

Evidence of a Large, International Network of HCV Transmission in HIV-Positive Men Who Have Sex With Men

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Background & Aims: Since 2000, there has been a marked rise in acute hepatitis C virus (HCV) in human immunodeficiency virus (HIV)-positive men who have sex with men (MSM). We conducted an international phylogenetic study to investigate the existence of an HCV transmission network among MSM. **Methods:** HIV-positive MSM diagnosed with recent HCV (n = 226) in England (107), The Netherlands (58), France (12), Germany (25), and Australia (24) between 2000 and 2006 were enrolled into a molecular phylogenetic study. Using real-time polymerase chain reaction (PCR), the NS5B region of the HCV genome (436 base pair) was amplified, sequenced, and compared with unrelated NS5B sequences. **Results:** NS5B sequences were obtained from 200 (89%) cases. Circulating HCV genotypes were 1a (59%), 4d (23%), 3a (11%), 1b (5%), and 2b/c (3%). Phylogenetic analysis revealed 156 (78%) sequences that formed 11 clusters (bootstrap value > 70%) containing between 4 and 37 individual sequences. Country mixing was associated with larger cluster size (17 vs 4.5 sequences; $P = .03$). “Molecular clock” analysis indicated that the majority (85%) of transmissions occurred since 1996. **Conclusions:** Phylogenetic analysis revealed a large international network of HCV transmission among HIV-positive MSM. The rapid spread of HCV among neighboring countries is supported by the large proportion (74%) of European MSM infected with an HCV strain co-circulating in multiple European countries, the low evolutionary distances among HCV isolates from different countries, and the trend toward increased country mixing with increasing cluster size. Temporally, this epidemic coincides with the introduction of highly active antiretroviral therapy and associated increases in sexual risk behaviors. International collaborative public health

efforts are needed to mitigate HCV transmission among this population.

Over the last 5 years, an increasing number of hepatitis C virus (HCV) outbreaks have been reported among human immunodeficiency virus (HIV)-positive men who have sex with men (MSM) in large urban centers in Europe,¹⁻³ the United States,^{4,5} and Australia.⁶ Longitudinal cohort studies have confirmed a recent rise in the incidence of HCV among HIV-positive MSM in England⁷ and The Netherlands,⁸ and phylogenetic analysis has revealed the presence of MSM-specific HCV transmission networks in the United Kingdom,² The Netherlands,⁸ and France.³ In contrast to the usual pattern of HCV transmission in such regions, with 80% of incident cases attributable to injecting drug use (IDU),⁹ the vast majority of MSM recently diagnosed with HCV deny IDU. This strongly suggests that the epidemiology of HCV in this population is changing, with permucosal (sexual) factors becoming a potentially important route of transmission.

Following the introduction of highly active antiretroviral therapy (HAART) for HIV and the consequent reduction in HIV-related morbidity and mortality, the number of people living with HIV is steadily increasing. Outbreaks of syphilis, lymphogranuloma venereum, *Shigella sonnei*, and HCV suggest a considerable and increasing burden of various sexually transmitted infections (STIs) among HIV-positive MSM.¹⁰⁻¹² A recent molecular

Abbreviations used in this paper: HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injecting drug use; MSM, men who have sex with men.

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epidemiologic study revealed that 10 hepatitis A virus outbreaks among MSM in 7 European countries between 1997 and 2005 actually represented 1 large outbreak among interconnected MSM communities, within which hepatitis A virus transmission has become endemic.¹³ These emergent high-risk sexual networks are susceptible to the introduction and spread of various infectious agents, such as HCV, for which sexual contact is not usually considered to be a primary mode of transmission.¹⁴

The emergence of HCV as an STI among HIV-positive MSM has potentially serious clinical implications because HIV coinfection profoundly influences the natural history of HCV and vice versa. HIV coinfection has been associated with lower rates of spontaneous HCV clearance, accelerated HCV-related liver disease, and less favorable HCV treatment outcomes. HCV coinfection has been associated with delayed CD4 cell recovery after initiation of HAART and increased HAART-associated hepatotoxicity.^{15–18} Recent findings among HIV-positive MSM with newly diagnosed HCV in New York suggested a fibrosis progression rate 5 times greater than that of HIV-negative individuals with recent HCV infection.¹⁹

