Title

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Journal

Clinical Infectious Diseases, 74(6)

ISSN

1058-4838

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Publication Date

2022-03-23

DOI

10.1093/cid/ciab596

Peer reviewed

Clinical Infectious Diseases

MAJOR ARTICLE

Identification of Genetically Related HCV Infections Among Self-Described Injecting Partnerships

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Background. The current opioid epidemic across the United States has fueled a surge in the rate of new hepatitis C virus (HCV) infections among young persons who inject drugs (PWIDs). Paramount to interrupting transmission is targeting these high-risk populations and understanding the underlying network structures facilitating transmission within these communities.

Methods. Deep sequencing data were obtained for 52 participants from 32 injecting partnerships enrolled in the U-Find-Out (UFO) Partner Study, which is a prospective study of self-described injecting dyad partnerships from a large community-based study of HCV infection in young adult PWIDs from San Francisco. Phylogenetically linked transmission events were identified using traditional genetic-distance measures and viral deep sequence phylogenies reconstructed to determine the statistical support of inferences and the direction of transmission within partnerships.

Results. Using deep sequencing data, we found that 12 of 32 partnerships were genetically similar and clustered. Three additional phylogenetic clusters were found describing novel putative transmission links outside of the injecting relationship. Transmission direction was inferred correctly for 5 partnerships with the incorrect transmission direction inferred in more than 50% of cases. Notably, we observed that phylogenetic linkage was most often associated with a lower number of network partners and involvement in a sexual relationship.

Conclusions. Deep sequencing of HCV among self-described injecting partnerships demonstrates that the majority of transmission events originate from outside of the injecting partnership. Furthermore, these findings caution that phylogenetic methods may be unable to routinely infer the direction of transmission among PWIDs especially when transmission events occur in rapid succession within high-risk networks.

Keywords. hepatitis C virus; phylogenetic; deep sequencing; injection drug use; molecular epidemiology.

The United States is in the midst of an opioid epidemic that has fueled a surge in hepatitis C virus (HCV) incidence, increasing by 294% from 2010 to 2015 among young persons who inject drugs (PWID) [1–3]. From 2003–2013, deaths associated with HCV rose above that of 60 other nationally notifiable infectious diseases combined [4]. Since 2013, national surveillance data indicated a 9.37% decline in the HCV-associated death rate with a further 6.56% rate decline observed from 2016–2017 [5, 6]. Moreover, national surveillance data have showed a substantial increase in the incidence of acute HCV infection throughout the United States from 2004 to 2014 [3] and a 71% increase in incidence compared to 2014 [7]. Despite the availability of direct acting antivirals (DAAs) this significant increase in new HCV infections has been attributed primarily to the opioid epidemic and associated injection drug use. For example, between 2015 and 2018 Northeastern Massachusetts experienced an outbreak of HIV and HCV attributable to syringe sharing of opioids and homelessness [8, 9]. A worrying trend is the emerging and rising epidemic among young adult PWID in nonurban areas [10], where an alarming 364% increase in new HCV infections occurred between 2006 and 2012 among Central Appalachia states [11]. This unprecedented US epidemic has created the impetus for the development of novel public health and treatment intervention strategies to target HCV transmission networks and interrupt transmission. Approaches aimed at using targeted interventions towards members of the /community who contribute most and are highly connected to other contacts within the population may be the most efficient way to interrupt HCV dissemination. However, implementation of public health interventions necessitates that the structure of contact and transmission networks is well defined and that the main drivers of transmission are understood, particularly in the setting of concentrated outbreaks where the conditions that drive outbreaks are often unknown.

In this study, we used a well-defined, sampled cohort of young adult PWIDs from San Francisco to reconstruct the

Received 10 March 2021; editorial decision 24 June 2021; published online 27 August 2021.
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Clinical Infectious Diseases® 2022;74(6):993–1003

https://doi.org/10.1093/cid/ciab596

HCV transmission network among self-described injecting partnerships using a deep sequencing approach [12].

METHODS

Study Population and Design

Study participants were recruited into the "Partner Study," a substudy of the U-Find-Out (UFO) study that represents a large prospective community-based epidemiologic study of young adult injectors at risk for HCV in San Francisco, California [13, 14]. From May 2006 to December 2016, UFO study participants were invited to recruit injecting partners to participate in this prospective substudy of HCV transmission within HCV-serodiscordant injection partnerships [15]. Injection partnerships or "dyads" were eligible for this study if: (i) individuals injected together in the same physical space at least 5 times in the prior month; (ii) they were discordant on HCV RNA or HCV RNA concordant positive with at least one of the partners identified as being acutely infected (HCV RNA positive/anti-HCV negative); and (iii) both members of the dyad had concordant answers to a diverse set of screening questions to validate their injecting activity with their injecting partner. Upon enrollment study participants were asked to return monthly for six-months for follow-up interviews. Re-enrollment for an additional six months occurred if the partnership members were still actively injecting together and remained HCV RNA discordant (meeting the same criteria as above). Partner study participants were allowed to enroll with a maximum of three concurrent injecting partners. The definition of an injecting partnership did not explicitly require that drugs or injecting equipment be shared.

HCV Testing

Anti-HCV antibodies were detected using a third generation EIA (EIA-3; Abbott Laboratories) and HCV RNA testing was performed quarterly using a transcription mediated amplification (TMA) technique (dHCV TMA assay component of the Procleix HIV-1/HCV assay, Gen-Probe Inc., San Diego, California) to detect early HCV infection in those who tested anti-HCV negative [14, 16].

Viral RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

HCV RNAs were extracted from 140 μL of plasma of patient samples following the manufacturers' protocol for the QIAamp viral RNA mini kit for plasma (Qiagen).

PCR Amplification of Core-NS2, HVR1, and NS5B

For each study participant, a RT-PCR amplification was performed across the Core-NS2 region (H77: 279–3542) or the HVR1 (H77: 381–1711 (1a), 381–1701 (3a)). In addition, a 389-base-pair fragment (H77: 8250–8638) of the NS5B region was attempted and amplified from samples. See Supplementary Methods for details on primers and PCR conditions used.

