ORIGINAL RESEARCH

Health Science Reports _Open Access_ WILEY

Patterns and correlates of hepatitis C virus phylogenetic clustering among people living with HIV in Australia in the direct-acting antiviral era: A molecular epidemiology study among participants in the CEASE cohort

Sofia R. Bartlett[1,2] 🟢 | Andrey Verich[3] | Joanne Carson[3] 🟢 |
Samira Hosseini-Hooshyar[3] | Phillip Read[4] 🟢 | David Baker[5] | Jeffrey J. Post[6,7,8] |
Robert Finlayson[9] | Mark Bloch[10] | Joseph S. Doyle[11,12] | David Shaw[13] |
Margaret Hellard[11,12] 🟢 | Maria Martinez[3] | Philippa Marks[3] |
Gregory J. Dore[3,14] | Gail V. Matthews[3,14] | Tanya Applegate[3] | Marianne Martinello[3]

[1]British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada

[2]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada

[3]The Kirby Institute, UNSW Sydney, Sydney, New South Wales, Australia

[4]Kirketon Road Centre, Sydney, New South Wales, Australia

[5]East Sydney Doctors, Sydney, New South Wales, Australia

[6]The Albion Centre, Sydney, New South Wales, Australia

[7]Department of Infectious Diseases, Prince of Wales Hospital, Sydney, New South Wales, Australia

[8]Prince of Wales Clinical School, University of New South Wales Sydney, Sydney, New South Wales, Australia

[9]Taylor Square Private Clinic, Sydney, New South Wales, Australia

[10]Holdsworth House Medical Practice, Sydney, New South Wales, Australia

[11]Department of Infectious Diseases, Alfred Health & Monash University, Melbourne, Victoria, Australia

[12]Burnet Institute, Melbourne, Victoria, Australia

Abstract

Background and Aims: In moving towards the elimination of hepatitis C virus (HCV) infection among people living with HIV, understanding HCV transmission patterns may provide insights to guide and evaluate interventions. In this study, we evaluated patterns of, and factors associated with HCV phylogenetic clustering among people living with HIV/HCV co-infection in Australia in the direct-acting antiviral era.

Methods: HCV RNA was extracted from dried blood spot (DBS) samples collected between 2014 and 2018 in the CEASE cohort study. The HCV Core-E2 region was amplified by a polymerase chain reaction and Sanger sequenced. Maximum likelihood phylogenetic trees (1000 bootstrap replicates) were used to identify patterns of clustering (3% genetic distance threshold). Mixed-effects logistic regression was used to determine correlates of phylogenetic clustering. Factors assessed were sexual risk behavior, education, injecting drug use, housing, employment, HIV viral load, age, sex, and sexuality.

Results: Phylogenetic trees were reconstructed for HCV subtype 1a ($n = 139$) and 3a ($n = 63$) sequences, with 29% (58/202) in a pair or cluster. Overall ($n = 202$), phylogenetic clustering was positively associated with younger age (under 40; adjusted odds ratio [aOR] 2.52, 95% confidence interval [CI] 1.20–5.29), and among gay and bisexual men ($n = 168$), was positively associated with younger age (aOR 2.61, 95% CI 1.10–6.19), higher education (aOR 2.58, 95% CI 1.09–6.13), and reporting high-risk sexual behavior

[13]Royal Adelaide Hospital, Adelaide, South Australia, Australia

[14]St Vincent's Hospital, Sydney, New South Wales, Australia

Correspondence
Marianne Martinello, The Kirby Institute, UNSW Sydney, Sydney, New South Wales, Australia.
Email: mmartinello@kirby.unsw.edu.au

(aOR 3.94, 95% CI 1.31–11.84). During follow-up, five reinfections were observed, but none were in phylogenetic clusters.

Conclusion: This study found a high proportion of phylogenetic relatedness, predominantly among younger people and gay and bisexual men reporting high-risk sexual behavior. Despite this, few reinfections were observed, and reinfections demonstrated little relationship with known clusters. These findings highlight the importance of rapid HCV treatment initiation, together with monitoring of the phylogeny.

KEYWORDS
gay and bisexual men (GBM), hepatitis C, HIV, molecular sequencing, phylogenetic analysis

1 | INTRODUCTION

Hepatitis C virus (HCV) infection is well established in populations of people who inject drugs (PWID).[1–3] An epidemic of HCV among gay and bisexual men who are living with HIV has more recently emerged,[4,5] with transmission related to both high-risk sexual and drug use behaviors linked mainly to sex practices involving drug use.[6] Molecular epidemiological studies examining phylogenetic clustering of recently acquired HCV infection in Australia have found that HIV co-infection and sexual acquisition of HCV were associated with phylogenetic clustering of HCV.[7,8] Given the high proportion of HCV phylogenetic clustering among gay and bisexual men living with HIV, both in Australia and internationally,[9,10] it has been hypothesized that HCV is transmitted within highly connected, but separate, sexual and drug use networks.[11] Because of the possible high degree of connectivity in these networks, the potential for sustained transmission, including HCV reinfection after treatment, has been flagged as a factor that may undermine HCV microelimination efforts.[12–15]