The aim of this study was to determine whether reported cases of acute HCV among HIV-positive MSM represent small isolated HCV outbreaks or whether these HCV outbreaks form part of a larger interconnected international transmission network. Using a phylogenetic approach, we analyzed HCV strains obtained from HIV-positive MSM who were recently diagnosed with acute HCV in Europe and Australia. If indeed a high-risk sexual network exists that facilitates the rapid and ongoing transmission of HCV among HIV-positive MSM, it would have important implications for clinicians and public health agencies responsible for control, education, therapy, and surveillance of HCV in those regions.

Materials and Methods

Study Population and Case Definition

We studied 226 HIV-positive MSM diagnosed with acute HCV infection in England, The Netherlands, Germany, France, and Australia. The majority of cases, except German, had been previously reported. Acute HCV infection was defined as a documented anti-HCV seroconversion within 12 months and/or clinical and biochemical criteria (acute hepatitis in individuals without preexisting liver disease, excluding other infective, metabolic, toxic, and drug causes for hepatitis, and a serum alanine aminotransferase level ≥ 10 times the upper limit of normal).²⁰ All HCV infections were diagnosed after 2000. Although recruitment of MSM varied for each country, all participants were HIV-positive MSM attending urban HIV treatment centers and/or participating in longitudinal cohort studies. English MSM were recruited from 2 large urban HIV clinics in London (n = 95) and

1 in Brighton (n = 12).² Dutch participants included notified cases from HIV and/or STI clinics in Amsterdam (n = 44) and Rotterdam (n = 9), as well as participants of the Amsterdam Cohort Studies among MSM (n = 5).^{1,8} German MSM (n = 25) were recruited from HIV clinics/practices in Berlin²¹ and French MSM (n = 12) from 2 large centers for infectious diseases and 1 liver unit in Paris.³ All Australian subjects (n = 24) were participating in the Australian Trial in Acute Hepatitis C, a prospective study of the natural history and treatment outcomes in acute HCV ongoing in 21 sites in Australia.⁶

Reverse-Transcriptase Polymerase Chain Reaction Method

Using the first available HCV RNA-positive sample for each participant, RNA isolation was performed on 100 μ L of serum using the TriPure method (Roche Diagnostics, Indianapolis, IN). Pellets containing the HCV RNA were resuspended in a volume of 50 μ L containing 10 mmol/L Tris buffer, pH = 8.0, and 10 U RNasin. Of each isolate, 3 μ L was used as input for a nested multiplex reverse-transcriptase polymerase chain reaction (RT-PCR), which targets the NS5B region of the HCV genome. This NS5B PCR was devised to amplify HCV RNA (436 base pairs) of genotypes 1–4; the exact conditions and primers have been described elsewhere.²² All sera and RNA isolates had been stored at -80°C .

RNA isolation and amplification of the 202 European samples were performed at the Health Service Laboratory in Amsterdam, The Netherlands. To prevent cross-country contamination, samples originating from different European countries were always handled separately. Using the same protocol, RNA isolation and amplification of the 24 Australian samples were performed at the University of New South Wales in Sydney, Australia.

Sequencing and Phylogenetic Analysis

Sequence reactions were performed as described by van de Laar et al (2005).²² NS5B PCR products were ethanol precipitated. Sense and antisense strands were separately cycle sequenced using the BigDye Terminator system (version 1.1; Applied Biosystems, Foster City, CA) and subsequently purified using DyeEx spin kits (Qiagen, Germantown, MD). Viral epidemic history was assessed by constructing phylogenetic trees of the case sequences plus available unrelated reference sequences from GenBank (www.ncbi.nlm.nih.gov) and the Los Alamos HCV Sequence Database (www.hcv.lanl.gov). Phylogenies were estimated by the maximum likelihood approach implemented in PAUP*4, the Hasegawa–Kishino–Yano substitution model plus a γ distribution model of among-site rate heterogeneity (HKY- Γ).²³ Statistical robustness of phylogenies was assessed by bootstrapping with 1000 replicates (bootstrap values > 70 represent robust clusters). Phylogenetic trees were visualized using Figtree v1.0 software (Edinburgh, UK). The date of the common an-