Illumina Deep Sequencing and Data Analysis

Purified PCR amplicons were fragmented and barcoded using NexteraXT DNA Library Prep Kit, as per manufacturer's protocol. Samples were pooled and sequenced on an Illumina MiSeq platform, using a 2 × 250 bp V2 reagent kit. Paired-end reads obtained from Illumina MiSeq were cleaned, *de-novo* assembled and variants called using an in-house bioinformatics pipeline extending from our prior deep sequencing pathogen studies [17, 18]. Refer to Supplementary Methods for more details on the deep sequencing analysis.

Phylogenetic Reconstruction and Cluster Analysis

Consensus sequences were aligned using MUSCLE [19] and phylogenetic tress were inferred using maximum likelihood analysis employing the best fit model of nucleotide substitution as implemented within IQ-TREE with 1000 bootstrap replicates [20]. In order to support the identification of local clusters additional reference sequences from North America were obtained from the HCV-GLUE sequence database (http://hcv-glue.cvr.gla.ac.uk/#/home). Clusters were identified using ClusterPicker v1.2.4 [21] with a bootstrap threshold of 90% and a maximum genetic distance threshold of 0.05 for Core-NS2 and 0.02 for NS5B [22, 23].

Deep Sequencing Phylogenetic Analysis

We utilized phyloscanner (version 1.8.0) [24] to analyze the phylogenetic relationships between and within hosts of all individuals simultaneously using mapped reads produced by Illumina deep sequencing (see Supplementary Methods for additional details).

Ethical Approval

All study protocols and procedures were reviewed and approved by the UCSF institutional review board and the institutional review board of Massachusetts General Hospital.

RESULTS

Study Cohort Characteristics

A total of 101 injecting partnerships reflecting 122 unique participants (some at-risk partners were co-enrolled under multiple index cases) were previously enrolled in the UFO partnership study [25] (Figure 1). Among the 101 injecting partnerships, 40 partnerships (56 participants) demonstrated evidence of incident HCV infection (Figure 1). Of those 56 participants comprising either member of the 40 partnerships in which incident HCV infection was observed, we successfully amplified the Core-NS2 region from 44 subjects (79%) and the NS5B region from 45 subjects (80%) (Table 1). For 37 subjects (66%), both regions successfully amplified, and for 52

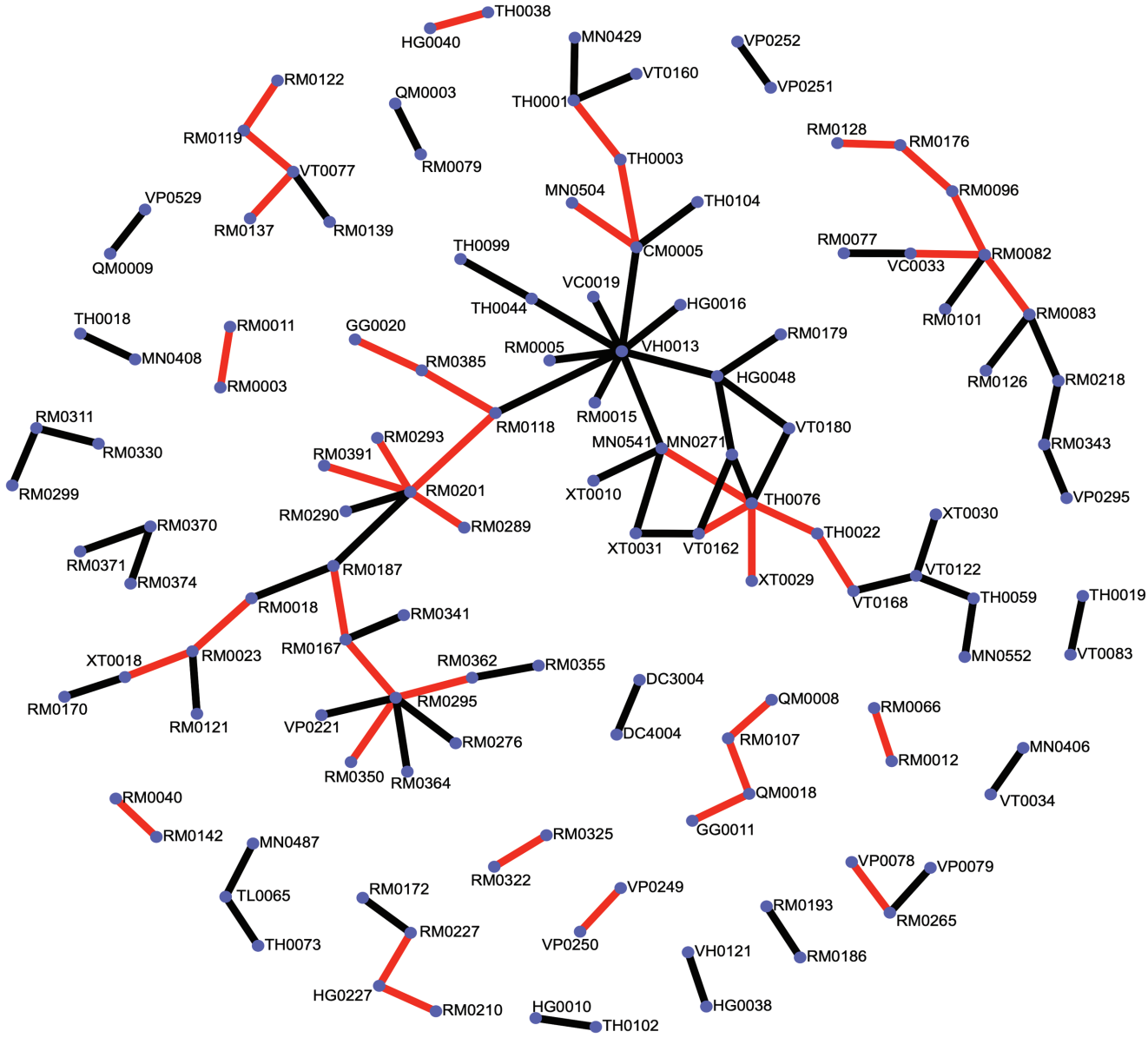

Figure 1. Overview of the study population within the UFO partnership study. In total, 101 partnerships were enrolled and denoted as lines between individuals. Black lines represent those injecting partnerships in which the at-risk partner did not seroconvert. Red lines between study participants reflects those where a new HCV infection was observed in the at-risk partner. Abbreviations: HCV, hepatitis C virus; UFO, U-Find-Out.

