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Patterns and Characteristics of Hepatitis C Transmission Clusters among HIV-Positive and HIV-Negative Individuals in the Australian Trial in Acute Hepatitis C

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Background. Injecting drug users remain the population at greatest risk of acquiring hepatitis C virus (HCV) infection, although a recent increase in cases of sexually transmitted HCV infection has been observed among human immunodeficiency virus (HIV)–infected individuals. The extent to which these separate epidemics overlap is unknown.

Methods. The Australian Trial in Acute Hepatitis C (ATAHC) enrolled 163 individuals (29% of whom were HIV infected) with recent HCV infection. E1/HVR1 sequences were used to construct phylogenetic trees demonstrating monophyletic clusters or pairs, and viral epidemic history and phylogeography were assessed using molecular clock analysis. Individual clusters were characterized by clinical and demographic characteristics.

Results. Transmission through injection drug use occurred for 73% of subjects, with sexual transmission occurring for 18% (92% of whom were HIV infected). Among 112 individuals with available E1/HVR1 sequences, 23 (20%) were infected with a strain of HCV identical to that of another subject, comprising 4 homologous clusters and 3 monophyletic pairs, the majority of which (78%) were HIV infected. Clusters contained individuals with both injection drug use–related and sex-related acquisition, and in all clusters (except for 1 female HIV-uninfected pair), individuals identified as men who have sex with men, irrespective of HIV status.

Conclusions. This large unique study of HIV-infected and HIV-uninfected individuals with recently acquired HCV infection demonstrates that clustering is common in the HIV-infected population and that it occurred almost invariably among men who have sex with men, irrespective of the actual mode of acquisition. These findings suggest the coexistence of both injection drug use and sexual risk behaviors for individuals in the same social networks and have implications for the development of public health messages.

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Transmission of hepatitis C virus (HCV) continues unabated in the developed world, driven largely by the high incidence of HCV infection among injection drug users (IDUs) [1–4]. Despite the rapid spread of HCV infection, characterization of transmission patterns has been difficult, because acute HCV infection is asymptomatic in the majority of infected individuals; because infection occurs most frequently among highly marginalized, at-risk populations; and because public health surveillance systems to detect new infections are often limited. However, the use of novel molecular epidemiological methods, including phylogenetic analysis, to examine transmission dynamics in IDU populations has extended our understanding in recent years [5–7].

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In addition to the ongoing epidemic among IDUs, a more recent dramatic increase in cases of acute HCV infection has been reported among populations of human immunodeficiency virus (HIV)–-infected men who have sex with men (MSM). The majority of these new infections have been associated with sexual (permucosal) risk exposure [8–11], with cases reported from Europe, the United States, and Australia [12, 13]. Analysis of behavioral risk factors associated with acquisition of HCV infection through permucosal exposure in HIV-infected MSM has established a number of sexual and (noninjection) drug use practices [8]. In addition, a phylogenetic study involving 226 HIV-infected MSM demonstrated evidence of a large European network of predominantly permucosal HCV transmission [14]. Molecular clock analysis indicated that the majority of infections were of recent origin (ie, they occurred after the introduction of highly active antiretroviral therapy [HAART] in the mid 1990s).

The relative contribution of biological versus behavioral factors behind this changing epidemiology in HIV-infected MSM is unclear. Furthermore, if the factors are predominantly behavioral, then it is unclear whether a similar increase in "permucosal" cases of acute HCV infection could be observed among HIV–uninfected MSM populations. Although a few studies have tried to address this issue [15, 16], most are limited by the discrete populations in which they have been conducted, and none have been able to explore transmission networks between HIV-infected and HIV-uninfected populations through phylogenetic analysis.

The Australian Trial in Acute Hepatitis C (ATAHC) was a prospective study of the natural history and treatment of recently acquired HCV infection, enrolling both HIV–uninfected and HIV-infected subjects [17]. The aim of this article was to explore, using demographic characteristics, risk behavior data, and phylogenetic analysis, the epidemiology of recently acquired HCV infection in Australia and the evidence of transmission networks involving linkage between HIV-uninfected and HIV-infected populations.

METHODS

This study was conducted according to the principles expressed in the Declaration of Helsinki and the International Conference on Harmonisation - Good Clinical Practice guidelines. All patients provided written informed consent for the collection of samples and subsequent analysis.

Study Design

ATAHC was a multicenter, prospective cohort study of the natural history and treatment of recent HCV infection and is described in detail elsewhere [17]. Paticipants with recent HCV infection included those who met the following eligibility criteria.