Molecular epidemiologic studies have begun to shed light on drivers behind the transmission of HCV that would not be possible to elucidate using either phylogenetic or epidemiologic techniques in isolation. In the setting of urban people who inject drugs who are living with HCV infection, it has been demonstrated that phylogenetic clustering of HCV is associated with social injecting networks, suggesting that having closely related viruses could indicate putative transmission links between hosts.[16] Additionally, phylogenetic studies of HCV/HIV co-infection have shown that phylogenetic clustering of HCV is observed among gay and bisexual men with HIV co-infection. However, many unrelated clusters comprising multiple HCV genotypes and subtypes are also observed[17,18] in these studies, suggesting multiple introductions of HCV into networks of HIV-positive gay and bisexual men occurred. While the direction of transmission cannot be determined by these molecular epidemiologic techniques, examining the patterns of phylogenetic clustering on a population level can provide many important insights that may otherwise continue to be concealed.

Evidence from Australia in the period after universal direct-acting antiviral (DAA) access began in March 2016 suggests that HCV reinfection rates among gay and bisexual men living with HIV were so far relatively low.[19,20] This was believed to be due to high coverage and uptake of DAAs among gay and bisexual men living with HIV.[21–23] However, the degree of phylogenetic clustering or network connectivity among specific populations before treatment scale-up is not known. Therefore, it remains possible that low HCV reinfection rates observed in these studies are also due to the make-up and structure of the networks through which HCV was transmitted. For example, populations with low levels of phylogenetic clustering could indicate that loosely connected sexual or drug use networks exist. Previous studies have found that HCV acquisition is associated with younger age,[24] female sex (vs. male sex),[25] recent injection drug use,[26] homelessness,[27] material deprivation,[14] and sexual behavior such as serosorting[28] or chemsex.[29] Investigation into the extent of HCV phylogenetic clustering and patterns and correlates of clustering with these previously identified factors is needed. Phylogenetic analyses of HCV sequences isolated from people in the period before and after DAA scale-up among gay and bisexual men living with HCV/HIV co-infection are particularly needed. Therefore, we set out to address this knowledge gap.

The research question addressed in this study is what are the patterns and correlates of HCV phylogenetic clustering among people living with HIV/HCV co-infection in Australia before and after the beginning of the direct-acting antiviral era?

The primary aim of this analysis was to investigate phylogenetic clustering of HCV subtypes and factors associated with clustering among people living with HIV/HCV co-infection at enrollment in the Control and Elimination of HCV from HIV-infected individuals within Australia (CEASE) study.[20]

2 | METHODS

2.1 | Study population and design

Data and specimens from the CEASE study were used for these analyses.[20] Briefly, CEASE is a prospective cohort study among people living with HIV/HCV coinfection in Australia. Adults

(≥18 years) living with HIV and with detectable anti-HCV antibody were enrolled between July 31, 2014 and March 22, 2017 at 14 sites across New South Wales, Queensland, South Australia, and Victoria. Participants with detectable HCV RNA were offered HCV treatment as per standard of care, and follow-up assessments were conducted between May 26, 2017 and May 31, 2018 for all participants.

2.2 | Study assessments and measures

At enrollment participants provided written informed consent, completed a questionnaire, had a dried blood spot (DBS; whole capillary blood collected via finger prick on a Whatman 903 Protein Saver Card [GE Healthcare] and allowed to air dry) sample collected, and underwent transient elastography with FibroScan®, (Echosens). DBS samples were stored at −80°C in individual double-seal gas-impermeable bags with at least 8 g of silica gel desiccant and a humidity indicator card (HIC). DBS cards were checked every 6 months and desiccant was replaced if any HIC indicated 40% or greater humidity accumulated. At follow-up, participants completed an abridged questionnaire, had a DBS sample collected, and underwent transient elastography with FibroScan®, (Echosens). Based on questionnaire responses, sexual risk behavior categories related to transmission potential were assigned for all participants for each time point as follows;

1. Low-risk sexual behavior: No regular or casual male partners; HIV-negative and HCV negative regular male partner-only (with or without anal intercourse); HIV-positive or HCV positive regular male partner only, condom use for all anal intercourse.

2. Intermediate or unknown-risk sexual behavior: Condomless anal intercourse with HIV-positive/unknown or HCV positive/unknown regular male partner; one or more casual male partner/s with condom use for all anal intercourse.