subjects at least one region amplified. Collectively, from these partnerships in which a new HCV infection was observed, we amplified and sequenced data from 32 partnerships. The composition of these partnerships was predominantly young white and of the opposite sex with females being the at-risk partner (Table 2). Among the partnership there was a high frequency of sharing injecting equipment and frequently injecting within the prior month (Table 2). The overall HCV genotype distribution among the 52 individuals were: 1a: 65% (n = 34), 1b: 2% (n = 1), 2a: 2% (n = 1), 2b: 6% (n = 3), 3a: 23% (n = 12) and 4a: 2% (n = 1). Maximum-likelihood phylogenetic trees of the Core-NS2 sequences (Supplementary Figure 1*A*) and NS5B sequences (Supplementary Figure 1*B*) illustrate that the viral sequences from injecting partnerships are well-representative of the breadth of genetic diversity observed across hundreds of North American HCV isolates.

Phylogenetic Clustering Using Consensus Sequences Identifies 14 Transmission Clusters

Phylogenetic analysis of consensus sequences of the Core-NS2 and NS5B regions from the 52 individuals revealed that 52% (27/52) of the participants grouped into 14 clusters. Specifically, phylogenetic analysis of the Core-NS2 region from 44 participants identified 12 clusters (C1-C12) of 25 individuals (Figure 2A). The median genetic distance within the 8 genotype 1a

Table 1. Sample Overview of the Hepatitis C Virus (HCV) Region Sequenced for Each Cluster (Labeled C#) and Individual and Their Genotype and Clinical Status Upon Enrollment Into the Study

		HCV Region Sequenced			
Cluster Name	ID[a]	**C-NS2**	**NS5B**	Genotype	Clinical Status at Enrollment
C1	RM0122	+	+	1a	Chronic
	RM0322	+	NA	1a	Acute
C2	RM0083	+	+	1a	Chronic
	RM0293	+	+	1a	Negative
C3	RM0176	+	+	1a	Negative
	RM0350	+	+	1a	Chronic
	RM0128	NA	+	1a	Acute
	RM0295[b]	+	NA	1a	Chronic
C4	**RM0012**	+	+	1a	Chronic
	RM0066	+	+	1a	Acute
C5	RM0003	+	+	1a	Acute
	RM0011	+	NA	1a	Chronic
C6	**RM0040**	+	+	1a	Chronic
	RM0142	+	NA	1a	Negative
C7	RM0265	+	+	1a	Negative
	VP0078	+	+	1a	Negative
C8	VP0249	+	NA	1a	Negative
	VP0250	+	+	1a	Negative
C9	RM0167	+	+	3a	Negative
	RM0187	+	+	3a	Acute
C10	RM0119	+	+	3a	Negative
	VT0077	+	+	3a	Acute
C11	CM0005	+	+	3a	Acute
	TH0003	+	+	3a	Negative
C12	**GG0020**	+	+	3a	Chronic
	RM0385	+	+	3a	Negative
C13	**RM0201**	+	+	1a	Negative
	RM0289	NA	+	1a	Negative
C14	HG0227	NA	+	2b	Negative
	RM0391	NA	+	2b	Chronic
C15[c]	**GG0011**	+	+	1a	Acute
	QM0018	+	+	1a	Negative
	HG0040	+	+	1a	Acute
	MN0504	ND	ND	ND	Negative
	MN0541	+	+	1a	Chronic
	QM0008	+	+	1a	Chronic
	RM0018	ND	ND	ND	Chronic
	RM0023	+	+	1a	Chronic
	RM0082	ND	ND	ND	Chronic
	RM0096	+	+	1a	Chronic
	RM0107	+	+	1b	Negative
	RM0118	ND	ND	ND	Chronic
	RM0137	NA	+	4a	Negative
	RM0210	+	NA	1a	Chronic
	RM0227	NA	+	3a	Chronic
	RM0325	+	+	1a	Chronic
	RM0362	+	+	1a	Chronic
	TH0001	+	+	1a	Chronic
	TH0022	+	+	3a	Negative
	TH0038	+	+	1a	Negative
	TH0076	NA	+	2a	Negative
	VC0033	NA	+	2b	Chronic
	VT0162	+	+	1a	Negative
	VT0168	+	NA	3a	Chronic
	XT0018	+	+	1a	Negative
	XT0029	+	+	3a	Chronic

Abbreviations: NA, not available due to polymerase chain reaction (PCR) failure; ND, not done due to lack of sample availability.

[a]Clusters whose participants are labeled in bold indicate those participants referred to as the index.

[b]Deep sequencing was not performed on RM0295. Sanger sequencing of a 450bp fragment covering E1 was previously performed.

[c]Cluster 15 was found by deep sequencing analysis alone.

Table 2. Baseline Characteristics of 32 Sequenced Injecting Partnerships (index and At-Risk Partners) in the U-Find-Out (UFO) Study

Baseline Characteristic	Median (IQR)
Age	23.7 (22.4 – 26.3)
Age difference of partnership (index—at-risk partner), y	2 (–2 to 5.75)
Race of at-risk partner: Non-white (%)	44%
Gender composition of partnership (%)	
Female at-risk partner / male index	43.75%
Male at-risk partner / female index	15.63%
Male / male	37.5%
Female / female	3.13%
Past month	
No. of days injected	20 (10 – 27.3)
No. of other injecting partners	4.5 (3 – 11.3)
Frequency of sharing injecting equipment	
Never	12.5%
Rarely	9.38%
Sometimes	9.38%
Usually	31.25%
Always	37.5%
Had a sexual relationship with partner	43.75%
Number of months injecting together	6 (2.4–12)
Number of months known each other	10 (5.16 – 24)

Abbreviation: IQR, interquartile range.

clusters was 0.00468 (interquartile range [IQR]: 0.00089–0.01) and within the 4 genotype 3a clusters was 0.00016 (IQR: 0–0.00056).