cestor for each HCV cluster was estimated using a molecular clock approach, which places the phylogenetic history of HCV onto a real timescale of months and years. Based on previous estimates,^{24,25} the inferred rate of nucleotide evolution for our NS5B sequences was 5.0×10^{-4} substitutions per site per year. Dates of origin for each cluster were subsequently calculated using a Bayesian Markov Chain Monte Carlo approach implemented in BEAST v1.3 (Oxford, UK).²⁶ In common with previous studies,^{2,27} a “coalescent based” reconstruction of epidemic history^{24,25} cannot be applied to these data because the proportion of sampled lineages is too high. However, lineage splits in the phylogenetic tree do represent past transmission events and reflect the actual spread of the virus within the population. Therefore, we used the molecular clock-based phylogenies to calculate the number of phylogenetic lineage splits in each cluster that took place after 1996, 1998, and 2000. These 3 specific years mark different events in the MSM community; the introduction of HAART, which successfully decreased HIV-related morbidity and mortality (1996); rise in sexual risk taking and resurgence of STI among MSM following success of HAART (1998); and the first reports of acute HCV in HIV-positive MSM (2000).

Nucleotide Sequence Accession Numbers

Sequences reported in this study have been submitted to GenBank and can be retrieved under accession numbers FJ386690 to FJ386850. The majority (39/49) of HCV cases from Amsterdam had previously been submitted: GenBank accession numbers DQ897975 to DQ898026 and EF123061 to EF123064.⁸

Results

Study Population

The demographics of the study population are shown in Table 1. The vast majority (96%) was diagnosed based on anti-HCV or HCV RNA seroconversion rather than a clinical/biochemical hepatitis. The median age at HCV diagnosis was 38 years (interquartile range [IQR], 33–42 years). The median CD4 count was 518 cells/ μ L (IQR, 359–695 cells/ μ L), and 62% of participants received HAART (Table 1). Because Dutch participants were anonymously collected through centralized health

services in Amsterdam and Rotterdam, clinical information regarding CD4 count and use of HAART were mostly unavailable. The number of MSM reporting IDU in the 12 months prior to HCV seroconversion was low on the European mainland (2.9%), intermediate in the United Kingdom (17%), and high in Australia (50%). Despite the relatively high percentage of English MSM reporting IDU, only 1 subject from the United Kingdom (1.6%) reported needle sharing.

RT-PCR and Sequencing

The HCV NS5B fragment was amplified from 200 of 226 (88%) HIV-positive MSM enrolled. Subsequent sequencing and genotyping were successful in all 200 patients. The HCV genotype distribution according to country of origin is given in Table 2. The majority of isolates was genotype 1a (59%) and genotype 4d (23%) with some genotype 1b, 2b/c, and 3a infections. The difficult-to-treat HCV genotypes 1 and 4 accounted for 158 of 176 (90%) of amplified sequences from Europe and 16 of 24 (67%) of amplified Australian sequences. HCV genotype 3a was relatively more prevalent (33%) among Australian MSM. Because HCV genotype 3a in Western countries has particularly been associated with IDU,²⁸ the relatively high proportion of HCV genotype 3a among Australian MSM could be explained by the larger proportion of Australian MSM reporting IDU. However, HCV genotype 3a was equally prevalent among MSM who denied IDU.

Phylogenetic Analysis

Phylogenetic trees were constructed for each HCV genotype separately. These phylogenetic trees included our 200 HCV NS5B case sequences plus 850 reference sequences of concordant HCV genotypes. Phylogenetic analysis revealed 11 strongly supported monophyletic clusters (bootstrap > 70) of MSM-specific strains, 6 homologous pairs of genetically similar MSM sequences, and 32 “singleton” MSM sequences that were more closely related to reference strains than to another MSM strain from our study. Overall, 168 of 200 (84%) MSM harbored an HCV strain that was most similar to HCV present in another MSM participant of this study.

MSM clusters ranged in size from 4 to 37 sequences and were predominantly of genotype 1a and 4d (Table 3).