3. High-risk sexual behavior: Condomless anal intercourse with one or more casual male partners, including group sex.

2.3 | HCV RNA sequencing

Samples were stored up to a maximum of four years between collection and RNA extraction/sequencing (sequencing performed in 2018). A 10 mm disc of capillary blood-soaked filter paper was punched out from each DBS card, and then placed in a sterile 5 ml screw top tube. Each tube had 1000 μl of easyMAG lysis buffer (bioMérieux) added, then were incubated on a rotary blood wheel for 1 h at room temperature. After incubation, samples were centrifuged for 10 min at 1000 g, and the supernatant was pipetted in to an easyMAG sample vessel (bioMérieux). Viral RNA was extracted from DBS using the NucliSens easyMAG (bioMérieux). Reverse transcription of viral RNA with random hexamers was performed using Invitrogen Superscript (Vilo IV). Following reverse transcription, Core-E2 from nucleotides 347–1750 (H77 reference sequence,

GenBank accession no. NC_004102) was amplified by polymerase chain reaction (PCR) as per previously described methods.[30] Sanger sequencing was performed at the Australian Genome Research Facility on the Applied Biosystems 3730xl DNA Analyzer. Sequence curation was performed using RECall (beta v3.05).[31]

2.4 | Core-E2 phylogenetic analysis

Subtypes were determined by aligning sequences in ClustalW[32] with the panel of subtype reference sequences classified by Smith et al.[33] After alignment, a maximum likelihood phylogenetic tree was then constructed in RAxML.[34] After the determination of subtypes, two separate alignments of Core-E2 sequences were constructed in ClustalW[32] for subtype 3a and subtype 1a, respectively. These included HCV reference sequences obtained from the Los Alamos National Laboratory HCV Database[35] and from previous sequencing studies conducted in Australia[7,36,37] to improve cluster resolution and aid in identification of local transmission patterns. PCR primer sequences and hypervariable region one (HVR1) were trimmed from both alignments to improve cluster resolution.[30] The final sequence length was 1300 base pairs. Maximum likelihood phylogenetic trees with 1000 bootstrap replicates were constructed in RAxML[34] for the two subtype-specific alignments. The alignments were constructed separately under the General Time Reversible model of nucleotide substitution with a gamma-shaped distribution of rate variation across sites, utilizing the CIPRES Science Gateway.[38] Clusters were identified using ClusterPicker[39] with a 3% mean maximum genetic distance cut off and 90% bootstrap support threshold. The genetic distance cutoff was selected based on previous phylogenetic analyses among HCV Core-E2 sequences obtained from people with acute HCV infection in Australia in the time period immediately before CEASE recruitment.[7,36] These analyses determined that 5% mean maximum genetic distance cutoff and 90% bootstrap support threshold provided optimal detection of phylogenetic pairs and clusters for people with acute HCV infection. As CEASE participants had an unknown duration of HCV infection, a more conservative cluster cutoff of 3% mean maximum genetic distance was chosen for these analyses. The resulting phylogenetic trees were visualized as both cladograms and phylograms, and had the clustering information and other clinical and demographic details mapped on to them using EvolView V3.[40]

2.5 | Statistical analyses

Clinical, demographic, and behavioral characteristics were described overall among the study cohort, then exploratory analysis was conducted to determine the relationship between injecting and drug use behaviors between gay and bisexual men, compared with heterosexual men and all women, who had an HCV sequence obtained at enrollment. Clinical, demographic, and behavioral characteristics were then described with respect to whether

participants were classified as unconnected or connected (either in a pair or a cluster). The selection of characteristics examined was determined a priori and was based on factors found to be associated with HCV acquisition or HCV transmission clusters in previous studies.[9,10,18,41–45] Statistical associations with phylogenetic clustering were assessed by χ^2, Fisher's exact test, t test, and Mann–Whitney U test, as appropriate. Additional factors were identified through univariate logistic regression analyses. All variables with $p < 0.20$ in univariate analyses were entered into the adjusted logistic regression model, with analysis performed among two populations: 1. Overall, and 2. gay and bisexual men. To account for potential unmeasured confounding introduced by sexual or drug use networks within neighborhoods that participants were recruited in mixed-effects logistic regression analysis was performed for the adjusted analysis, with a random intercept for the study site where the participant was recruited. For all analyses, statistically significant differences were assessed at $p < 0.05$; p values are two-sided. All analyses were performed using STATA software (version 14; StataCorp L.P.).

2.6 | Patient consent statement

The CEASE study protocol was approved by St Vincent's Hospital, Sydney Human Research Ethics Committee (primary study committee), as well as by the institutional review board or independent ethics committee at each participating site and was conducted according to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines and local regulatory requirements. The study was registered with ClinicalTrials.gov (NCT02102451). The CEASE Study protocol and participant information and consent forms all contained explicit provision for the determination of genetic sequences of HCV in participant samples, and assessment of phylogenetic relationships between the viruses. All study participants provided written informed consent before study procedures.