Phylogenetic analysis of the NS5B region from 45 participants identified 9 clusters at a maximum genetic threshold of 0.02 (Figure 2B); 7 of these were detected in the prior Core-NS2 analysis, and 2 were newly found (as Core-NS2 sequence data were not available). The median genetic distance within the 5 genotype 1a clusters was 0.006378 (IQR: 0.001276–0.01148). The median genetic distance within the 4 genotype 3 clusters was 0.006378 (IQR: 0.003189–0.007653), and for the 1 additional genotype 2 cluster was 0.007653. Cluster 2 was the only cluster that was not found between both sequenced regions due to a low bootstrap support value of 61 within the NS5B region.

Deep Sequencing Reveals an Additional Cryptic Genetic Linkage Within the Population

To determine whether incorporation of within-host sequence diversity could improve the resolution of genetically linked clusters we utilized a phylogenetic framework containing both within- and between-host diversity across sliding windows. For each partnership we determined the minimum subgraph distance, defined as the shortest patristic distance between any nodes of one individual within a partnership, across all windows spanning the Core to NS2 region. The distribution of the minimum subgraph distances over the partnerships demonstrated that the majority of index-partner pairs were either phylogenetically closely related (minimum subgraph distance < 0.05 substitutions per site) or distantly related with intermediate distances being rare (Figure 3A). Further inspection of the distribution of subgraph distances found that partnerships could be segregated into those phylogenetically linked and closely related versus phylogenetically unlinked and distantly related (Figure 3B). Partnerships that were phylogenetically close had a median subgraph distance of 0.000001 compared to a median of 0.302 (IQR: 0.109–0.881) of those phylogenetically unrelated partnerships. Analysis of the distribution of subgraph distances across NS5B (Supplementary Figure 2) revealed a remarkably similar pattern as shown for the Core to NS2 data (Figure 3B).

Together, analyses of the within-host diversity from all deep sequenced participants mirrored our findings using consensus sequences (Figure 2). However, one additional partnership (GG0011 and QM0018), not classified as phylogenetically linked (Figure 2), was found to group in 100% of deep sequence phylogenies where a minor viral variant in QM0018 consistently intermingled with the viral population of GG0011 (Figure 3B). Transmission linkage was independently confirmed within this partnership using the Centers for Disease Control and Prevention's (CDC's) global hepatitis outbreak and surveillance technology (GHOST) tool [26] (Supplementary Figure 3).

Time of Sampling Between Index and Partner Samples Does Not Impair the Ability to Detect Genetically Related Infections

We further analyzed whether the time interval between the collection of index and partner samples would influence the ability to detect a genetically related infection. For those phylogenetic linked partnerships, the median time interval between collection of both index and partner samples was 28 days (IQR: 7–50.5 days) while for phylogenetic unlinked partnerships the median time interval between collection of samples was 35.5 days (IQR: 16.7–255). Thus, we did not observe any significant relationship between duration of sampling between index and partner samples and the inference of phylogenetic linkage ($P = .301$; Figure 4).

Inferring Transmission Direction Is Challenging Among PWIDs Due to the Close Genetic Relationship Between Partnerships

We concentrated on 9 of 12 clustered partnerships in which we had knowledge on the direction of transmission (based on prior negative HCV testing or stage of infection) and evaluated the accuracy of using deep sequencing data to infer transmission direction. Using Core to NS2 sequence data the fraction of pairs with the correct transmission direction (index → partner) was 25%, whereas in 37.5% of partnerships the incorrect direction of transmission was inferred. Three partnerships were classified as linked but no transmission direction could be inferred. In an attempt to increase the accuracy of the inferred transmission direction we examined different window widths and found consistent results (Supplementary Figure 4). With NS5B data we examined 7 partnerships plus the partnership of RM0128 and