First positive anti-HCV antibody detected ≤6 months before enrolment and either

a. acute clinical HCV infection, defined as symptomatic seroconversion illness or an alanine aminotransferase level >10 times the upper limit of normal, with exclusion of other causes of acute hepatitis, at most 12 months before the initial positive anti-HCV antibody; or

b. asymptomatic HCV with seroconversion, defined by a negative anti-HCV antibody test result in the 2 years prior to the initial positive anti-HCV antibody test result (this broader seroconversion window was used to identify participants across a wide range of estimated dates of infection, given that the period defining acute HCV infection and response to therapy in this setting is still unclear).

Enrolment was encouraged for all people who met the entry criteria, regardless of whether HCV treatment was required, and participants with detectable HCV RNA levels at screening were assessed for treatment. HCV treatment consisted of pegylated interferon-alfa-2a for 24 weeks (plus ribavirin for HIV-HCV–coinfected participants).

HCV Virological Assessment

HCV RNA levels were assessed at screening with a qualitative HCV RNA assay (TMA assay; Versant, Bayer; lower limit of detection, 10 IU/mL) and, if the results were positive, a quantitative HCV RNA assay (Versant HCV RNA 3.0; Bayer; lower limit of detection, 615 IU/mL). The HCV genotype (Versant LiPa2; Bayer) was determined for all HCV RNA–positive participants at screening.

Behavioral Risk Assessment

The most likely mode of transmission was determined by the clinician at the initial screening assessment. In addition, participants completed a self-administered questionnaire to obtain information on a variety of parameters, including risk and IDU behaviors.

HCV RNA Sequencing

RNA was extracted from serum samples collected at the screening using the QIAmp Viral RNA extraction kit (QIAGEN). The region encoding envelope glycoprotein 1 (E1) and the hypervariable region 1 (HVR1) of E2 was amplified by a real-time nested reverse-transcriptase polymerase chain reaction (RT-PCR), as described by Pham et al [18].

Determination of Viral Clusters

Sequences with 0%–2.2% divergence were regarded as coming from a potential common source [18]. These sequences were

then assessed for monophyletic clusters or pairs by constructing phylogenetic trees using the neighbor-joining method with p-distance algorithm. Bootstrap values of > 70 indicate robust clusters or pairs.

Assessing Viral Epidemic History

Viral epidemic history was assessed by constructing phylogenetic trees, using the maximum likelihood approach implemented in BEAST, the Hasegawa-Kishino-Yano substitution model plus a γ distribution model of among site rate heterogeneity (HKY-Γ) [19]. Markov Chains Monte Carlo sampling was performed for at least 1×10^7 generations, sampling every 1000 generations. Phylogenetic trees were visualized using Figtree software, version 1.2.3 (available from http://tree.bio.ed.ac.uk). The date of the most common recent ancestor for each HCV cluster was estimated using a molecular clock approach. On the basis of previous estimates, the inferred rate of nucleotide evolution for E1/HVR-1 sequences was 1.5×10^{-3} (lower bound) to 8.6×10^{-2} (upper bound) [20], with a mean rate of 3.65×10^{-3} substitutions per site per year [21]. To select the best-fitting molecular clock model, all possible combinations of the relaxed and strict molecular clock models were analyszd using Tracer software, version 1.4.1 (http://tree.bio.edu.ac.uk). All models that failed to converge or achieve sufficient chain mixing (effective sample size, >100) before 30×10^6 generations were excluded [22].

Phylogeography

To examine the specific pathway of location changes (migration events) between Australian states, a viral dispersal map from sequence phylogenies was constructed on the basis of reported approaches using the Slatkin and Maddison phylogenetic method [22–26]. First, the phylogenies for all sequences were constructed as described above. The trees were rooted using the closest group as an outgroup. Each HCV sequence was labeled according to its geographic location (Australian state). Quantitative features of phylogeographic analysis (migration matrix) were not analyzed in this study. Instead, simple qualitative phylogeographic dispersal, such as the degree of geographic dispersal and the most plausible origin of the current sample, were analyzed, as previously described for the global spread of HCV 1a and 1b [22].

BEAST, a standard Bayesian phylogenetic analysis program of DNA sequences, was used to generate phylogenetic trees. Two important tree characteristics—the size and the "bushiness" of the phylogenies—were calculated from the tree diagrams. The mean difference among the sequences was calculated as AvgDL (average difference between leaves). AvgDL provides a measure of the size of the evolutionary tree and, thus, the age of the evolutionary process.

RESULTS

Baseline characteristics of ATAHC participants, stratified by mode of acquisition, are shown in Table 1. Overall, 167 subjects were enrolled, with 4 failing to return after screening, resulting in a total study population of 163 subjects.