3 | RESULTS

3.1 | Participants that had an HCV Core-E2 sequence generated from enrollment samples

Overall, 291 participants had current HCV infection (detectable HCV RNA) at enrollment. Sequences from the Core-E2 region of the HCV genome were able to be generated from 84% (243/291) of participant specimens (Table 1). Among those who had a Core-E2 sequence obtained, median age was 48 years and 95% (231/243) were male (Table 1). Among participants who had a Core-E2 sequence obtained, there were differences in the prevalence of sexual and drug use behaviors among gay and bisexual men compared to heterosexual men and all women (Table 2). Among gay and bisexual men, 44% (86/195) reported current injecting drug

use, whereas 29% (14/48) of heterosexual men and all women reported current injecting drug use. While 50% (97/195) of gay and bisexual men likely acquired HCV through injecting drug use, 73% (35/48) of heterosexual men and all women likely acquired HCV through injecting drug use.

3.1.1 | Characteristics of participants in Core-E2 phylogenetic tree reconstruction

Among 243 Core-E2 sequences from specimens collected at enrollment, 202 were included in phylogenetic tree reconstruction and clustering analysis (Table 3); 139 subtype 1a sequences (Figures 1 and S1) and 63 subtype 3a sequences (Figures 2 and S1). Due to the small numbers of

TABLE 1 Enrollment demographic and clinical characteristics of participants who had a Core-E2 sequence obtained among participants in the CEASE study who had specimen collected with detectable hepatitis C virus (HCV) viremia

Characteristics, n (%)[1]	Participants with Core-E2 sequence obtained $N = 243$
Age, median (IQR)	48 (42, 54)
Male sex	231 (95)
Gay and bisexual men[2]	195 (80)
Completed higher education	136 (57)
Stable housing	217 (89)
Full or part-time employed	97 (40)
HIV viral load below <50 copies/ml	197 (81)
HIV viral load undetectable	162 (67)
Mode of HCV acquisition[10]	
Injecting drug use	132 (54)
Sexual exposure	79 (33)
Other	32 (13)
HCV subtype	
1a	142 (58)
1b	21 (9)
2	8 (3)
3a	64 (26)
3b/k	2 (1)
4	5 (2)
6	1 (1)
Injecting drug use	
Never[3]	45 (19)
Ever[4]	97 (40)
Current[5]	100 (41)

TABLE 1 (Continued)

Characteristics, n (%)[1]	Participants with Core-E2 sequence obtained N = 243
Sexual risk behavior[6]	N = 195
Low risk[7]	48 (25)
Intermediate or unknown risk[8]	37 (19)
High risk[9]	110 (56)

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; LOD, level of detection; n, number.

[1]Column percentages.

[2]Cis-gendered men who self-identified their sexuality as gay or bisexual.

[3]Never: No history of injection drug use.

[4]Ever: History of injection drug use, with no use in the 6 months before the study visit.

[5]Current: Injection drug use within 6 months of the study visit.

[6]Among gay and bisexual men only (n = 195)

[7]Low risk sexual behavior: No regular or casual male partners; HIV-negative, regular male partner-only living without HCV infection (with or without anal intercourse); HIV-positive or HCV positive regular male partner only, condom use for all anal intercourse.

[8]Intermediate or unknown-risk sexual behavior: Condomless anal intercourse with HIV-positive/unknown or HCV positive/unknown regular male partner; 1 or more casual male partner/s with condom use for all anal intercourse.

[9]High-risk sexual behavior: Condomless anal intercourse with 1 or more casual male partners, including group sex.

[10]Mode of HCV acquisition was clinician assigned.

TABLE 2 Comparison of injecting drug use and mode of HCV acquisition between gay and bisexual men and heterosexual men and all women in the CEASE study who had a Core-E2 hepatitis C virus (HCV) sequence obtained at enrollment

Total n (%)[1]	Overall N = 243	Gay and bisexual men[2] N = 195	Heterosexual men and all women[3] N = 48
Injecting drug use			
Never[4]	45 (19)	36 (18)	9 (19)
Ever[5]	97 (40)	72 (37)	25 (52)
Current[6]	100 (41)	86 (44)	14 (29)
Unknown	1 (0)	1 (1)	0 (0)
Mode of HCV acquisition[7]			
Injecting drug use	132 (54)	97 (50)	35 (73)
Sexual exposure	79 (33)	74 (38)	5 (10)
Other	32 (13)	24 (12)	8 (17)

Abbreviations: HCV, hepatitis C virus; n, number.

[1]Column percentages.

[2]Cis-gendered men who self-identified their sexuality as gay or bisexual

[3]Cis or trans-gendered women who self-identified their sexuality as gay, bisexual or heterosexual, and cis or trans-gendered men who self-identified their sexuality as heterosexual.

[4]Never: No history of injection drug use.

[5]Ever: History of injection drug use, with no use in the 6 months before the study visit.

[6]Current: Injection drug use within 6 months of the study visit.

[7]Mode of HCV acquisition was clinician assigned.