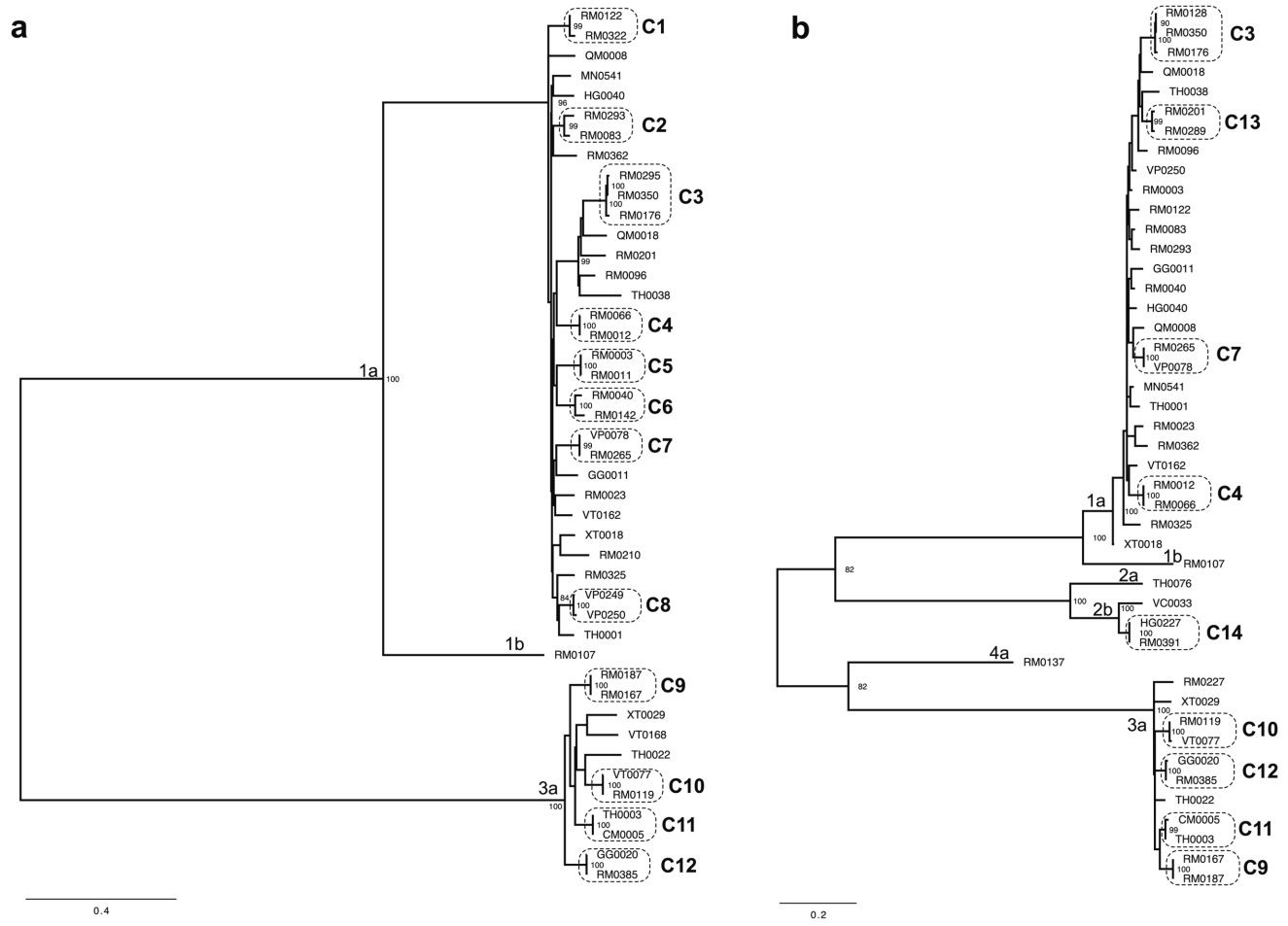

Figure 2. Maximum-likelihood phylogenetic tree showing phylogenetic clusters within the UFO partnership study for (*A*) Core-NS2 and (*B*) NS5B. Phylogenetic clusters defined by bootstrap analysis and genetic distance threshold are highlighted by a dashed line and labeled C1–14. Bootstrap supports values are only shown for nodes over 70%. Genotypes and subtypes are labeled respectively. Scale bar indicates the number of nucleotide substitutions per site. Abbreviation: UFO, U-Find-Out.

RM0176 and found that the correct transmission direction was inferred in only one partnership, whereas the remaining sample pairs displayed complex phylogenetic relationships.

Onwards Transmission of a Genetically Related Infection Is Supported by Dyadic and Sexual Behavior

To explore whether any factors are associated with the transmission of genetically related infections within our partnerships versus infections originating outside of the partnerships we examined the injecting networks between PWIDs (Figure 5). Of the 16 clusters represented, 7 are composed of dyads only (ie, only 1 partner was enrolled by each at-risk person) (Figure 5A), whereas 9 correspond to non-dyads (ie, >1 partner was enrolled by an at-risk person, or a chain of enrollment occurred, for a total of 3–7 connected persons) (Figure 5B). Of the 32 partnerships we confirmed the index and the partner are genetically similar in 12 partnerships. Three clusters (C1, C2, and C14) represent novel putative links outside of self-described partnerships that share genetic similarity. In addition, 1 cluster (C3) has expanded to include 2 other HCV-infected individuals not previously reported to be injecting together (RM0176 from Core to NS2 and RM0350 from NS5B).

Deeper investigation of the phylogenetically linked partnerships revealed that they were predominantly found within those dyads (5 of 7 partnerships [71%]) compared to 5 of 22 (23%) of sample pairs in more complex networks (>2 PWIDs) (Figure 6A). In this cohort phylogenetic linkage within an injecting relationship may be predicated on the size and structure of that relationship, such that dyads are more likely to harbor a virus that is genetically similar compared to those within larger injecting networks (P = .03; odds ratio [OR]: 8.5 [1.3–57.9]). Moreover, of those 12 injecting partnerships who share a genetically similar virus, 83.3% of were also in a sexual relationship, compared to 21% of those not phylogenetically linked and reported to be in a sexual relationship between each other (P = .0008; OR 19 [3–116] Figure 6B). Also, note that no significant differences were observed when examining the number of

