Methamphetamine injecting is associated with phylogenetic clustering of hepatitis C virus infection among street-involved youth in Vancouver, Canada*

Short title: HCV phylogenetics in street-involved youth

Author order: Evan Cunningham**¹, Brendan Jacka¹, Kora DeBeck^{2,3}, Tanya A Applegate¹, P. Richard Harrigan², Mel Krajden⁵, Brandon DL Marshall⁶, Julio Montaner^{2,4}, Viviane Dias Lima^{2,4}, Andrea Olmstead⁵, M-J Milloy^{2,4}, Evan Wood^{2,4}, and Jason Grebely¹

¹Viral Hepatitis Clinical Research Program, The Kirby Institute, UNSW Australia, Sydney NSW, Australia, ²British Columbia Centre for Excellence in HIV/AIDS, St Paul's Hospital, Vancouver BC, ³School of Public Policy, Simon Fraser University, Vancouver, BC, Canada, ⁴Division of AIDS, Department of Medicine, Faculty of Medicine, University of British Columbia, ⁵BC Centre for Disease Control, Vancouver BC, ⁶Department of Epidemiology, Brown University School of Public Health, Providence, RI.

**Corresponding Author

**Viral Hepatitis Clinical Research Program
The Kirby Institute
UNSW Australia
NSW, Australia, 2052
ecunningham@kirby.unsw.edu.au**

ABSTRACT

Background: Among prospective cohorts of people who inject drugs (PWID), phylogenetic clustering of HCV infection has been observed. However, the majority of studies have included older PWID, representing distant transmission events. The aim of this study was to investigate phylogenetic clustering of HCV infection among a cohort of street-involved youth. **Methods:** Data were derived from a prospective cohort of street-involved youth aged 14-26 recruited between 2005 and 2012 in Vancouver, Canada (At Risk Youth Study, ARYS). HCV RNA testing and sequencing (Core-E2) were performed on HCV positive participants. Phylogenetic trees were inferred using maximum likelihood methods and clusters were identified using ClusterPicker (Core-E2 without HVR1, 90% bootstrap threshold, 0.05 genetic distance threshold). **Results:** Among 945 individuals enrolled in ARYS, 16% (n=149, 100% recent injectors) were HCV antibody positive at baseline interview (n=86) or seroconverted during follow-up (n=63). Among HCV antibody positive participants with available samples (n=131), 75% (n=98) had detectable HCV RNA and 66% (n=65, mean age 23, 58% with recent methamphetamine injection, 31% female, 3% HIV+) had available Core-E2 sequences. Of those with Core-E2 sequence, 14% (n=9) were in a cluster (one cluster of three) or pair (two pairs), with all reporting recent methamphetamine injection. Recent methamphetamine injection was associated with membership in a cluster or pair ($P=0.009$).

Conclusion: In this study of street-involved youth with HCV infection and recent injecting, 14% demonstrated phylogenetic clustering. Phylogenetic clustering was associated with recent methamphetamine injection, suggesting that methamphetamine drug injection may play an important role in networks of HCV transmission.

KEYWORDS: injection drug use; street-involved youth; phylogenetics; methamphetamine; clustering; phylogenetic clustering

1. INTRODUCTION

The burden of hepatitis C virus (HCV) infection among people who inject drugs (PWID) is high, with an estimated midpoint prevalence of 67% globally (Nelson et al., 2011). Further, HCV incidence remains high (7 to 25 cases per 100 person-years), particularly among young PWID and street-involved, at-risk youth (Grebely et al., 2014; Hadland et al., 2014; Page et al., 2013).

Ongoing HCV transmission occurs, in part, because prevention measures that have been successful in preventing HIV infection, such as needle and syringe programs (NSP) and opioid substitution therapy (OST) have had less success in preventing HCV transmission (Nolan et al., 2014; Turner et al., 2011; White et al., 2014). As such, novel insights to inform strategies to prevent HCV transmission are needed.

Street-involved youth and recent initiates to injecting are at a particularly high risk of HCV infection (Page et al., 2013). Among young drug users, factors associated with HCV infection include: age >18 years, unstable housing (Kim et al., 2009), and recent injection drug use (Hadland et al., 2014). However, traditional epidemiological studies evaluating factors associated with HCV incidence measure the risk of acquisition of the infection, not transmission.