Core-E2 sequences obtained from non-1a and 3a subtypes (n = 37), phylogenetic tree reconstruction and clustering analysis were not attempted for these. Among those included in the phylogenetic clustering analysis, the median age was 45 years, with 58% (116/202) completing higher education and 90% (180/202) having stable housing. HIV viral load was below <50 copies/ml among 67% (134/202) of participants in the phylogenetic reconstruction, and 42% (83/202) had full or part-time employment. Among gay and bisexual men, the proportion of people in the high sex risk behavior category was 56% (95/195).

3.1.2 | Factors associated with clustering among HCV Core-E2 sequences from enrollment samples

Among sequences from enrollment specimens included in the phylogenetic analysis, 29% (58/202) were phylogenetically linked to one or more other CEASE enrollment sequences. In overall unadjusted analyses, age 40 years or younger, identifying as a gay or bisexual man, completing higher education, acquiring HCV infection through sexual exposure, having HCV subtype 3a infection, and current injecting drug use, were associated with phylogenetic clustering (Table 3) In unadjusted analyses among gay and bisexual men, age 40 years or younger, completing higher education, having stable housing, being full or part-time employed,

acquiring HCV infection through sexual exposure, current injecting drug use, and reporting high-risk sexual behavior were associated with phylogenetic clustering (Table 4). In overall adjusted analyses using mixed-effects model with a random intercept for site where participant was recruited from, age 40 years or younger was associated with phylogenetic clustering of Core-E2 sequences (Table 3). There was not considerable variation between subjects, with the estimated standard deviation in the random intercept for the final model being <0.001 (standard error 0.19). Among gay and bisexual men, age 40 years or younger, completion of higher education and reporting high-risk sexual behavior were associated with phylogenetic clustering (Table 4).

3.1.3 | Participants that had an HCV Core-E2 sequence obtained from postenrolment samples

Specimens obtained from follow-up timepoints where HCV RNA was detected were subjected to PCR amplification of the Core-E2 region, with an additional 12 sequences obtained (Table 5). Among these, four sequences were from participants who did not receive HCV treatment and had persistent chronic HCV infection. There were six sequences obtained from participants who received treatment after enrollment but did not achieve sustained virological response (SVR). The remaining two

TABLE 3 Mixed-effects logistic regression of factors associated with phylogenetic clustering among hepatitis C virus (HCV) subtype 1a and 3a Core-E2 sequences (at 3% genetic distance and 90% bootstrap support thresholds) obtained at enrollment among participants from the CEASE study

Characteristic total n (%)[1]	Overall N = 202	Unclustered N = 144	Clustered N = 58	Membership in cluster n ≥ 2					
				Unadjusted			Adjusted		
				Odds ratio	95% CI	p value	Odds ratio	95% CI	p value
Age ≤40 years (vs. >41 years)	45 (22)	23 (16)	22 (40)	3.21	1.61–6.43	0.001	2.52	1.2–5.29	0.02
Male sex (vs. female sex)[a]	194 (97)	137 (96)	57 (99)	2.08	0.24–18.20	0.51	–	–	–
Gay and bisexual men (vs. heterosexual men and all women)	168 (84)	114 (80)	54 (94)	3.55	1.19–10.59	0.02	2.34	0.72–7.55	0.156
Completed higher education (vs. completed only high school or less)	116 (58)	74 (52)	42 (73)	2.48	1.28–4.81	0.007	1.91	0.92–3.95	0.08
Stable housing (vs. unstable housing)	180 (90)	126 (88)	54 (94)	1.93	0.62–5.97	0.25	–	–	–
Full or part-time employed (vs. unemployed or other[2])	83 (42)	52 (36)	31 (54)	2.03	1.10–3.77	0.25	–	–	–
HIV viral load below <50 copies/ml (vs. ≥50 copies/ml)	134 (67)	95 (66)	39 (68)	1.04	0.54–1.98	0.91	–	–	–
Mode of HCV acquisition[3]									
Injecting drug use	111 (55)	86 (60)	25 (44)	Ref	–	–	Ref	–	–
Sexual exposure	67 (34)	39 (28)	28 (49)	2.47	1.27–4.77	0.007	2.27	0.42–3.19	0.5
Other	24 (12)	19 (14)	5 (9)	0.91	0.31–2.67	0.86	0.81	0.93–4.67	0.73
HCV subtype									
1a	139 (69)	103 (72)	36 (63)	Ref	–	–	Ref	–	–
3a	63 (32)	41 (29)	22 (38)	1.23	0.90–1.71	0.19	1.52	0.75–3.09	0.25
Injecting drug use									
Ever[4]	73 (37)	59 (41)	14 (25)	Ref	–	–	Ref	–	–
Never[5]	39 (20)	26 (19)	13 (23)	2.11	0.87–5.10	0.10	1.16	0.42–3.19	0.74
Current[6]	89 (45)	58 (41)	31 (54)	2.25	1.09–4.66	0.03	2.09	0.93–4.67	0.07

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; LOD, level of detection; n, number.

[a]Not included in adjusted model due to collinearity.

[1]Column percentages.

