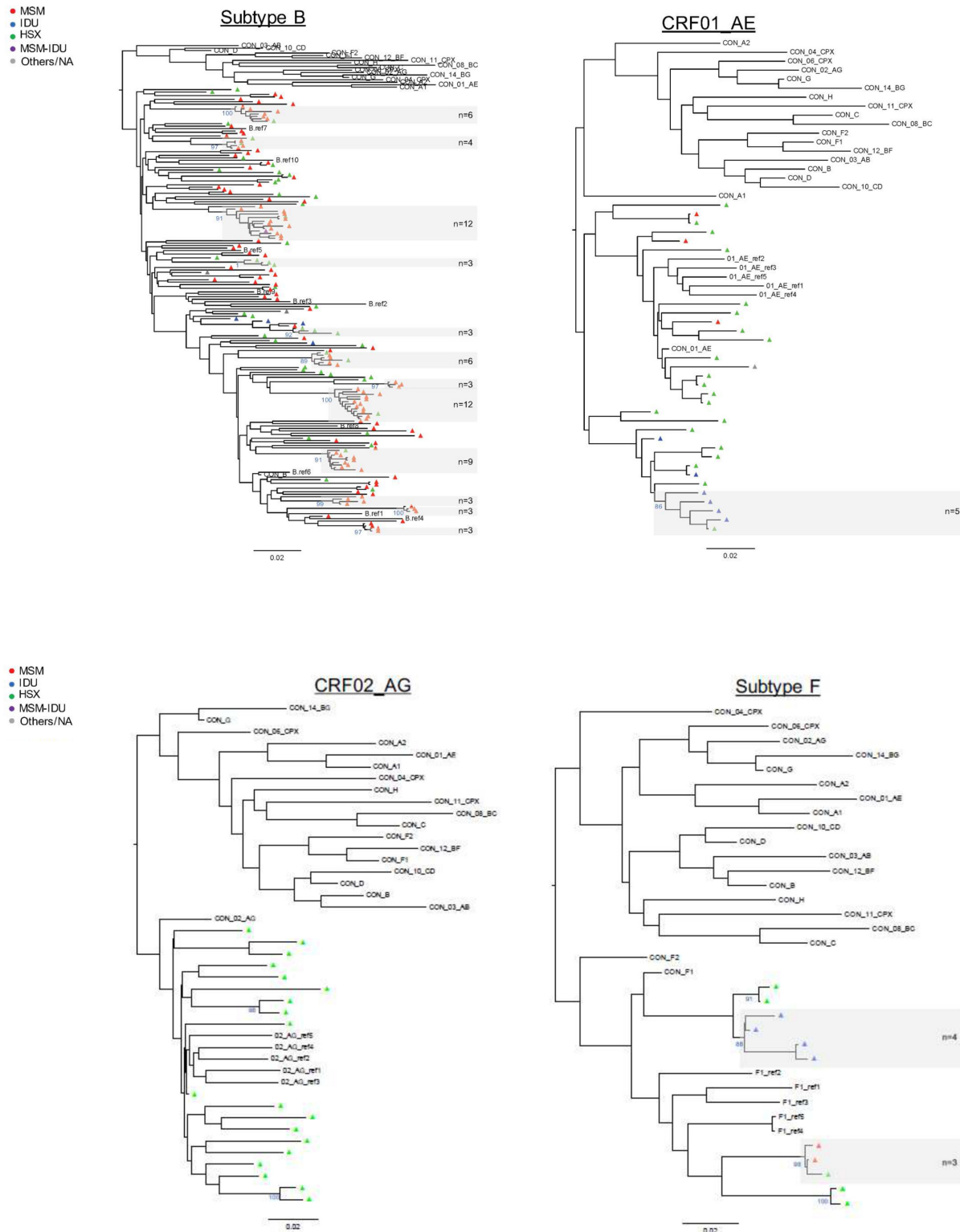
Cluster Size	Pairs (n = 19)	Triplets (n = 7)	≥4 individuals (i.e. large clusters) (n = 8)
<b>Age</b> (median, range)	36 (22–64)	31 (22–40)	33 (25–53)
<b>Risk Factor</b>			
HSX exclusive	42.1% (n = 8)	28.6% (n = 2)	0
MSM exclusive	31.6% (n = 6)	42.9% (n = 3)	0
Mixed MSM/HSX	21.1% (n = 4)	14.3% (n = 1)	75% (n = 6)
IDU†	5.3% (n = 1)	14.3% (n = 1)	25% (n = 2)
<b>Subtype</b>			
B	57.9% (n = 11)	85.7% (n = 6)	75% (n = 6)
CRF01_AE	15.8% (n = 3)	0% (n = 0)	12.5% (n = 1)
CRF02_AG	10.5% (n = 2)	0% (n = 0)	0
F	10.5% (n = 2)	14.3% (n = 1)	12.5% (n = 1)
D	5.2% (n = 1)	0% (n = 0)	0