One hundred nineteen subjects (73%) had IDU identified as the most likely route of infection, 29 (18%) were most likely infected through sexual exposure, and in 15 cases (9%), "other" or "unknown" routes of infection were identified (Table 1). Of the 29 sexually acquired cases, 4 involved HIV–uninfected women, all of whom had a known HCV–infected partner and no history of IDU. Of the 25 men exposed sexually, only 2 were HIV uninfected, whereas 23 (92%) were HIV infected. Of the 2 HIV-uninfected men, 1 was infected through same-sex (MSM) exposure with partner(s) of unknown status, and 1 was exposed through sexual contact with a woman known to be HCV infected. All 23 HIV-infected subjects were sexually exposed through MSM exposure. Of the 15 cases with other routes of exposure identified, 2 were suspected infections through medical procedures, 1 was associated with occupational exposure, 2 were associated with nonoccupational needlesticks, 1 was associated with assault, and 4 cases had unknown routes of exposure. In 3 cases, either MSM sexual exposure ($n = 2$) or IDU ($n = 1$) were disclosed by the subject on answering the risk assessment survey but were not identified by the clinician on the primary case report form.

Clinical, demographic, and behavioral characteristics, stratified by mode of acquisition, are shown in Table 1. Individuals exposed through sexual contact were more often male and HIV infected. Aside from expected differences in drug use characteristics, other notable differences between individuals with sexual and IDU-related acquisition were observed, including differences in education level, employment, prison experience, social functioning, and depression. Clinical characteristics, however, were generally similar between the groups (Table 1.)

Participants were stratified further on the basis of both HIV status and mode of acquisition (IDU vs sexual exposure) (Table 2). Because the group of HIV-uninfected subjects with sexual acquisition comprised only 6 cases, this group was excluded from analysis, leaving 3 groups; the HIV-uninfected IDU group ($n = 96$), the HIV-infected IDU group ($n = 23$), and the HIV-infected sexual acquisition group ($n = 23$). Characteristics of these 3 groups are given in Table 2. Marked differences were seen between the HIV-uninfected IDU group and the HIV-infected sexual acquisition group, similar to those described in Table 1. Indices of social stability, such as higher education, home ownership, and employment, were all highest for the sexual acquisition group, followed by the HIV-infected IDU group and then the HIV–uninfected IDU group. The social functioning score, a global indicator of social stability in which

Table 1. Participant Characteristics, Stratified by Mode of Acquisition

Characteristic	Overall (n = 163)	IDU acquisition group (n = 119)	Sexual acquisition group (n = 29)	Other (%) (n = 15)
Male sex	117 (72)	82 (69)	25 (86)	10 (67)
Age, mean years± SD	34.3 ± 9.9	31.9 ± 8.9	41.2 ± 9.9	40.4 ± 8.9
White race	149 (91)	108 (91)	28 (97)	13 (87)
IDU in previous 6 months,	99 (61)	92 (77)	3 (10)	4 (27)
Any history of IDU	124 (76)	112 (94)[a]	7 (24)	5 (33)
HIV infection	50 (31)	23 (19)	23 (79)	4 (27)
Estimated duration of infection at screening medianweeks(IQR)	25 (16-38)	25 (17-39)	25 (12-33)	21 (15-37)
Duration of infection <24 weeks	81 (50)	59 (50)	13 (45)	9 (60)
Presentation of recent HCV				
Acute clinical (symptomatic)	67 (41)	46 (39)	12 (41)	9 (60)
Acute clinical (ALT >400 IU/mL)	32 (20)	22 (18)	9 (31)	1 (7)
Asymptomatic seroconversion	64 (39)	51 (43)	8 (28)	5 (33)
Documented HCV seroconversion illness	67 (41)	46 (39)	12 (41)	9 (60)
Peak ALT level, median IU/mL (IQR)	468 (175-1206)	412 (139-963)	661 (254-1729)	567 (156-1788)
HCV RNA level, median $\log_{10}$ copies/mL (IQR)	5.6 (4.2–6.6)	5.6 (4.2–6.4)	6.1 (4.6–6.8)	4.4 (4.1–5.5)
HCV RNA level >400,000 IU/mL	44 (27)	29 (24)	13 (45)	2 (13)
HCV genotype				
Genotype 1	75 (46)	54 (45)	13 (45)	8 (53)
Genotype 3	56 (34)	41 (34)	12 (41)	3 (20)
Tertiary education or greater	66 (40)	43 (36)	16 (55)	7 (47)
Privately owned accommodation	39 (24)	23 (19)	11 (38)	5 (33)
Current full-time or part-time employment	63 (39)	32 (27)	21 (72)	10 (67)
Ever been in prison	25 (16)	22 (19)	1 (3)	2 (13)
Current methadone or buprenorphine treatment	17 (10)	16 (13)	0 (0)	1 (7)
Social functioning score >14	60 (37)	53 (45)	4 (14)	3 (20)
Current depression	25 (16)	23 (20)	0 (0)	2 (13)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; SD, standard deviaion.