[2]Other included unemployment benefits, disability pension, retirement fund, self-employed, dependent on others, or student allowance.

[3]Mode of HCV acquisition was clinician assigned.

[4]Ever: History of injection drug use, with no use in the 6 months before the study visit.

[5]Never: No history of injection drug use.

[6]Current: Injection drug use within 6 months of the study visit.

sequences were from participants who cleared their HCV infection after treatment but subsequently had an HCV reinfection. Only one sequence obtained at follow-up was linked to another sequence from a different (untreated) participant at enrollment.

4 | DISCUSSION

This study characterized phylogenetic clustering among people living with HIV/HCV co-infection pre and post scale-up of DAA therapy in Australia. A high proportion of sequences were observed to be closely related to other participant sequences before DAA availability and broad access. There appeared to be little impact of phylogenetic transmission clusters on reinfection, with very few reinfections identified following DAA scale-up. Overall, these findings suggested that where HCV treatment uptake is high, even with a high proportion of phylogenetic clustering and on-going risk behavior, DAA therapy is highly effective and low levels of HCV reinfection are observed.

The proportion of phylogenetic clustering observed in this study was 29% (58/202), indicating that one in three participants were infected with HCV subtype 1a or 3a variants that were highly related to each other. While phylogenetic clustering is not a direct marker of transmission, it does give an indication of how

IDU Category
★ Recent
★ Not Recent
★ No history

Sex
■ Male
■ Female
■ Transgender

Sexual Orientation
▲ Gay/Lesbian
▲ Heterosexual/Straight
▲ Bisexual

MSM Sex-Risk Category
● High
● Intermediate or unknown
● No or low
○ Non-applicable
○ Missing Data

FIGURE 1 Radial cladogram phylogenetic tree of Core-E2 sequences (HCV genotype [GT] 1a H77 reference position 347–1750) obtained from 139 CEASE participants at study enrollment between 2014 and 2016 with subtype 1a infection. This Maximum likelihood tree was inferred with RAxML under the general time-reversible model of nucleotide substitution with a gamma rate distribution.

closely related the networks through which HCV is being transmitted in the community are.[46,47] The proportion of phylogenetic clustering observed here is consistent with that observed in previous molecular epidemiologic studies of chronic HCV infection among people living with HIV.[41,42,44,45,48–50] This study supports previous findings indicating that HCV is transmitted in Australia within networks of people living with HIV, particularly among gay and bisexual men.[37,43,51]

In this study, among gay and bisexual men, HCV phylogenetic clustering was associated with younger age, completion of higher education, and high-risk sexual behavior. Among the study population overall, younger age was associated with phylogenetic clustering of HCV. Markers of material and social privilege, such as completing higher education, and phylogenetic clustering between people living with HCV/HIV co-infection suggest the need to tailor HCV prevention messaging in Australia to very

FIGURE 2 Radial cladogram phylogenetic tree of Core-E2 sequences (HCV genotype [GT] 1a H77 reference position 347–1750) obtained from 63 CEASE participants at study enrollment between 2014 and 2016 with subtype 3a infection. This Maximum likelihood tree was inferred with under the general time-reversible model of nucleotide substitution with a gamma rate distribution.

specific population demographic groups. Further, people with lower levels of education may have lower levels of health literacy,[52] resulting in fewer of them presenting to services to get tested or treated,[53] and being underrepresented in studies such as this. This may result in phylogenetic clustering being skewed towards people with higher levels of education due to them presenting to services more frequently. While current injecting drug use and being in the high sexual risk behavior category were both associated with clustering in the unadjusted analyses, only the high sexual risk behavior category was independently associated with clustering among gay and bisexual men. Injecting drug use probably plays some role in HCV transmission among people living with HIV, however, this study suggests that being in the high sexual risk behavior category remains the greatest independent predictor of phylogenetic clustering among gay and bisexual men living with HIV.

TABLE 4 Mixed-effects logistic regression of factors associated with phylogenetic clustering among hepatitis C virus (HCV) subtype 1a and 3a Core-E2 sequences (at 3% genetic distance and 90% bootstrap support thresholds) obtained at enrollment among gay and bisexual participants from the CEASE study