Phylogenetic studies can model underlying transmission patterns that cannot be determined using epidemiological studies. Phylogenetic studies have the ability to identify transmission networks, allowing for the identification of factors that are associated with phylogenetic clustering of HCV infection. Recently, in a cohort of long-term PWID from Vancouver, Canada, factors independently associated with phylogenetic clustering of HCV included age <40 years, HIV co-infection, HCV seroconversion, and recent syringe borrowing (Jacka et al., 2014). However, previous work evaluating phylogenetic clustering among PWID is limited in that the majority of participants were older and had chronic HCV infection at the time of sampling; therefore, factors associated with clustering may have been related to more distant transmission events. Further data

is needed to understand HCV transmission among younger individuals with recent HCV infection, given that such information would better reflect current HCV epidemic dynamics. The aim of this study was to investigate phylogenetic clustering of HCV infection and associated factors among a cohort of street-involved youth in Vancouver, Canada between 2005 and 2012.

2. METHODS

2.1 Study population and design

Data were derived from a prospective cohort of street-involved youth recruited between 2005 and 2012 in Vancouver, Canada (At Risk Youth Study, ARYS). Eligibility criteria included age 14-26 years, self-report use of illicit drugs other than or in addition to marijuana in the past month, and ability to provide informed consent. Beginning in 2005, youth who fit the above profile were recruited in the Greater Vancouver region through methods described in detail elsewhere (Wood et al., 2006). Enrolment was conducted through targeted street-based outreach, and efforts to have street youth recruit their peers. Given that ARYS is an open prospective cohort, new participants were continuously enrolled in the cohort over the study period to replace those who died or were lost to follow-up. Participants provided written informed consent prior to entering the study. The University of British Columbia/Providence Health Care Research Ethics Board approved this study.

For the current study, all participants who: 1) were HCV antibody-positive at enrolment; or 2) demonstrated HCV seroconversion (defined by an HCV antibody negative test at enrolment followed by an HCV antibody positive test at a subsequent study visit) between 2005 and 2012, with an available sample for HCV RNA testing and sequencing who were recent injectors were eligible for inclusion in the analytic sample.

2.2 Study assessments

At enrolment and semi-annually, participants completed an interviewer-administered questionnaire. Data on socio-demographic characteristics, as well as information pertaining to drug use patterns and risk behaviours were collected. Nurses collected blood samples for HIV and hepatitis C virus serology, and also provided basic medical care and referrals to appropriate health care services. Participants received \$20 for each study visit.

2.3 HCV RNA testing and sequencing

HCV RNA was quantified using an in-house PCR as described elsewhere (Meng and Li, 2010). Sequencing was attempted on all samples with detectable HCV RNA. Complementary DNA (cDNA) was generated using SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies) with random hexamers. A fragment of the HCV genome covering Core, Envelope-1 and the hypervariable region-1 (HVR1) was amplified using a method previously described (Jacka et al., 2014). Purified amplicons were sequenced using the Sanger method and sequence chromatograms processed using RECall: a fully automated sequence analysis pipeline (Jacka et al., 2014). Reverse transcription, PCR and sequencing reaction and thermal cycling conditions are described in Supplementary Information¹.

2.4 Phylogenetics

Among participants with an available sequence, phylogenetic trees of the Core-E2 segment not including the hypervariable region-1 (HVR1) were inferred using maximum-likelihood analysis implemented in RAxML through the CIPRES Science Gateway under the General Time Reversible model of nucleotide substitution with substitution rate heterogeneity, as previously described (Jacka

et al., 2014). Reference sequences obtained from the Los Alamos National Laboratory HCV database were included to support identification of “local” clusters, and were aligned to study sequences using ClustalW (Jacka et al., 2014). The robustness of the resulting tree was assessed by bootstrapping with 1000 replicates, and clusters were identified using ClusterPicker software with a bootstrap threshold of 90% and a maximum genetic distance threshold of 0.05 (Jacka et al., 2014).

2.5 Statistical analyses

The primary study outcome was phylogenetic clustering of HCV infections (defined by two or more participants with HCV genome sequence satisfying bootstrap and genetic distance threshold requirements). The proportion of participants in a pair ($n=2$ participants within genetic distance and bootstrap thresholds) or cluster ($n\geq 3$ participants) were identified. Characteristics of participants in a pair or cluster were compared to those without membership in a cluster or pair using Fisher’s exact tests. Hypothesized factors were determined a priori on the basis of factors previously shown to be associated with HCV clustering or HCV acquisition. These factors included: sex (Patrick et al., 2001), age (Roy et al., 2009) and drug use (including crack cocaine smoking, injecting heroin, injecting cocaine, and injecting methamphetamines in the last 6 months (all yes vs. no) according to the survey prior to the sample collection (Hadland et al., 2014). For participants infected at baseline, data was derived from the baseline survey while data for participants who seroconverted were taken from the survey done at the time of the sample collection immediately following seroconversion.