[a]Six percent of subjects identified as having IDU as risk factor by the clinician failed to disclose this on a self-administered survey.

higher values reflect greater instability, demonstrated that 50% of HIV-uninfected individuals had scores >14, compared with 22% of HIV-infected IDU cases and 13% of HIV-infected sexual acquisition cases.

Phylogenetic Analysis

A phylogenetic tree was constructed using 116 E1/HVR1 sequences from 112 screening samples and 4 cases of reinfection (Figure 1A). Of the 163 samples from enrolled subjects, sequences could not be obtained for 11 screen samples (6.7%) because of technical difficulties and for an additional 40 screen samples (24.5%) because of the low level or absence of viremia (virus load, <615 IU/mL). The majority of genotypes were 1a (53 [47%]) and 3a (50 [44%]), with smaller numbers of 1b (3), 2a/b (6), and 4a (1). Of the 112 sequences subjected to phylogenetic analyses, 77 were obtained from HIV–uninfected subjects and 35 were from HIV-infected subjects, representing 68% and 70% of each group, respectively. Phylogenetic analysis using a bootstrap value of >98% identified 4 clusters and 3 homologous pairs of virus, containing a total of 23 viruses (20% of the total sequenced) (Figure 1A). Of these 23 viruses, 18 were from HIV-infected subjects (51% of HIV-infected subjects sequenced) and 5 were from HIV-uninfected subjects (8% of HIV–uninfected subjects sequenced; $P < .001$). The vast majority (19 of 23) of these viruses were in genotype 1a clusters and involved both IDU-acquired (n = 15) and sexually acquired (n = 7) cases. Details of subjects falling into the clusters and pairs by HIV status and mode of acquisition are given in Table 3. HIV-infected clusters contained individuals with both sexual and IDU-related acquisition (clusters 4–6). One separate pair of HIV–uninfected IDUs was identified (cluster 2). Clusters 1 and 3 contained both HIV-infected and HIV-uninfectedindividuals. Cluster 1 contained 1 HIV–uninfected patient who acquired HCV infection through IDU, one HIV–uninfected subject with "other" identified as the most likely route of transmission, and 1 HIV-infected subject with HCV reinfection acquired through sexual exposure. In cluster 3, 2 HIV-infected IDUs clustered with an HIV–uninfected MSM infected through sexual

Table 2. Participant Characteristics Stratified by HIV Status and Mode of Acquisition

	IDU acquistion group		Sexual acquisition group
Characteristic	HIV-uninfected subjects (n = 96)	HIV-infected subjects (n = 23)	HIV-infected patients (n = 23)
Male sex	59 (61)	23 (100)	23 (100)
Age, mean years ± SD	30.2 + 8.5	38.9 + 7.1	41.9 + 10.0
White race	85 (89)	23 (100)	22 (96)
MSM as risk group for HIV acquisition	NA	23(100)	23 (100)
Tertiary education or greater			
Yes	27 (28)	16 (70)	13 (57)
No	69 (72)	7 (30)	10 (43)
Clinical characteristics			
Estimated duration of infection at screening, weeks			
Median (IQR)	27 (20-41)	16 (9-20)	25 (9-33)
<24 weeks	41 (43)	18 (78)	10 (43)
Presentation of recent HCV infection			
Acute clinical (symptomatic)	35 (36)	11 (48)	8 (35)
Acute clinical (ALT level, >400 IU/mL)	17 (18)	5 (22)	9 (39)
Asymptomatic seroconversion	44 (46)	7 (30)	6 (26)
Documented HCV seroconversion illness	35 (36)	11 (48)	8 (35)
Peak ALT level prior to enrolment			
Median level, IU/mL (IQR)	382 (135-1115)	586 (260-746)	661 (286-1628)
>400 IU/L, n (%)	44 (46)	15 (65)	16 (70)
HCV RNA level			
Log_{10} HCV RNA, median (IQR)	5.5 (4.2–6.3)	6.0 (3.9–6.8)	6.3 (4.7–6.8)
>400,000 IU/mL, n (%)	20 (21)	9 (39)	11 (48)
Demographic characteristics			
Privately owned accommodation	17 (18)	6 (26)	9 (39)
Current full-time or part-time employment	20 (21)	12 (52)	18 (78)
Any history of imprisonment	21 (22)	1 (4)	1 (4)
Current methadone or buprenorphine treatment	11 (11)	0 (0)	0 (0)
Social functioning score, >14	48 (50)	5 (22)	3 (13)
Current depression	20 (21)	3 (13)	0 (0)
Any history of IDU	91 (95)	21 (91)	7 (30)
IDU in previous 6 months	75 (78)	17 (74)	3 (13)

NOTE. Data are no. (%) of subjects, unless otherwise indicated. ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men; NA, not applicable; SD, standard deviation

exposure. Although these clusters 1 and 3 contained participants with differing HIV status, further exploration revealed that both HIV-infected subjects in cluster 3 identified their sexual orientation as MSM, as did 2 subjects in cluster 1—namely, the HIV–uninfected subject with IDU-acquired HCV infection and the HIV-uninfected subject who acquired HCV infection through an "other" route. Thus, irrespective of the most likely mode of HCV acquisition and HIV status, all subjects included in clusters or pairs in this analysis were MSM (with the exception of the 1 pair of female HIV-uninfected IDUs).