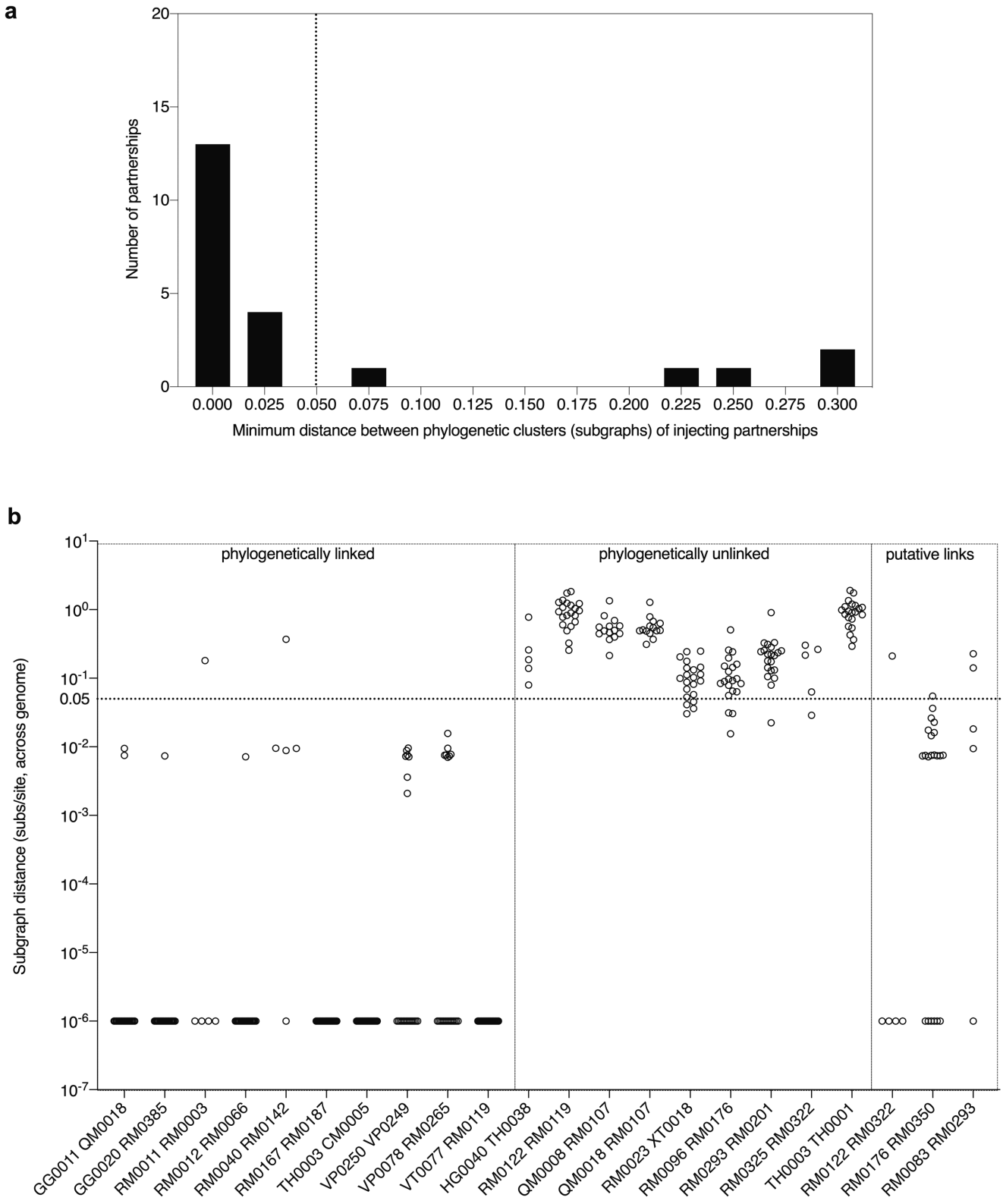

Figure 3. Deep sequence phylogenetic data from injecting partnerships. *A*, Histogram shows the distribution between injecting partnerships of the minimum subgraph distance obtained using phyloscanner, this analysis included data from 19 self-described partnerships and 3 newly identified putative partnerships. Majority of clustered participants had minimum subgraph distance <0.05 substitutions per site (indicated with a dotted line). *B*, Subgraph distances calculated from deep sequencing phylogenies for Core to NS2 stratified in those phylogenetically linked and unlinked partnerships. Subgraph distances (y-axis) summarized for all analyzed deep sequence phylogenies for 19 self-described injecting partnerships in which index and at-risk partners have Core to NS2 or HVR sequence data available. Dotted line indicates the distance threshold of 0.05 substitutions per site to define those partnerships classified as phylogenetically close and linked and those phylogenetically distant and unlinked. RM0295 is not depicted as deep sequencing data was not available. Three clusters denoted as putative links as found in Figure 2 are also shown.