3. RESULTS

In total, 945 participants were enrolled in ARYS between 2005 and 2012. Of those, 9% ($n=86$) were HCV antibody positive at enrolment. Among those antibody negative at enrolment ($n=859$), 7% ($n=63$) demonstrated HCV seroconversion during follow-up. Among all HCV positive participants ($n=149$), 100% were recent injectors. Among HCV antibody positive participants with available samples ($n=131$), 75% ($n=98$) had detectable HCV RNA, with 66% ($n=65$) having

available Core-E2 sequences. Factors previously shown to be associated with an inability to obtain a sequence in those with detectable HCV RNA include HCV RNA <10,000 IU/mL and sample volume <200µL (Jacka et al., 2014).

The participant characteristics for those with available Core-E2 sequences (n=65) are outlined in Table 1. Participants had a median age of 23 years (Q1-Q3: 21-25), 31% (n=20) were female, 60% (n=39) reported recent (last 6 months) methamphetamine injection, and 3% (n=2) were HIV-infected. HCV genotype prevalence was: G1a: 42% (n=27), G3a: 51% (n=33), G2b 5% (n=3) and G1a 3% (n=2).

As shown in the phylogenetic tree inferred using maximum-likelihood analysis (n=65, Supplementary Figure 1), 14% (n=9) were in a cluster (n=3) or pair (n=6), including one cluster of three participants and three pairs (Figure 1). In one cluster (Figure 1a), a young female demonstrated phylogenetic clustering with two older males. This is in contrast to the other three clusters, where similar ages were observed between the participants in each cluster.

Comparisons of those clustering (n=9) and not clustering (n=56) are demonstrated in Table 1. The two groups had similar median age (23 years vs. 22 years), female sex (32% vs 22%), and HIV co-infection (4% vs 0%). There were no differences in the proportion of individuals clustering among those with crack use, cocaine injecting, heroin injecting or speedball injecting. However, a greater proportion of individuals in a cluster reported recent methamphetamine injection (past 6 months) as compared to those not in a cluster (100% vs 54%, $P=0.009$).

4. DISCUSSION

In this study of street-involved youth in Vancouver, Canada, with recent injecting and HCV infection, 14% of participants demonstrated phylogenetic clustering of HCV infection. Methamphetamine injecting in the last six months was significantly associated with phylogenetic clustering. These findings could be used to help inform strategies to curb HCV transmission

engaging individuals who are at a higher risk of HCV transmission, such as methamphetamine injectors, with appropriate harm reduction and treatment strategies.

The prevalence of phylogenetic clustering observed in this analysis is lower than previous studies in Vancouver, Canada (31%; Jacka et al., 2014), Ottawa, Canada (36%; Pilon et al., 2011) and Melbourne, Australia (37%; Aitken et al., 2004). The lower proportion with clustering in this study compared to previous studies might be due to several different reasons. First, there was a lower prevalence of HCV among at-risk PWID in this study, partly related to a decreased HCV incidence observed among PWID in Vancouver over the past two decades (Grebely et al., 2014). Further, participants were sampled more broadly throughout the Greater Vancouver region compared to previous studies of long-term PWID in the Vancouver Downtown Eastside neighbourhood (a restricted geographical area known for having a highly concentrated population of PWID). Lastly, the follow-up period for the current study (2005-2012) was shorter than for previous work (1996-2012) and there has been less time for the formation of larger clusters. These factors would all have contributed to the lower proportion of observed clustering in this study.

The finding that methamphetamine injection was associated with phylogenetic clustering of HCV is novel. However, previous data have demonstrated that methamphetamine injection was independently associated with acquisition of HCV infection (Grebely et al., 2014; Hadland et al., 2014; Miller et al., 2009). Methamphetamine use in any form among street-involved youth in Vancouver has increased from 2.5% in 1999 to 10.5% in 2011, consistent with global trends (Lianping et al., 2013). It has been shown that the availability of methamphetamine in Vancouver has increased, with the proportion of respondents reporting that they could obtain these drugs within 10 minutes rising from 13% in 2005 to 41% in 2011 (Lianping et al., 2013). Methamphetamine use has also been shown to be an independent predictor of initiation of injecting (Werb et al., 2013). These points are concerning, since it puts the growing number of methamphetamine users, injection or otherwise, at a higher risk of

both acquisition and transmission of HCV. This is of particular importance given that, in contrast to drugs such as heroin where opioid agonist therapy is available, methamphetamine has no pharmacological treatment and prevention strategies for this group are important. Additionally, the association between phylogenetic clustering and methamphetamine injecting may partly be due to an increased frequency of injecting among methamphetamine users. Recent findings highlight a recent and growing issue among street-involved youth as injection methamphetamine use becomes a more serious problem due to increasing rates of transitions from other drugs such as heroin and crack (Marshall et al., 2011). As a result of this switch, new and/or denser networks may be forming among individuals injecting methamphetamines.