Molecular Clock Analysis

The degree of geographic dispersal and the plausible origin of HCV isolates in clusters or pairs were estimated on a real time scale using Markov Chain Monte Carlo approach to calculate the year of the most common recent ancestor (Figure 1B). The hierarchy presented in the phylogeographic tree indicated that the earliest divergence events occurred in New South Wales around 1989, and all recent migration events for monophylogenetic clusters or pairs occurred after 1998, after the introduction of HAART. Most sequences originated from New South Wales and Victoria with the exception of early migration (1991) of a genotype 3a virus from New South Wales to South Australia (SA). The number of migration events prior to the cluster formation ranged from 2 to 7 (mean ± standard deviation, = 4.7 ± 1.9).

In addition, we examined whether there was evidence for different timings of transmission networks in HIV-uninfected

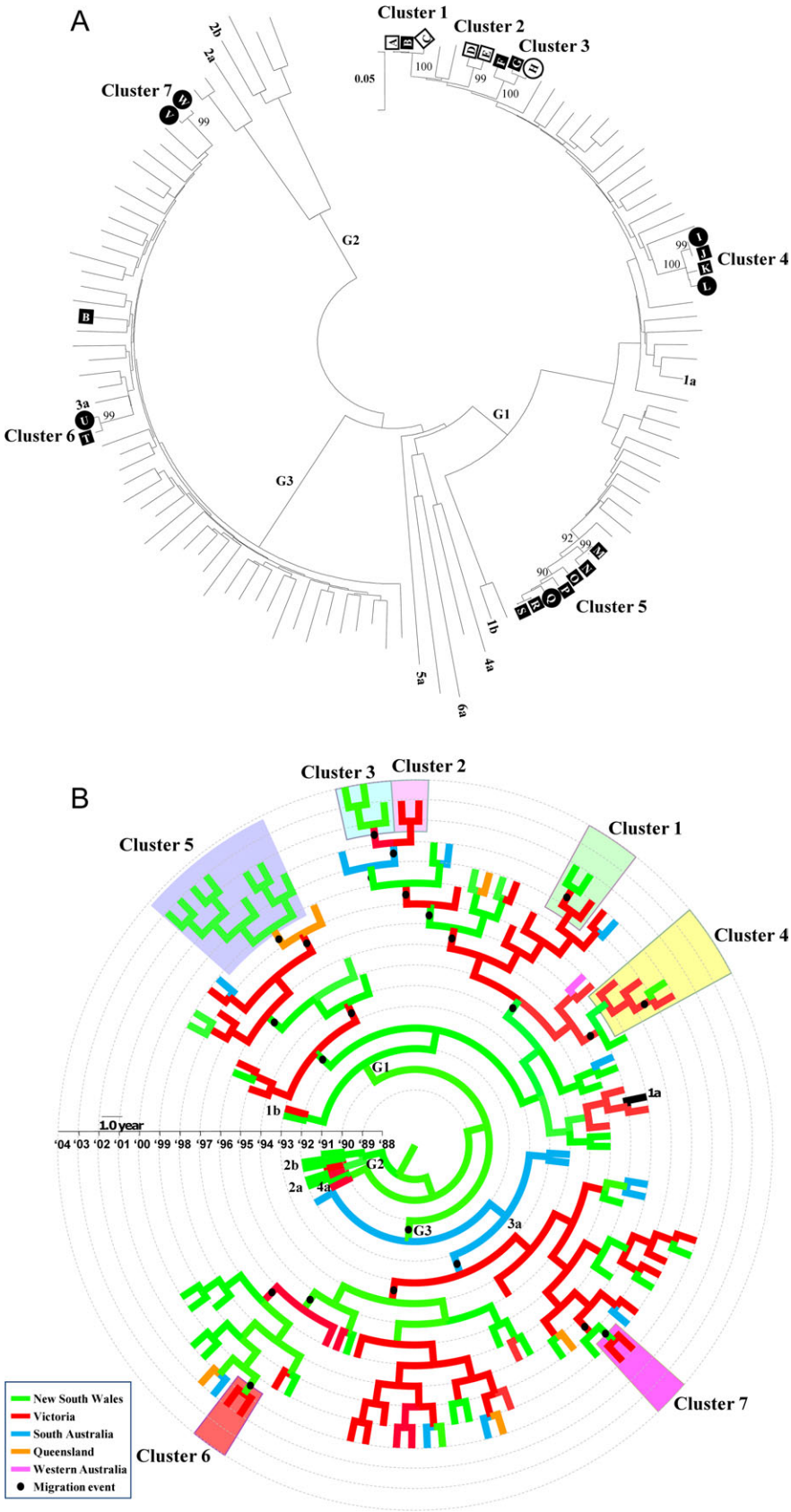

Table 3. Clinical and Demographic Characteristics of 23 Clustering Subjects

Subject	Cluster/pair	Center	Sex	GT	HIV infection	Mode ofacquisition	MRCA	State of origin
A	1	NSW	Male	1a	No	IDU	1996	VIC
B	1	NSW	Male	1a	Yes	IDU		
C	1	VIC	Male	1a	No	Other		
D	2	VIC	Female	1a	No	IDU	2002	SA
E	2	VIC	Female	1a	No	IDU		
F	3	NSW	Male	1a	Yes	IDU	2002	SA
G	3	NSW	Male	1a	Yes	IDU		
H	3	NSW	Male	1a	No	Sexual		
I	4	NSW	Male	1a	Yes	Sexual	1999	NSW
J	4	VIC	Male	1a	Yes	IDU		
K	4	VIC	Male	1a	Yes	IDU		
L	4	VIC	Male	1a	Yes	Sexual		
M	5	NSW	Male	1a	Yes	IDU	2000	QLD
N	5	NSW	Male	1a	Yes	IDU		
O	5	NSW	Male	1a	Yes	IDU		
P	5	NSW	Male	1a	Yes	IDU		
Q	5	NSW	Male	1a	Yes	Sexual		
R	5	NSW	Male	1a	Yes	IDU		
S	5	NSW	Male	1a	Yes	IDU		
T	6	VIC	Male	3a	Yes	IDU	2002	NSW
U	6	VIC	Male	3a	Yes	Sexual		
V	7	VIC	Male	3a	Yes	Sexual	2001	NSW
W	7	VIC	Male	3a	Yes	Sexual		

NOTE. GT, genotype; HIV, human immunodeficiency virus; IDU, injection drug use; MRCA, most recent common ancestor; NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC,Victoria.

and HIV-infected ATAHC subjects using markers of evolutionary tree diversity. Bushiness models were constructed with subject sequences using BEAST software. AvgDL was calculated for both HIV-infected subjects and HIV-uninfected subjects (Table 4). The AvgDL in HIV-infected subjects was significantly lower than that in HIV-uninfected subjects for both genotype 1 (13.00 vs 19.98; $P < .001$) and genotype 3 (11.62 vs 20.42; $P < .001$) evolutionary trees.