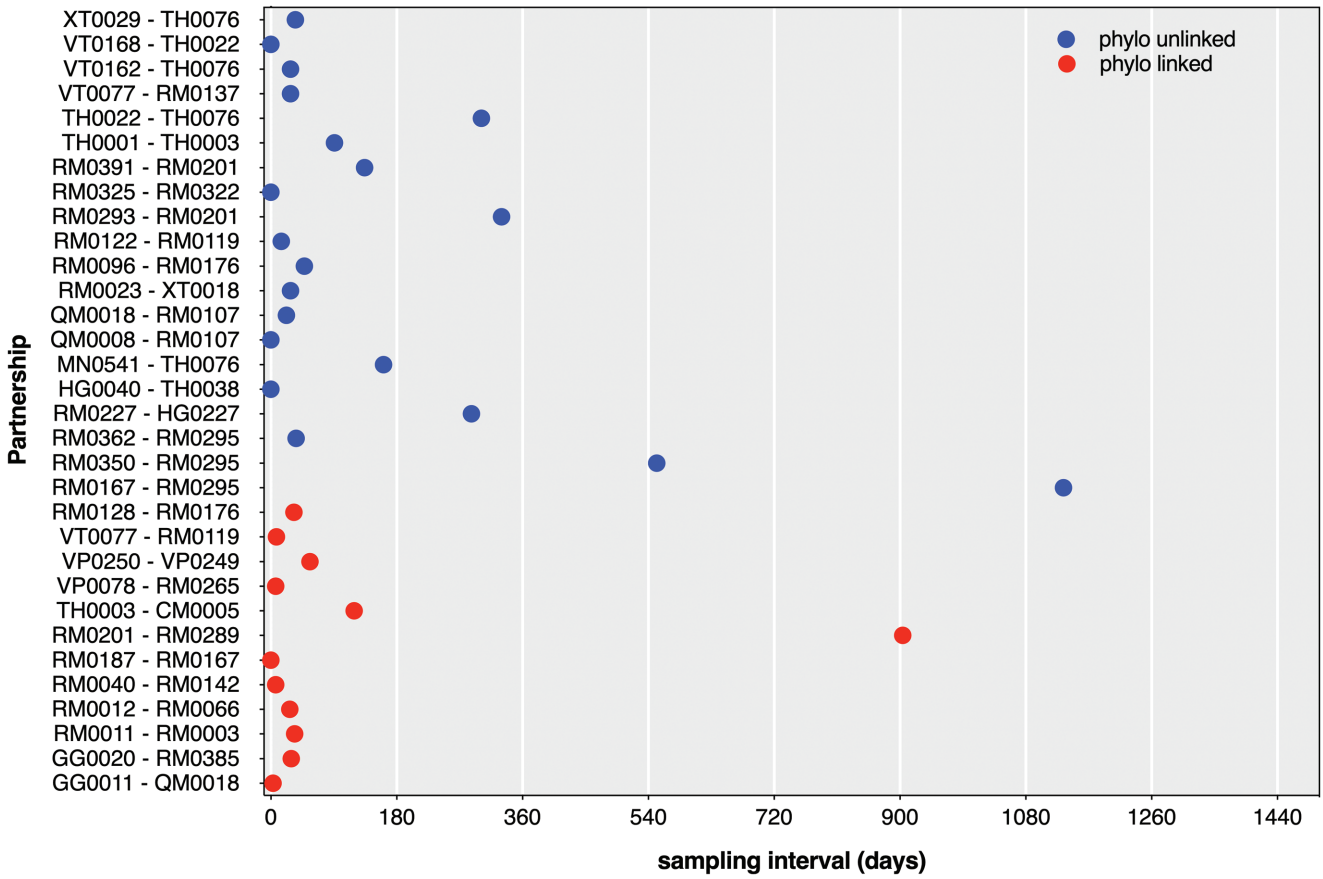

Figure 4. Time between collection of index and partner samples among 32 sequenced self-described partnerships. Blue dots indicate those partnerships that are shown by phylogenetic means to be unlinked; red dots indicate those partnerships that are phylogenetic linked. Data are plotted in days between the collection time of the index and partner samples.

additional injecting partners in the phylogenetically linked and unliked partnerships (Supplementary Table 1).

DISCUSSION

In this study we combine high-resolution deep sequencing and phylogenetics with clinical data to investigate the nature of HCV transmission within injecting partnerships. Among these PWIDs we found evidence of clustering suggestive of potential transmission events in only 52% (n = 27) of participants. Within partnerships we found that 63% (n = 20) did not have a genetically related infection. On the other hand, when a partnership was confirmed to be phylogenetically close it was very genetically similar (<1% divergence in most cases). This may imply direct transmission or that transmission occurred via unsampled intermediates in quick succession. We found evidence of novel putative transmission links between individuals outside of self-described partnerships and that deep sequencing could enhance the resolution of transmission linkage but not accurately resolve the direction of transmission between infected individuals. Collectively, these findings highlight that HCV prevention efforts focused solely on partnerships and social networks may be inadequate for understanding the true dynamics of HCV transmission.

The rate of clustering observed in this study was higher than that observed in previous PWID studies [23, 27–30] and may be due to the nature of recruiting self-described injecting partnerships, and is more similar to the clustering rate of 54% in Sack-Davis et al who enrolled injecting partnerships [31]. Prior studies have explored the factors associated with phylogenetic clustering and found support for greater clustering with participants of a younger age, HIV coinfection, recent HCV seroconversion, and recent syringe borrowing [23]. Within this study cohort a number of behavioral characteristics were independently associated with phylogenetic clustering, such as injecting more days together in the past month and always sharing injection equipment [25]. Moreover, although the role of HCV viremia was not directly related to increased odds of phylogenetic clustering, the index partner being in the HCV-seronegative viremic phase (acute infection) was associated with an increased risk of transmission among partnerships [25]. Furthermore, sexual relationships within the UFO cohort have also been associated with increased sharing of syringes and injecting