Table 1. Patient Demographics of HIV-Positive Men Who Have Sex With Men on Diagnosis of Acute Hepatitis C Virus Infection

	Number	Median age, y	Median CD4 cells/ μ L	HAART (%)	IDU (%)
United Kingdom	107	35 (IQR: 32–39)	510 (IQR: 341–703)	61/102 (60)	10/60 (17)
The Netherlands	58	40 (IQR: 35–46)	na	4/9 (44) ^a	2/58 (3)
Germany	25	38 (IQR: 30–41)	429 (IQR: 343–628)	15/25 (60)	1/25 (4)
France	12	40 (IQR: 34–44)	604 (IQR: 569–697)	9/12 (75)	0/12 (0)
Australia	24	40 (IQR: 34–46)	596 (IQR: 417–807)	18/24 (75)	12/24 (50)
Overall	226	38 (IQR: 33–42)	518 (IQR: 359–695)	107/172 (62)	25/179 (14)

^aData on HAART are known for the Rotterdam cases only.

Table 2. HCV Genotype Distribution of Acute HCV Infections Among HIV-Positive Men Who Have Sex With Men in England, The Netherlands, Germany, France, and Australia Based on Sequencing Part of the NS5B Region

	UK n (%)	NL n (%)	GE n (%)	FR n (%)	AUS n (%)	Total n (%)
Isolates	107	58	25	12	24	226
Untyped	20 (19)	1 (2)	3 (12)	2 (17)	0 (0)	26 ^a (12)
Genotyped	87 (81)	57 (98)	22 (88)	10 (83)	24 (100)	200 (88)
Genotype						
1a	53 (61)	31 (54)	17 (77)	1 (10)	16 (67)	118 (59)
1b	6 (7)	3 (5)	1 (5)	—	—	10 (5)
2b	2 (2)	1 (2)	—	—	—	3 (2)
2c	2 (2)	—	—	—	—	2 (1)
3a	9 (10)	3 (5)	—	1 (10)	8 (33)	21 (11)
4d	15 (17)	19 (33)	4 (18)	8 (80)	—	46 (23)

NOTE. Sequencing part of the NS5B region (436 base pairs).

UK, England; NL, The Netherlands; GE, Germany; FR, France; and AUS, Australia.

^aNineteen of 26 (83%) could be genotyped using VERSANT HCV genotype assay (LIPA); 17 of 19 (89%) were infected with difficult-to-treat HCV genotypes 1 and 4.

The genotype 1a phylogeny is depicted in Figure 1 and shows 7 monophyletic clusters, 3 homologous pairs, and 12 singleton MSM sequences. Five of the 7 clusters contained sequences from more than 1 European country; the 2 remaining clusters (clusters 9 and 10) contained only Australian isolates. All MSM sequences of HCV genotype 4d were part of 2 monophyletic clusters containing 34 (cluster 2) and 12 (cluster 6) sequences, respectively (Figure 1). Phylogenies of less prevalent HCV genotypes are not shown (available on request). The genotype 3a phylogeny revealed 1 MSM-specific cluster (cluster 8) that contained 5 English and 1 French sequence, plus 1 homologous pair of Australian sequences (pair F), and 13 unrelated MSM sequences. The genotype 1b tree contained 2 unrelated MSM sequences, 2 homologous pairs of MSM sequences (an English pair D and a

Dutch pair E), and 4 nearly identical English MSM strains (cluster 11) that were part of a larger paraphyletic cluster together with 12 European and American reference strains. All genotype 2 HCV sequences appeared unrelated to any other MSM study strain. Because half of the Australian MSM reported IDU, we compared sequences of Australian MSM who reported IDU with those who denied IDU. Australian clusters 9 and 10 both contained MSM reporting and denying IDU. The 2 homologous pairs of Australian MSM (pairs C and F) both consisted of MSM reporting IDU, and, of the 10 unrelated MSM sequences, 4 did and 6 did not report IDU.