				Membership in cluster $n \geq 2$					
	Overall	Unclustered	Clustered	Unadjusted			Adjusted		
Characteristic total n (%)[1]	N = 168	N = 114	N = 54	Odds ratio	95% CI	p value	Odds ratio	95% CI	p value
Age ≤40 years (vs. >41 years)	40 (24)	19 (17)	21 (39)	3.18	1.52–6.64	0.002	**2.61**	**1.1–6.19**	**0.03**
Completed higher education (vs. completed only high school or less)	104 (62)	63 (56)	41 (76)	2.55	1.24–5.27	0.011	**2.58**	**1.09–6.13**	**0.03**
Stable housing (vs. unstable housing)	151 (90)	100 (88)	51 (95)	2.38	0.65–8.66	0.19	4.37	0.95–20.18	0.059
Full or part-time employed (vs. unemployed or other[2])	76 (46)	45 (40)	31 (58)	2.07	1.07–3.99	0.03	0.69	0.27–1.72	0.42
HIV viral load below <50 copies/ml (vs. ≥50 copies/ml)	112 (67)	76 (67)	36 (67)	1.03	0.52–2.05	0.94			
Mode of HCV acquisition[3]									
Injecting drug use	81 (49)	59 (52)	22 (41)	Ref	–	–	Ref	–	–
Sexual exposure	64 (39)	37 (33)	27 (50)	1.96	0.97–3.93	0.06	1.54	0.62, 3.83	0.348
Other	23 (14)	18 (16)	5 (10)	0.74	0.25–2.25	0.6	0.66	0.19, 2.31	0.51
HCV subtype									
1a	117 (70)	82 (72)	35 (65)	Ref	–	–			
3a	51 (30)	32 (28)	19 (35)	1.18	0.83–1.67	0.35			
Injecting drug use									
Never[5]	35 (21)	22 (20)	13 (25)	Ref	–	–	Ref	–	–
Ever[4]	56 (34)	43 (38)	13 (25)	0.51	0.2–1.29	0.16	0.69	0.23–2.04	0.497
Current[6]	76 (46)	48 (43)	28 (52)	0.99	0.43–2.26	0.98	1.1	0.37–3.25	0.86
Sexual risk behavior									
Low risk[7]	43 (26)	37 (33)	6 (12)	Ref	–	–	Ref	–	–
Intermediate or unknown risk[8]	30 (18)	23 (21)	7 (13)	1.88	0.56–6.28	0.31	1.37	0.38–4.98	0.63
High risk[9]	95 (57)	54 (48)	41 (76)	4.68	1.8–12.15	0.002	**3.94**	**1.31–11.84**	**0.01**

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; LOD, level of detection; n, number.

[1]Column percentages.

[2]Other included unemployment benefits, disability pension, retirement fund, self-employed, dependent on others, or student allowance.

[3]Mode of HCV acquisition was clinician assigned.

[4]Ever: History of injection drug use, with no use in the 6 months before the study visit.

[5]Never: No history of injection drug use.

[6]Current: Injection drug use within 6 months of the study visit.

[7]Low risk sexual behavior: No regular or casual male partners; HIV-negative, regular male partner-only living without HCV infection (with or without anal intercourse); HIV-positive or HCV positive regular male partner only, condom use for all anal intercourse.

[8]Intermediate or unknown-risk sexual behavior: Condomless anal intercourse with HIV-positive/unknown or HCV positive/unknown regular male partner; 1 or more casual male partner/s with condom use for all anal intercourse.

[9]High risk sexual behavior: Condomless anal intercourse with 1 or more casual male partners, including group sex.

Despite consistent levels of sexual risk behavior among CEASE participants who identified as gay and bisexual men between enrollment and follow-up,[21] it does not appear to have increased the risk that people who have an HCV reinfection will be part of a phylogenetic cluster. Overall, there were five reinfections observed during follow-up; of the two which were able to have HCV sequences obtained, neither belonged to phylogenetic clusters. Although the CEASE study participants are not a random sample of the population, they are likely to be somewhat representative of people living with HIV who are retained in care in urban centers, which is thought to be about 85% of people living with HIV in Australia.[54] Therefore this finding may be generalizable to the greater population of people

TABLE 5 Characteristics of participants in the CEASE study who had HCV viremia HCV at follow-up 1 timepoint and an HCV Core-E2 sequence was able to be obtained

Participant ID	Sex, age	Gay or bisexual man	Clinical status at follow-up 1	HCV subtype at enrollment	HCV subtype at follow-up 1	Phylogenetic cluster status at enrollment	Phylogenetic cluster status at follow-up 1
1208-61239-010	Male, 66	Yes	Reinfected	1a	1a	Unlinked	Unlinked
1208-61202-007	Male, 51	Yes	Non-SVR*	3a	3a	Unlinked	Linked to own enrollment sequence
1208-61202-107	Male, 53	Yes	Non-SVR*	1a	1a	Unlinked	Linked to own enrollment sequence
1208-61203-025	Male, 43	Yes	Non-SVR*	1a	1a	Unlinked	Linked to own enrollment sequence
1208-61240-003	Male, 54	Yes	Untreated	1a	1a	Sequence not obtained	Linked
1208-61215-015	Male, 61	Yes	Untreated	3a	3a	Unlinked	Linked to own enrollment sequence
1208-61202-077	Male, 47	Yes	Reinfected	1a	1a	Unlinked	Unlinked
1208-61215-010	Male, 32	Yes	Non-SVR*	1a	1a	Unlinked	Unlinked
1208-61215-004	Male, 50	Yes	Non-SVR*	3a	3a	Unlinked	Unlinked
1208-61215-023	Male, 45	Yes	Non-SVR*	3a	3a	Unlinked	Unlinked
1208-61215-007	Male, 39	Yes	Untreated	2b	2b	Unlinked	Unlinked
1208-61215-039	Male, 54	Yes	Untreated	3a	3a	Unlinked	Unlinked

Abbreviations: HCV, hepatitis C virus; SVR, sustained virological response.