† Category 'IDU' also includes men who are both MSM and IDU.

Abbreviations: HSX, heterosexual; IDU, injection drug use; MSM, men who have sex with men.

doi:10.1371/journal.pone.0151478.t002





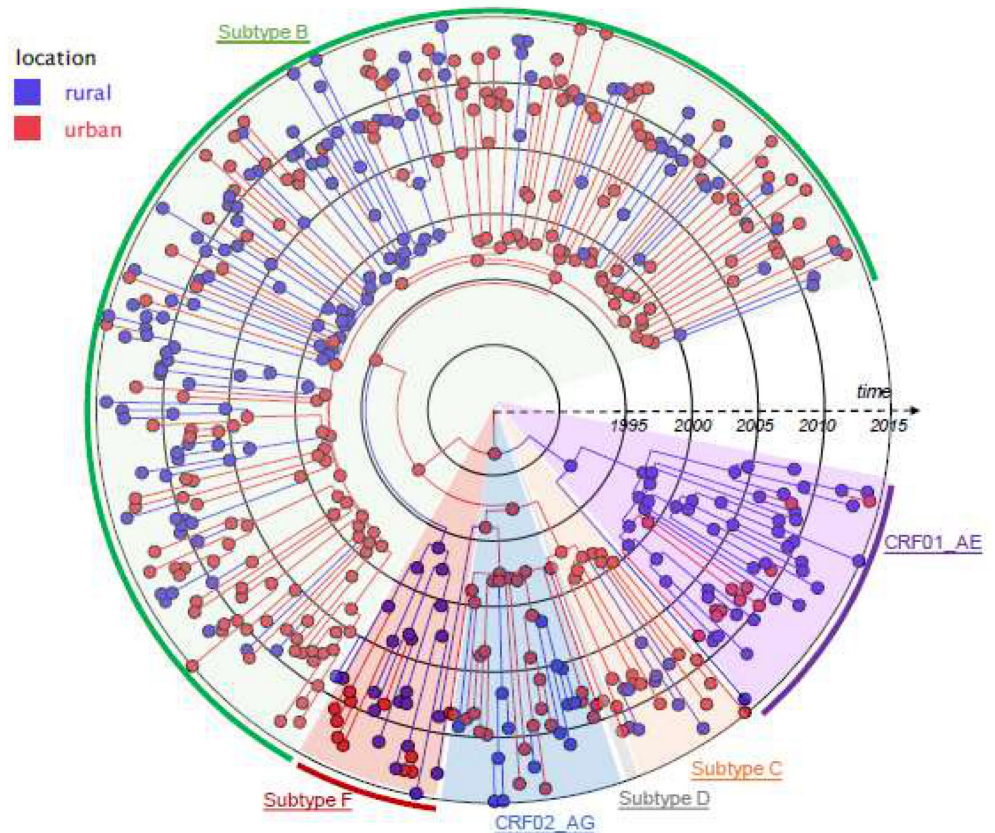
**Fig 3. Maximum likelihood phylogenetic trees (by subtype).** All clusters identified by network analysis were supported by a bootstrap value above 80%. The bootstrap value of the larger clusters is indicated in blue at the root of each corresponding clusters and non-dyad clusters are highlighted in grey with the cluster size indicated for each cluster. Tips are colored by their reported risk factor in red (men who have sex with men, MSM), green (heterosexual, HSX), blue (injection drug use, IDU), purple (MSM plus IDU) and grey (unknown/others), respectively. All trees (Subtype B, CRF\_01AE, CRF\_02AG and subtype F) include a set of reference consensus sequences from the main subtypes and CRF forms, and a set of reference sequences from the same subtype.