DISCUSSION

Our study demonstrates several important findings regarding HCV transmission networks, based on detailed characterization of persons with recently acquired HCV infection enrolled in a prospective study recruiting both HIV-uninfected and HIV-infected individuals through the same Australian network. First, although IDU was the most common mode of acquisition (73%), sexual exposure accounted for 18% of cases, of which 80% involved HIV-infected MSM. Sexual transmission among HIV-uninfected individuals was unusual but was almost always in the context of contact with a known HCV infected partner (one exception being an HIV-uninfected MSM with multiple partners of unknown status).

Second, patients with IDU-acquired and sexually acquired cases differed markedly on all indices of social stability, with individuals infected through IDU representing a far more socially disadvantaged group, although this was less so for HIV-infected IDUs (all of whom were MSM). The lack of

Figure 1. *A,* Phylogenetic analysis of 116 E1/HVR-1 sequences (657 bp) from 112 hepatitis C virus (HCV) RNA–positive patients demonstrating clustering of subjects enrolled in the Australian Trial in Acute Hepatitis C. Clusters are numbered 1–7. Spheres represent HCV sexual transmission, squares represent injection drug use–related transmission, and diamonds represent other modes of transmission. Filled symbols represent human immunodeficiency virus (HIV)–infected patients, and unfilled symbols represent HIV-uninfected patients. Patients ($n = 23$) in clusters are labeled from A to W. Cases with HCV reinfection did not cluster. HCV genotypes (G1, G2, and G3) are indicated along the major branches; reference HCV subtype sequences are also indicated. The percentage bootstrap values in which the major groupings were observed among 1000 replicates are indicated; only values > 70% are shown. The branch lengths are proportional to the evolutionary distance between sequences, and the distance scale, in nucleotide substitutions per position, is shown. *B,* Phylogeographic tree of all E1/HVR-1 sequences on a real time scale of 1 year spanning 1988–2004. Geographic status is indicated by color (*green,* New South Wales; *red,* Victoria; *blue,* South Australia, SA; *orange,* Queensland, QLD and *pink,* WA, Western Australia). Black dots represent migration events for each monophyletic cluster or pair. All 7 clusters or pairs are shaded and labeled 1–7.