The phylogenies of the predominant HCV genotypes 1a and 4d demonstrate that the majority of MSM clusters contains sequences from different countries. Country mixing occurred in 7 of the 11 clusters, and these clusters were significantly larger when compared with country-specific clusters (no country mixing) (17 vs 4.5 sequences, respectively; $P = .03$) (Table 3). There was a nonstatistically significant trend to increased country mixing with increased median cluster size when comparison was made between country-specific clusters, clusters containing isolates from 2 countries (intermediate degree of country mixing), and clusters containing isolates from 3 or more countries (high degree of country mixing) (4.5, 11.5, and 19 sequences, respectively; $P = .08$ by Kruskal-Wallis). This trend was statistically significant when the 6 homologous pairs of MSM sequences, which represent possible newly evolving clusters, were included in our analysis (2, 11.5, and 19 sequences, respectively; $P = .005$ by Kruskal-Wallis). Overall, 131 of 200 (66%) of MSM were infected with an HCV lineage that circulated in more than 1 country. This percentage increased to 74% (130/176) when Australian MSM were excluded. Although Australian MSM do cluster together, only 1 Australian MSM was infected with a strain that also circulates in Europe (cluster 5).

Table 3. Identified Hepatitis C Virus Clusters Among HIV-Positive MSM With Acute HCV Infection After 2000 in England, The Netherlands, France, Germany, and Australia

Cluster (n)	Number of sequences per country					Country mixing
	UK	NL	GE	FR	AUS	
1 Genotype 1a (37)	32	5	—	—	—	Yes
2 Genotype 4d (34)	3	19	4	8	—	Yes
3 Genotype 1a (19)	1	17	1	—	—	Yes
4 Genotype 1a (17)	11	—	6	—	—	Yes
5 Genotype 1a (12)	1	2	8	—	1	Yes
6 Genotype 4d (12)	12	—	—	—	—	No
7 Genotype 1a (6)	—	5	1	—	—	Yes
8 Genotype 3a (6)	5	—	—	1	—	Yes
9 Genotype 1a (5)	—	—	—	—	5	No
10 Genotype 1a (4)	—	—	—	—	4	No
11 Genotype 1b (4)	4	—	—	—	—	No

UK, England; NL, The Netherlands; GE, Germany; FR, France; and AUS, Australia.