doi:10.1371/journal.pone.0151478.g003

and included 9, 11 and 12 individuals respectively, had tMRCAs of 2006 (95% Confidence Interval [CI] 2001–2011), 1998 (95%CI 1992–2003) and 1999 (95%CI:1993–2003) respectively.

### Migration Analysis

Based on visual inspection (Fig 1b), there was no clear relationship between clustering individuals and their area of residence, suggesting a high degree of geographic mixing of the population within South-East Austria. To further examine the relationship between geography and HIV transmission, we next investigated the spatio-temporal dynamics of the observed epidemic specifically between urban and rural regions of South East Austria by applying a Bayesian phylogeographic inference using discrete non-reversible models to our geo-referenced sequences. In this phylogeographic reconstruction, each node was annotated with a geographic location (Fig 4). The resulting phylogeny demonstrates inferred ancestral nodes from either rural (in blue) or urban areas (in red). Branches linking red and blue nodes represent location state transitions (viral lineage migrations) between the rural and urban regions. We found that the sampled subtype B HIV epidemic was mainly initiated in the urban areas of Graz (in red) as illustrated by the most probable location state of the descendant nodes with 57.1% (95%CI



**Fig 4. Time-scaled phylogeographic reconstruction of the 259 HIV-1 sequences from South-East Austria.** Ancestral nodes are colored according to the inferred most probable location state with the color codes corresponding to the urban areas (Graz City and suburban Graz) in red and the rural areas in blue. Branch colors represent the most probable location of the parental node of each branch. Tips are colored according to the observed sampling location of each sequence. Each clade is highlighted in unique color: green (subtype B), red (subtype F), blue (CRF02\_AG), gray (subtype D), orange (Subtype C) and purple (CRF01\_AE).

doi:10.1371/journal.pone.0151478.g004

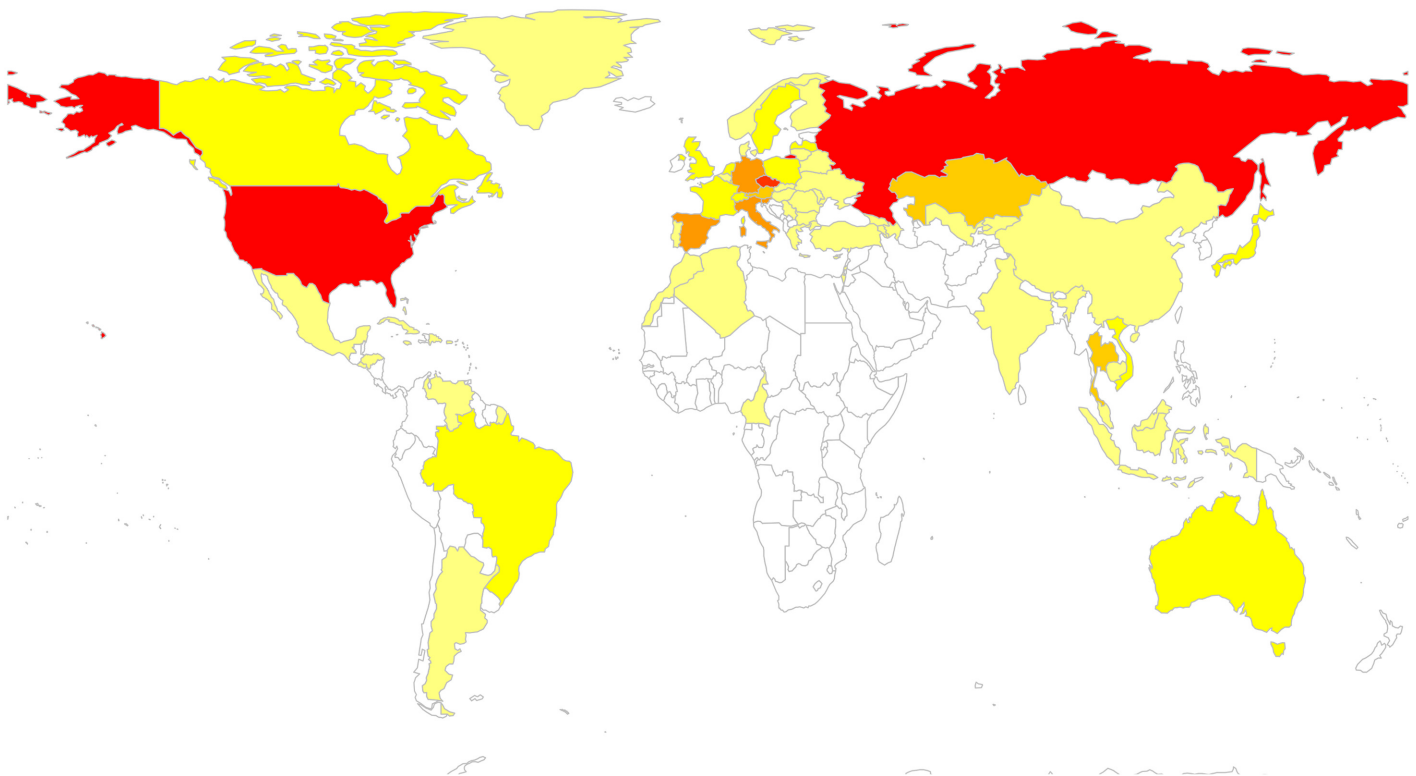
54%-60.1%) of all viral lineages movements originating from urban Graz (Fig 4). For the HIV-1 subtype F and CRF01\_AE epidemic, all of the sampled viruses and the inferred ancestral nodes corresponded to rural areas.