Table 4. Analysis of Phylogenetic Tree Evolutionary Characteristics, by Human Immunodeficiency Virus (HIV) Infection Status

	No. of Leaves		Average Distance between leaves (AvgDL) ± SD	
Genotype	1a	3a	1a	3a
HIV status				
HIV infected	25	17	13.00 ± 3.93	11.62 ± 3.77
HIV uninfected	33	38	19.98 ± 7.20	20.42 ± 7.62
P			< .001	

NOTE. Two-way analysis of variancewas used to determine the association between HIV infection status and mean distance between leaves (AvgDL). SD, standard deviation.

heterosexual HIV-infected individuals with IDU- acquired HCV infection in ATAHC is consistent with the extremely low prevalence of HIV infection in Australian IDU populations [27]. Clinical variables, however, were generally similar for patients with IDU-acquired and sexually acquired cases, including genotype distribution and seroconversion symptoms. The higher baseline HCV RNA level in the sexually acquired group likely reflects the significant proportion of persons in this group (80%) who were HIV infected. It is well recognized that average HCV RNA levels are ~1 log higher for HIV-infected versus HIV-uninfected individuals [28]. Similarly, the greater proportion of cases diagnosed via asymptomatic seroconversion in the IDU group may reflect differing patterns of testing in IDU and HIV-infected populations.

Our phylogenetic analysis confirms data from international cohorts demonstrating that clustering of recently acquired HCV cases is common in HIV-infected populations [14]. The majority (51%) of our HIV-infected subjects had a virus that was homologous with another subject, compared with only 8% of HIV-uninfected subjects. A large European network proposed that frequent clustering of HIV-infected cases represented recent transmission networks occurring predominantly since the advent of HAART [14]. Our molecular clock analysis confirms the time frame for these events. Furthermore, our phylogeographic analysis was able to demonstrate that New South Wales was the state where these HCV clusters originated and was also the state where most clusters or pairs were found. Geographic dispersal of clusters and pairs was demonstrated to be independent from mode of transmission and HCV genotype. Finally, results from our bushiness analysis demonstrate that the virus in the HIV-infected ATAHC population was significantly less evolved (ie, younger) than that in the HIV-uninfected population, supporting the more recent transmission of virus in this population.

Conclusions from the European phylogenetic study suggested that these transmission networks were a new phenomenon observed in HIV-infected men exposed to HCV through sexual (permucosal) risk. However, our data clearly show that clusters contain HIV-infected individuals exposed both from IDU and from sexual activity and suggests that social networks exist in groups of HIV-infected MSM that contain both IDUs and non-IDUs. Because use of noninjection drugs has been recognized as a risk factor for sexually acquired HCV infection [8], and because we have demonstrated that HIV-infected MSM are similar regardless of their IDU status, it is highly likely that social mixing of HIV-infected individuals with all types of drug-taking behaviors is occurring and fuelling the ongoing epidemic. Interestingly, we observed only 3 instances of HIV-uninfected individuals falling into HIV-infected clusters (clusters 1 and 3). All of these individuals were identified as MSM, although 1 was infected through IDU and another through unknown (but probable sexual) exposure. The absence of any HIV-uninfected heterosexual IDUs involved in these clusters again suggests that these networks of HCV transmissions are predominantly defined by sexual orientation rather than risk behavior.

In summary, our study has a number of findings important to our current knowledge of the epidemiology of HCV transmission. IDU remains overwhelmingly the most common mode of infection among HIV-uninfected populations but is less common than sexual transmission among HIV-infected populations. In this group, both IDU-related and sexual exposures occur and are involved as mechanisms of transmission in the same social networks, which appear to be based on sexual orientation rather than specific risk-taking behavior. These findings further extend the debate on the role of sexual and IDU-related transmission of HCV in both HIV-infected and HIV-uninfected MSM populations and have implications for the appropriate targeting of future public health messages.