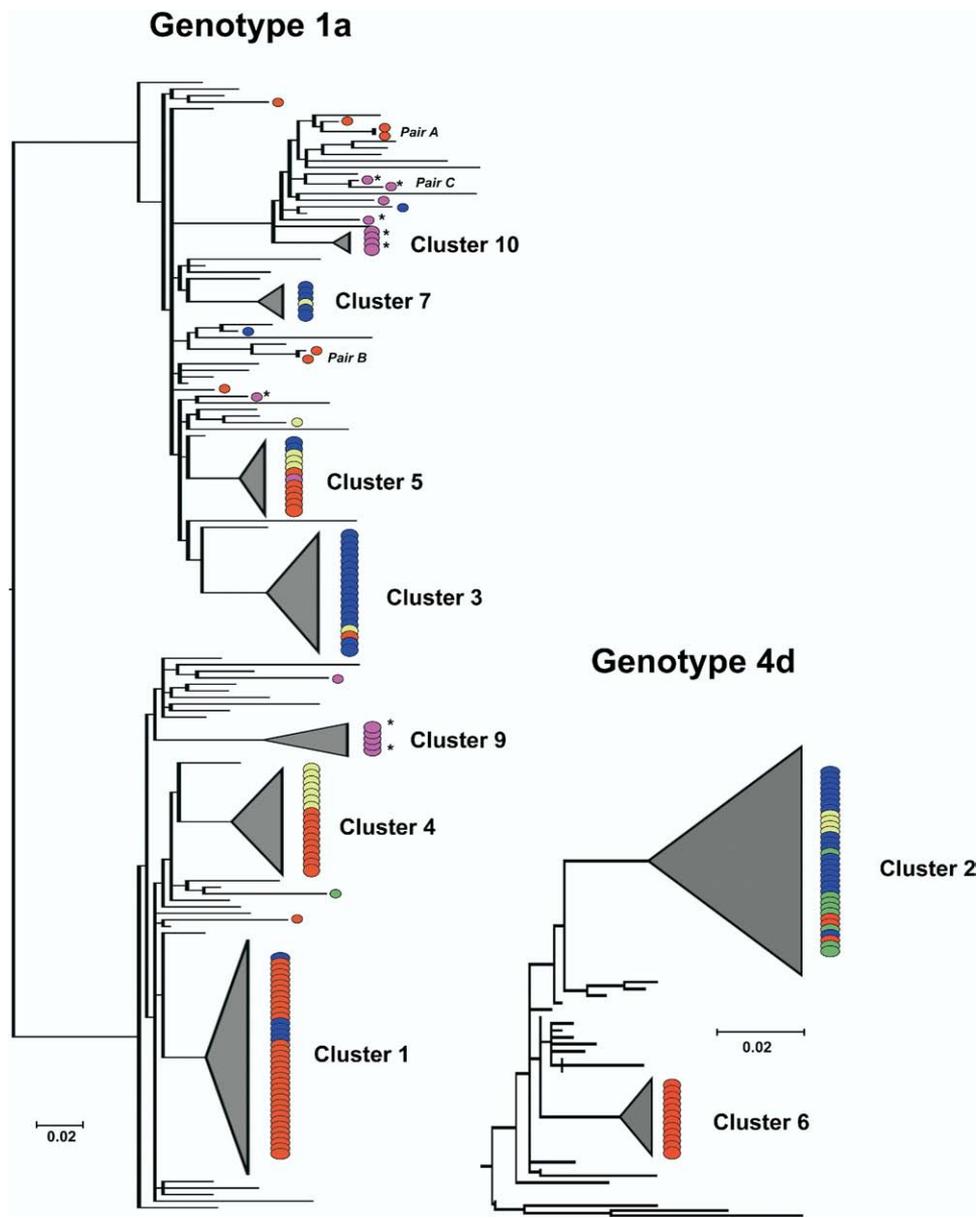


Figure 1. NS5B phylogenetic trees of HCV genotypes 1a (left) and 4d (right). Monophyletic clusters are shaded, country of origin coded: (red) England, (blue) The Netherlands, (yellow) Germany, (green) France, (purple) Australia. Australian MSM with reported IDU are marked IDU*.

For each MSM-specific HCV cluster, we used a Bayesian Markov Chain Monte Carlo approach to calculate the year of the most common recent ancestor in the MSM community. The calculated year of origin varied from 1975 (cluster 2) to 2001 (cluster 10). Because of the high rate of chronicity and long asymptomatic course of HCV infection, the year of the most recent common ancestor does not directly reflect the rate of spread of the virus within a population. Viral spread is reflected by the temporal distribution of lineage splits in each cluster. Except for clusters 8 and 9, the vast majority of lineage splits (85%-100%) occurred since 1996, regardless of the year of cluster origin. Overall, the percentage of lineage splits across all clusters decreased from 85% (n = 120) since 1996 to 78% (n = 111) since 1998 and 63% (n = 90) since 2000 (Figure 1).

Discussion

It has been estimated that parenteral risk factors, particularly IDU, account for 80% of HCV transmission in developed countries.⁹ However, the increasing number of HIV-positive MSM diagnosed with acute HCV infection in Europe, Australia, and the United States suggests that the epidemiology of HCV transmission in this population is changing. Using a phylogenetic approach, our study reveals the existence of a large European MSM-specific transmission network, linking the independently reported national HCV outbreaks in England, The Netherlands, Germany, and France. Based on the individual cohorts, evaluation of risk factors, which did not form part of this study, the majority of these infections relates to permucosal risk factors in the context of (traumatic)

sexual practices.^{1-3,8} A second MSM-specific transmission network, possibly related to both IDU and sexual behavior, was identified in Australia. Solely based on phylogenetic evidence, this Australian network at present shows very limited overlap with the network that exists in Europe.