**Intersection between the Global HIV Epidemic and South East Austria.** One hundred and seven of the 259 sequences (41.3%) from this collection had a putative inferred linkage with a sequence from outside Austria. These sequences were linked with sequences from 69 other countries. The 14 Austrian CRF01\_AE sequences who had a putative inferred link to a non-Austrian sequence, were linked to 158 CRF01\_AE sequences (predominantly from south-east Asia), and 1215 subtype A sequences (predominantly from large transmission clusters from Eastern Europe) (Fig 5). The majority of linked sequences were subtype B (76%), and originated from MSM (64%) (S1 Fig).

## Discussion

We reconstructed the HIV-1 transmission network in South-East Austria by analyzing 259 sequences of persons newly diagnosed with HIV-infection between 2008 and 2014. Network analysis revealed a high rate of inferred clustering within the cohort, consisting mainly of MSM and injection drug users, and a large number of international connections.

The sequences included in this analysis represent an estimated 7.4% of the new HIV diagnoses across Austria in this time period, and over 95% of the new HIV diagnoses in South-East Austria [7,8]. We were therefore able to reconstruct a relatively complete network of individuals



**Fig 5. Intersection between the Global HIV Type 1 Endemic and South East Austria.** A total of 107 (41.3%) of 259 sequences obtained in South-East Austria shared a putative link with sequences from foreign countries/regions. In this heat map with a gradient from 1 to 40 linkages, countries are colored by the number of putative links from yellow to red (i.e. red is 40 or more linkages). Unlinked countries are colored in white.

doi:10.1371/journal.pone.0151478.g005

newly diagnosed with HIV in South-East Austria between 2008 and 2014. We found a high degree of clustering, with putative transmission linkages for 45.6% (118/259) of the sequences.