This study has several limitations. The small number of participants with phylogenetic clustering limited the ability to perform adjusted analyses. Thus, we were unable to account for potential confounders such as geographic proximity. As such, the association between methamphetamine and clustering should be interpreted with caution, given we cannot determine if this is an independent association. Further, this analysis was not intended to identify participants linked by HCV transmission; therefore, there may be additional un-sampled individuals involved in the transmission cluster, and the direction of HCV transmission cannot be determined. Third, this cohort specifically recruited street-involved youth in Vancouver and the generalizability of these findings may not be applicable to populations of PWID in other setting. Lastly, all behavioural characteristics are self-reported and therefore may be subject to response biases.

In conclusion, phylogenetic clustering in this cohort was low (14%) and methamphetamine injecting was found to be associated with membership in a cluster or pair (all participants in a pair/cluster reported methamphetamine injecting). These findings provide novel information that may aid in the targeting and use of HCV prevention and treatment strategies. In order to effectively design treatment as prevention programs, it is necessary to understand factors associated with HCV transmission so that limited resources can be directed in such a way as to have the largest positive

impact through the implementation of public health and treatment as prevention interventions at the population level. Given the role of methamphetamine injecting in ongoing HCV acquisition (Grebely et al., 2014; Hadland et al., 2014; Miller et al., 2009) and transmission, specific targeting of education, prevention and treatment strategies to methamphetamine injectors could be explored as potential options for prevention of HCV infection. Further research is needed to better understand how HCV is being transmitted among methamphetamine injectors, as well as the risks of reinfection after treatment among this group, and to evaluate targeted strategies for street-involved youth to prevent HCV transmission.

FIGURE LEGEND

Figure 1. Maximum-likelihood phylogenetic tree showing all clusters identified. Circles represent females and squares represent males. Numbers at tips represent participant ages.

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Table 1. Characteristics of participants with available HCV Core-Envelope 2 segment sequence in the ARYS cohort, 2005-2012, Vancouver, Canada, not in a cluster (n=56), in a cluster (n=9), and combined (n=65).

Characteristics	Overall (n=65)	Not in a cluster (n=56)	In a cluster (n=9)	<i>P</i>
Female sex (vs. male sex)	20 (31%)	18 (32%)	2 (22%)	0.710
Age (median (Q1-Q3))	23 (21-25)	23 (21-25)	22 (22-25)	0.761
High school education or higher (vs. less than high school)*	45 (69%)	38 (68%)	7 (78%)	0.710
Unstable housing (vs. stable) [†]	52 (80%)	44 (79%)	8 (89%)	0.674
Neighbourhood: Downtown South	28 (43%)	23 (41%)	5 (56%)	0.323
Years injecting (median (Q1-Q3))	6 (3-9) [^]	5 (3-8)	8 (4-11)	0.343
HCV acute/recent (vs. not)	40 (62%)	34 (61%)	6 (67%)	1.000
HIV infection (vs. none) [†]	2 (3%)	2 (4%)	0 (0%)	1.000
Currently enrolled in methadone treatment (vs. yes)	6 (9%)	6 (11%)	0 (0%)	0.584
Syringe borrowing (vs. none) [†]	16 (24%)	14 (25%)	2 (22%)	1.000
Crack use (vs. none) [†]	39 (60%)	36 (64%)	3 (33%)	0.140
Cocaine injecting (vs. none) [†]	23 (35%)	20 (36%)	3 (33%)	1.000
Heroin injecting (vs. none) [†]	39 (60%)	34 (61%)	5 (56%)	1.000
Speedball injecting (vs. none) [†]	10 (15%)	9 (16%)	1 (11%)	1.000
Methamphetamine injecting (vs. none) [†]	39 (60%)	30 (54%)	9 (100%)	0.009
Genotype				
1a	27 (42%)	23 (45%)	4 (44%)	1.000
1b	2 (3%)	2 (4%)	0 (0%)	1.000
2b	3 (5%)	3 (5%)	0 (0%)	1.000
3a	33 (51%)	28 (55%)	5 (56%)	1.000

Percentages indicate column percentages; *At the time of enrolment; [†] In the last 6 months prior to sample; [^] Data unavailable for 7 participants. Abbreviations: HIV = human immunodeficiency virus; HCV hepatitis C virus; ARYS = At Risk Youth Study

Figure 1.