Increased permucosal transmission of HCV implies that HCV is emerging as an STI, which is rapidly affecting an increasing proportion of HIV-positive MSM communities in Europe, Australia, and probably the United States. Our molecular analysis revealed 11 robust HCV transmission clusters among HIV-positive MSM in Europe and Australia, which included 86% of the European and 42% of the Australian MSM infections we studied. Such clustering is typical of multiple independent, parallel chains of transmission, each seeded by a single source of infection. Three strong lines of evidence suggest that, once introduced, each strain rapidly spreads to neighboring countries via a joint transmission network: (1) the large proportion (74%) of European MSM that is infected with an HCV strain that circulates in multiple European countries, (2) the observed low evolutionary distances among HCV isolates from different countries, and (3) the fact that the degree of country mixing tends to increase with increasing cluster size. Because MSM communities across Europe are large and interconnected, HCV transmission might become endemic among HIV-positive MSM, similar to what has been observed for hepatitis A virus and hepatitis B virus.^{13,29} Recently, an increasing incidence of non-IDU-acquired HCV was reported in HIV-negative MSM in Brighton, United Kingdom, raising the possibility of the infection bridging between HIV-positive and HIV-negative MSM populations.³⁰

Using an evolutionary analysis of HCV lineages circulating among European and Australian MSM, we calculated that the majority (85%) of transmission has occurred since 1996, with 63% of sampled lineage splits occurring after 2000. This observed discontinuity in the course of HCV infection among HIV-positive MSM over time would imply occasional introduction and transmission of HCV in the MSM population between 1975 and 1996, followed by a more rapid expansion of HCV transmission among HIV-positive MSM since 1996. Interestingly, recent longitudinal cohort studies from London and Amsterdam support our finding of a marked increase in HCV transmission since 2000. Before 2000, HCV incidence among HIV-positive MSM in both cities did not exceed 1 per 1000 person-years (PY) follow-up and, despite occasional introductions in the MSM community, sexual transmission of HCV remained rare before 2000.^{8,31} After 2000, the estimated annual HCV incidence in HIV-positive MSM attending HIV/genitourinary medicine clinics in London and Brighton has risen by 20% each year, from 7 per-1000 PY follow-up in 2002 to 12 per 1000 PY follow-up in the first 6 months of 2006.⁷ In

Amsterdam, HCV incidence rose 10-fold to 8.7 per 1000 PY in the period 2000–2003 compared with 0.8 per 1000 PY in the period 1984–1999.⁸ Significantly, in 2007, 15.5% of HIV-positive MSM attending the Amsterdam STI clinic during an anonymous survey tested positive for HCV infection.³² Our evolutionary analysis suggests that there has been a significant change in the pattern of HCV transmission since the late 1990s that has given rise to the spread of HCV in HIV-positive MSM all over Europe.

The reason for a change in HCV transmission pattern since the late 1990s remains unclear, but it probably relates to biologic and behavioral factors. It is striking that permucosal transmission of HCV is almost exclusively described in HIV-positive MSM. HIV coinfection might facilitate HCV transmission by increasing both viral infectiousness because of higher HCV viral loads in serum and semen and viral susceptibility through impaired immunologic control of HCV.^{33,34} However, HIV-positive MSM diagnosed with acute HCV infection in our study had relatively preserved CD4 counts, with median CD4 counts exceeding 500 cells/ μ L. Moreover, high rates of HCV acquisition soon after diagnosis with primary HIV infection among MSM in the United Kingdom argue for behavioral risk factors rather than a role for HIV infection itself.³⁵ Biologically, our data also argue against a change in the virulence of HCV because the identified cocirculating strains in this population belong to several different genotypes and subtypes. The timescale of the current HCV epidemic coincides with the introduction of HAART, which has been associated with a rise in sexual risk behavior and increased STI rates among MSM. Less concern about HIV given the availability of HAART and the belief that HAART reduces HIV transmission has been associated with increased sexual risk taking in MSM.³⁶ Furthermore, unprotected anal intercourse within the context of serosorting (ie, engaging in sexual risk only with HIV seroconcordant partners) is now considered a “harm reduction” strategy by reducing HIV transmission in those having unprotected anal intercourse.³⁷ However, this practice places these men at increased risk of HIV superinfection and STIs. Ulcerative STIs might potentiate sexual transmission of HCV through mucosal lesions; in particular, lymphogranuloma venereum and syphilis have been associated with acute HCV in HIV-positive MSM.^{1,3} In a recent case-control study, HCV/HIV coinfecting MSM were more likely to report unprotected anal intercourse, traumatic sexual techniques (eg, fisting and group sex), a history of STI, and the recreational use of party drugs compared with HIV monoinfected controls.² The popularity of the Internet, cheaper international travel, growing MSM tourism, and organized sex parties have probably extended sexual networks and increased the connectivity of high-risk MSM communities in different parts of the world. In a large survey among British residents, 62% of MSM outside a committed relationship reported a sexual