*Non-SVR includes treatment nonresponse, relapse and virological breakthrough among those who had treatment but had detectable HCV RNA at follow-up 1 timepoint.

living with HIV in Australia, or other high-income countries with similar epidemiology of HIV and HCV infection.

While this study is a large sample of people living with HCV/HIV co-infection, it does have some limitations. First, it was recruited through sites providing clinical care, therefore people not engaged in clinical care would not have been be sampled in this study. Second, due to the high degree of genetic variability within HCV genotypes and subtypes, phylogenetic trees were constructed separately for each subtype. As the numbers of certain subtypes and genotypes in the cohort were so small (1b, 2, 3b/3k, 4, and 6), we did not attempt to construct phylogenetic trees for these sequences. Excluding these participants from the phylogenetic clustering analysis may have slightly under or overestimated the proportion of phylogenetic clustering. Additionally, because so few heterosexual men or women were included in this study, stratified analysis of factors associated with phylogenetic clustering were not able to be assessed for this subpopulation.

Overall, these findings suggest that despite sustained drug and sexual transmission risk behaviors and a high proportion of HCV phylogenetic clustering, HCV micro-elimination among people living with HIV is extremely feasible with high HCV treatment uptake. DAA treatment was successful among people living with HCV/HIV co-infection, with low levels of re-infection detected. Sustaining high HCV testing and treatment uptake among people living with HCV/HIV co-infection is necessary to ensure networks do not become susceptible to viral re-emergence. Continued monitoring of patterns and correlates of phylogenetic clustering will aid in detecting early signals of viral re-emergence in networks and can be useful to distinguish between reinfection and treatment failure. Robust surveillance and monitoring, including sequencing of HCV reinfection cases, may be required to ensure Australia stays on track to eliminate HCV as a public health threat by 2030.

AUTHOR CONTRIBUTIONS

Sofia R. Bartlett: Conceptualization; data curation; formal analysis; investigation; writing—original draft; writing—review & editing. **Andrey Verich**: Formal analysis; investigation; writing—review & editing. **Joanne Carson**: Data curation; investigation; writing—review & editing. **Samira Hosseini-Hooshyar**: Data curation; investigation; writing—review & editing. **Phillip Read**: investigation; writing—review & editing. **David Baker**: Investigation; writing—review & editing. **Jeffrey J. Post**: Investigation; writing—review & editing. **Robert Finlayson**: Investigation; writing—review & editing. **Mark Bloch**: Investigation; writing—review & editing. **Joseph S. Doyle**: Investigation; writing—review & editing. **David Shaw**: Investigation; writing—review & editing. **Margaret Hellard**: investigation; writing – review & editing. **Maria Martinez**: data curation; investigation; writing – review & editing. **Philippa Marks**: Data curation; funding acquisition; investigation; writing—review & editing. **Gregory J. Dore**: Conceptualization; funding acquisition; investigation; writing—review & editing. **Gail V. Matthews**: Conceptualization; funding acquisition; investigation; supervision; writing —review & editing. **Tanya Applegate**: Conceptualization; funding acquisition; investigation; supervision; writing—original draft; writing—review & editing. **Marianne Martinello**: Conceptualization; investigation; methodology; supervision; writing—review & editing.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

S.R.B. has received speakers' honoraria and participated in medical advisory board programs with Gilead Sciences and AbbVie (all personal payments given as unrestricted donation to BC Centre for Disease Control Foundation), and received research funding from Gilead Sciences through her institution. G.J.D. is a consultant/advisor and has received research grants from Abbvie, Bristol Myers Squibb, Gilead, Merck, Janssen and Roche. JSD has received consultancies to his institution from Gilead, Abbvie and Merck. J.S.D. and M.H. receives investigator-initiated research funding from Gilead Sciences, Abbvie, Merck, and BMS. P.R. has received speaker's honoraria from Gilead Sciences and Abbvie, and research funding from Gilead Sciences. No commercial entities nor the supporting/funding source had any involvement in study design, data collection, data analysis, interpretation of data, writing of the report, or the decision to submit the report for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

DISCLAIMER

The views expressed in this publication do not necessarily represent the position of the Australian Government. The content is solely the responsibility of the authors.

TRANSPARENCY STATEMENT

Sofia Bartlett affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ORCID

Sofia R. Bartlett (ID) http://orcid.org/0000-0001-9444-0186
Joanne Carson (ID) http://orcid.org/0000-0001-8043-0200

Phillip Read 🆔 http://orcid.org/0000-0001-5422-3777

Margaret Hellard 🆔 http://orcid.org/0000-0002-5055-3266

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.