Nearly a third of HIV positive males identified HSX contact as their only risk for HIV acquisition, which may be considered a high proportion when compared to other settings in Europe and the United States [23–27]. Interestingly, of the clustering males that reported only HSX risk, 47% (9/19) clustered closely with MSM (either as pairs or within larger MSM clusters). This suggests misrepresentation of risk that may be related to perceived stigma in South-East Austria that prevents males from self-identifying as MSM, or may be related to a group of “missing” individuals with two risk factors (i.e. HSX/MSM [Bisexual], or MSM/IDU) bridging these individuals to MSM HIV-1 transmission networks. Local transmission networks of HIV infected persons are never complete because i) sampling of the entire network is often impossible, such that the inferred network will not include HIV-infected persons not yet diagnosed. Further, a putatively inferred linkage does not represent a true transmission link, as additional unsampled individuals could have been members of the transmission chain linking the two observed putatively linked individuals [2]. Therefore we can't rule out that there might be “missing” individuals bridging our sampled HIV-1 infected MSM and HSX males. Our results correlate well with characteristics of the overall Austrian HIV epidemic. Interestingly heterosexual contact was the most frequently reported mode of HIV acquisition until 2008, and only recently surpassed by men who sex with men (MSM) sexual contact [8]. In part, these findings may be explained by stigma and discrimination that may limit individuals from self-identifying as MSM. Austria, while influenced by Roman Catholicism, has slowly become more liberal with laws and social opinions concerning sexual orientation and gender identity. Lesbian, gay, bisexual, and transgender (LGBT) persons in Austria are, however, still facing some legal challenges not experienced by non-LGBT residents [28]. In a survey conducted by the European Union in 2012, collecting online-information from over 93,000 LGTB persons, 48% of respondent from Austria felt discriminated against or harassed on the grounds of sexual orientation in the last 12 months with an average number of 1,078 harassment incidents per 1000 respondents [28]. More than that, only 26% of Austrian LGBT respondents reported that they reveal their sexual orientation or gender identity to most people in their private and professional lives, while 29% reported that they never reveal their LGBT lifestyle [28].

Bayesian phylogeographic analyses also provided insights into the spatiotemporal dynamics of the South-East Austrian HIV epidemic, suggesting a key role for the densely populated region of Graz as a hub for transmission of new HIV infections throughout the region. These results are consistent with our recent evaluation of French individuals with primary HIV infection which revealed an early spread of the HIV epidemic from Paris to the rest of the Country [29]. Interestingly, these analyses also showed that the subtype F and CRF01\_AE local epidemics were more likely to have started (and persisted) in rural areas (Fig 4).

Previously, Frentz and co-workers evaluated 1,330 clustering pol sequences from 26 European countries collected between 2002 and 2007 and found that transmission of HIV-1 in Europe is predominantly occurring between individuals from the same country as only 25.8% of sequences clustered with individuals from foreign countries (10.2% with individuals from neighboring countries only and 15.6% with individuals from countries without a common border) [28]. In contrast, our study found that more than 40% of sequences had putative linkages with sequences from countries outside Austria, with the majority of linkages to Eastern European countries without a common border. The main explanation for these differing findings can be found in the size of the network tested. While Frentz and colleagues evaluated linkage within a limited pool of 4,260 pol sequences only, our study evaluated linkage using nearly 150,000 sequences of the Los Alamos National Laboratory HIV sequence database. In contrast to Frentz et al., our findings may therefore indicate that infections through travelling between

countries may be more frequent and a more international approach that is not restricted to national borders is needed for surveillance of HIV-1 epidemics.

In conclusion, analysis of HIV1 sequences from South-East Austria revealed a highly clustered transmission network with a large number of international connections. Higher rates of clustering in MSM, and a high proportion of HSX males clustering within MSM networks may inform prevention, testing and linkage to care strategies. Future studies are needed to further expand that network to other parts of Austria and other countries across Europe.

## Supporting Information

**S1 Fig. Characteristics of the 107 sequences from the South-Eastern Austrian Cohort putatively linked with sequences from foreign countries/regions by A. Subtype, and B. Risk Factor.** HSX: heterosexual; MSM: Men who have sex with men; IDU: Injection Drug Users. (PDF)

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### Previous presentation at scientific meetings

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## Author Contributions

Conceived and designed the experiments: MH AC HK SL SM. Performed the experiments: HK BH KW ES. Analyzed the data: AC MH HK SM. Contributed reagents/materials/analysis tools: HK AC SM MH BH ES. Wrote the paper: MH AC HK SM BH KW ES SL.

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