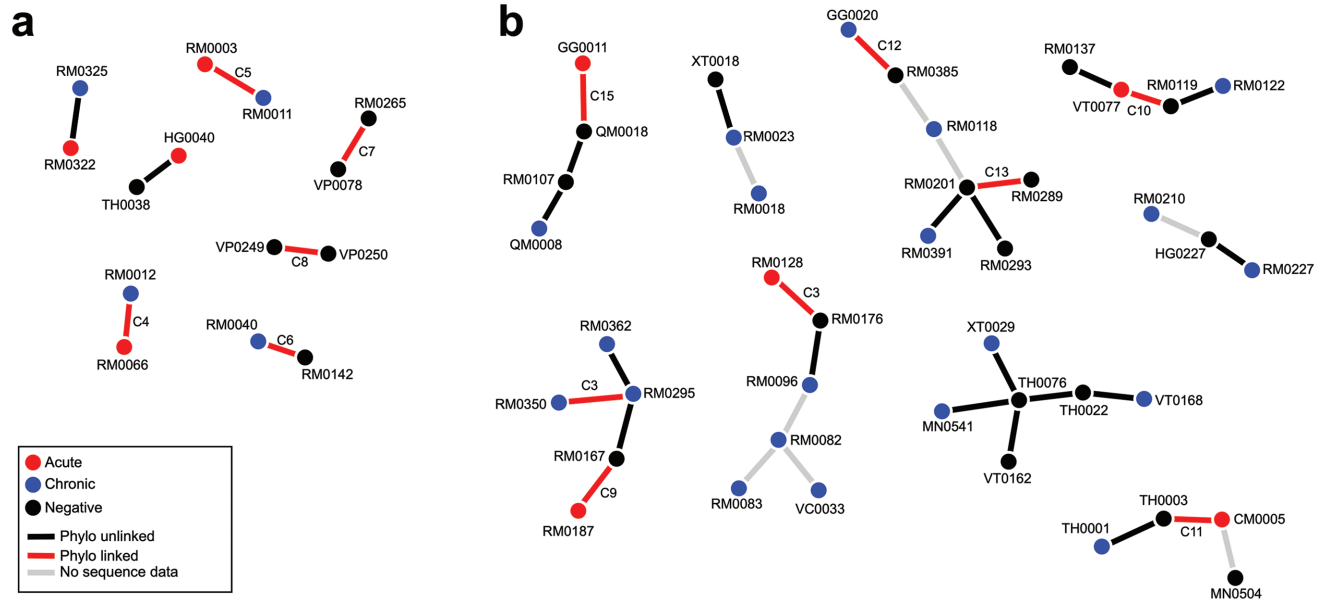

Figure 5. Network representation of the UFO partnerships in which a new HCV infection occurred. Circles and connecting lines denote an injecting partnership in which a new infection occurred. Colored circles denote stage of infection (baseline RNA status) at time of enrollment; blue indicates that the participant was in the chronic stage of infection; red indicates that the participant was in the acute infection window as defined by anti-HCV negative and HCV RNA positive test results; and black indicates that the participant was HCV negative upon study enrollment. Colored lines denote different category membership; red indicates that an injecting partnership was confirmed by sequencing and phylogenetic analysis; black indicates that an injecting partnership was not confirmed by sequencing and phylogenetic analysis; gray lines indicate that an injecting partnership could not be evaluated due to a lack of sequence data for a participant. Phylogenetically defined clusters are labeled as indicated prior and correspond to those as depicted in Table 1 and Figure 2. *A*, Dyadic injecting partnerships in which HCV-infected individuals are only enrollment with one at-risk partner. *B*, Larger injecting networks in which infected individuals are linked to multiple index and partners. Abbreviations: HCV, hepatitis C virus; UFO, U-Find-Out.

equipment [32], and being in a sexual relationship with one's partner was associated in an unadjusted statistical analysis with having a phylogenetically linked transmission event [25].

The topological relationship between sequences can potentially be used to infer the direction of transmission [33–35]. In our case we found that inferring the direction of transmission was more challenging as the virus was heavily intermingled within closely related individuals. In the Core-to NS2 analysis the direction of transmission was inconsistent in 70% of cases with at best only 3 partnerships demonstrating sufficient

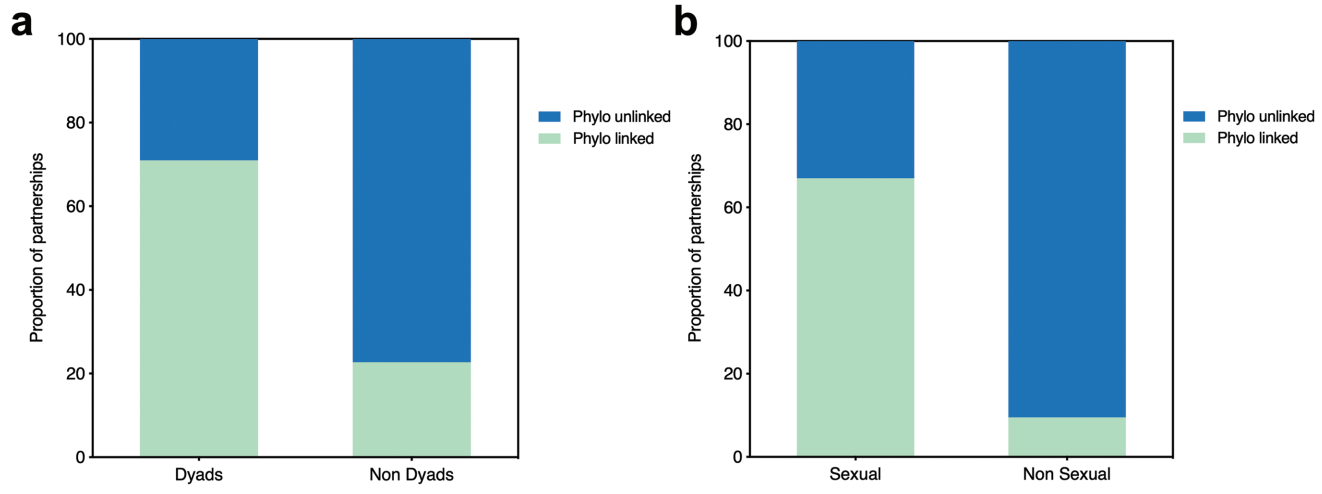

Figure 6. Phylogenetic relatedness of injecting partnerships, cluster size and sexual relationship. *A*, Proportion of injecting partnerships comparing those PWIDs who are in a dyadic relationship and those that share more than two at-risk partners plotted as a function of their phylogenetic status (linked vs unlinked). *B*, Proportion of injecting partnerships comparing whether an index and at-risk partner are engaged in a sexual or non-sexual relationship and their determined phylogenetic status. Abbreviation: PWID, person who injects drugs.

evidence for transmission directionality. Thus, among PWIDs the topological signal for direction of transmission may be inherently difficult to disentangle with high confidence as only 4 of 9 pairs (44%) were accurately inferred.

There were several limitations in this study. First, sequence data could not be obtained for all members of the injecting partnerships and that our study scope was limited to surveillance in enrolled partnerships in which there was a new HCV infection, and not the wider general community. Second, HCV clearance and reinfection is also possible but a previous study using the same cohort found that the incidence of re-infection was relatively low [36]. Third, behavioral differences may account for some non-linkage between injecting partnerships as a prior study in the same cohort found that those individuals that know that their partner is HCV positive are more likely to practice safer injection practices. Yet the sharing of injecting equipment was still common even after the HCV status of the partner was known [37]. Fourth, the collection of data via self-reporting can be vulnerable to social desirability bias and may have led to inaccurate or incomplete reporting of partner-specific data. Although self-reported drug and risk behaviors have been shown to be sufficiently reliable [38] the concordance between specific risk behaviors occurring within injecting dyads may vary [39].

Despite these limitations, our results highlight that HCV transmission in injecting networks is complex and multifaceted with most new infections not being seeded directly from the index case but rather from outside of the reported injection partnership. These results warrant further genomic surveillance among high-risk groups to better understand the topography of HCV transmission networks and guide prevention and treatment modalities. Such necessary steps may help mitigate public health disasters such as that in Scott County, Indiana, where HCV was cryptically spreading before the emergence of the large opiate driven outbreak of HIV [40].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank Matthew Hall for his assistance with Phyloscanner.

Financial support. This work was supported by the National Institutes of Health (grant numbers U19 AI082630 and U24 DA044801 to T. M. A. and R01 DA016017 to K. P.).

Potential conflicts of interest. M. D. M. reports receiving grant K01DA037802, outside the submitted work. K. P. reports payments for expert testimony regarding history of Pre-exposure prophylaxis research (not related to this research). All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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