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References

1. Maher L, Li J, Jalaludin B, Chant KG, Kaldor JM. High hepatitis C incidence in new injecting drug users: a policy failure? Aust N Z J Public Health **2007**; 31:30–5.
2. Page K, Hahn JA, Evans J, et al. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis **2009**; 200: 1216–6.
3. Judd A, Hickman M, Jones S, et al. Incidence of hepatitis C virus and HIV among new injecting drug users in London: prospective cohort study. BMJ **2005**; 330:24–5.
4. Robotin MC, Copland J, Tallis G, et al. Surveillance for newly acquired hepatitis C in Australia. J Gastroenterol Hepatol **2004**; 19:283–8.
5. Cochrane A, Searle B, Hardie A, et al. A genetic analysis of hepatitis C virus transmission between injection drug users. J Infect Dis **2002**; 186:1212–1.
6. Mathei C, Van Dooren S, Lemey P, Van Damme P, Buntinx F, Vandamme AM. The epidemic history of hepatitis C among injecting drug users in Flanders, Belgium. J Viral Hepat **2008**; 15:399–408.
7. Pybus OG, Cochrane A, Holmes EC, Simmonds P. The hepatitis C virus epidemic among injecting drug users. Infect Genet Evol **2005**; 5:131–9.
8. Danta M, Brown D, Bhagani S, et al. Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours. AIDS **2007**; 21:983–91.
9. Gilleece YC, Browne RE, Asboe D, et al. Transmission of hepatitis C virus among HIV-positive homosexual men and response to a 24-week course of pegylated interferon and ribavirin. J Acquir Immune Defic Syndr **2005**; 40:41–6.
10. Serpaggi J, Chaix ML, Batisse D, et al. Sexually transmitted acute infection with a clustered genotype 4 hepatitis C virus in HIV-1-infected men and inefficacy of early antiviral therapy. AIDS **2006**; 20:233–40.
11. Gotz HM, van Doornum G, Niesters HG, den Hollander JG, Thio HB, de Zwart O. A cluster of acute hepatitis C virus infection among men who have sex with men–results from contact tracing and public health implications. AIDS **2005**; 19:969–74.
12. Matthews GV, Hellard M, Kaldor J, Lloyd A, Dore GJ. Further evidence of HCV sexual transmission among HIV-positive men who have sex with men: response to Danta et al. AIDS **2007**; 21:2112–3.
13. Fierer DS, Uriel AJ, Carriero DC, et al. Liver fibrosis during an outbreak of acute hepatitis C virus infection in HIV-infected men: a prospective cohort study. J Infect Dis **2008**; 198:683–6.
14. van de Laar T, Pybus O, Bruisten S, et al. Evidence of a large, international network of HCV transmission in HIV-positive men who have sex with men. Gastroenterology **2009**; 136:1609–17.
15. Richardson D, Fisher M, Sabin CA. Sexual transmission of hepatitis C in MSM may not be confined to those with HIV infection. J Infect Dis **2008**; 197:1213–4, author reply 4–5.
16. Jin F, Prestage GP, Matthews GV, et al. Prevalence, incidence and risk factors for hepatitis C in homosexual men: data from two cohorts of HIV negative and HIV positive men in Sydney, Australia. Sex Transm Infect **2009**; 86:25–8.
17. Dore GJ, Hellard M, Matthews G, et al. Effective treatment of injecting drug users with recently acquired hepatitis C virus infection. Gastroenterology **2010**; 138:123–135.
18. Pham S, Bull R, Bennett W, Dore G, Lloyd A, White P. Frequent multiple HCV infections among injecting drug users in a prison setting. Hepatology **2010**; 52:1564–72.
19. Drummond AJ, Ho SY, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. PLoS Biol **2006**; 4:e88.
20. Herring BL, Tsui R, Peddada L, Busch M, Delwart EL. Wide range of quasispecies diversity during primary hepatitis C virus infection. J Virol **2005**; 79:4340–6.
21. Kato N, Abe K, Mori K, Ariumi Y, Dansako H, Ikeda M. Genetic variability and diversity of intracellular genome-length hepatitis C virus RNA in long-term cell culture. Arch Virol **2009**; 154:77–85.
22. Magiorkinis G, Magiorkinis E, Paraskevis D, et al. The global spread of hepatitis C virus 1a and 1b: a phylodynamic and phylogeographic analysis. PLoS Med **2009**; 6:e1000198.
23. Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics **1989**; 123:603–13.
24. Wallace RG, Hodac H, Lathrop RH, Fitch WM. A statistical phylogeography of influenza A H5N1. Proc Natl Acad Sci U S A **2007**; 104:4473–8.
25. Paraskevis D, Pybus O, Magiorkinis G, et al. Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach. Retrovirology **2009**; 6:49.
26. Nakano T, Lu L, Liu P, Pybus OG. Viral gene sequences reveal the variable history of hepatitis C virus infection among countries. J Infect Dis **2004**; 190:1098–108.
27. National Centre in HIV Epidemiology and Clinical Research. HIV/AIDS, Hepatitis C and Sexually Transmissable Infections in Australia Annual Surveillance Report. Sydney, NSW: National Centre In HIV Epidemiology and Clinical Research, The University of New South Wales, **2008.**
28. Sherman KE, Shire NJ, Rouster SD, et al. Viral kinetics in hepatitis C or hepatitis C/human immunodeficiency virus-infected patients. Gastroenterology **2005**; 128:313–27.