Table 4. Estimated Year of Origin for Each Identified Hepatitis C Virus Cluster and the Calculated Number of Lineage Divergent Events Over Time^a

Cluster	Genotype	Number of sequences	Year of origin (95% CI)	Number (%) of lineage splits since		
				1996	1998	2000
1	1a	37	1985 (1975–1993)	30 (83)	27 (75)	22 (61)
2	4d	34	1975 (1961–1989)	28 (85)	26 (78)	21 (64)
3	1a	19	1993 (1985–1999)	17 (94)	17 (94)	13 (72)
4	1a	17	1989 (1978–1998)	15 (94)	14 (88)	11 (69)
5	1a	12	1997 (1990–2001)	11 (100)	10 (91)	9 (82)
6	4d	12	1996 (1990–2000)	11 (100)	10 (91)	7 (64)
7	1a	6	1997 (1989–2003)	5 (100)	4 (80)	4 (80)
8	3a	6	1984 (1974–1993)	0 (0)	0 (0)	0 (0)
9	1a	5	1984 (1972–1994)	0 (0)	0 (0)	0 (0)
10	1a	4	2000 (1995–2004)	3 (100)	3 (100)	3 (100)

^aYear of origin and number of lineage splits could not be calculated for HCV cluster 11 because these 4 English MSM were part of a larger paraphyletic cluster that also contained HCV reference strains from Europe and the United States.

partner overseas in the last 2 holidays. Among MSM, travelling has been associated with increased risk for unprotected anal intercourse, especially among those with positive HIV status.^{38,39}

Australian sequences lie in 2 distinct country-specific HCV clusters, as might be expected given its relative geographic separation. In Australia, but also in Europe, it is plausible that HCV initially entered the MSM community through incidental transfer from the injection drug user population. In contrast to the European cases, IDU explains a significant proportion of HCV infections within the Australian MSM population. Importantly, however, MSM reporting IDU clustered with MSM who denied ever injecting, emphasizing that parenteral as well as permucosal risk factors contribute to the transmission of HCV among Australian MSM. Interestingly, 1 Australian MSM was infected with an HCV strain that also circulates in Europe, implying at least some HCV transmission between the 2 continents.⁴⁰ These results highlight the potential of HCV to rapidly infect other MSM populations. Importantly, whereas small HCV outbreaks among HIV-positive MSM have been observed,^{4,5} US health authorities have not yet reported spread of HCV on a scale similar to that in Europe or Australia. Phylogenetic analysis of HCV strains circulating in the United States would provide valuable information on the global spread of HCV in this population, especially because different European MSM have reported sexual contact in the United States during the acute phase of HCV infection.¹

In conclusion, Europe and Australia are currently facing a new epidemic of sexually transmitted HCV, which is rapidly infecting an increasing proportion of HIV-positive MSM through large international transmission networks. The first cases of HCV superinfection and reinfection have already been described.^{41,42} HCV/HIV coinfection negatively influences the natural history of HCV,^{16–18} in particular because HCV is acquired after HIV¹⁹ and at an older age (>40 years).⁴³ Therefore, the

current epidemic among HIV-positive MSM has serious clinical implications, exacerbated by the high frequency of difficult-to-treat HCV genotypes 1 and 4 in MSM in Europe and Australia. Regional collaborative measures are necessary, which should include targeted prevention, education of patients and clinicians, screening, and therapy of HCV. Annual HCV antibody screening should become part of the routine care of all HIV-positive MSM and should be considered for MSM with negative or unknown HIV status who report high-risk behaviors.⁴⁴ In particular, those participating in mucosal traumatic sex practices, group sex, unprotected anal intercourse with casual partners, IDU, and those diagnosed with ulcerative STIs should be more frequently screened. Early detection of HCV will allow early effective treatment and identification of risk factors, which may mitigate ongoing transmission.²⁰ In immunocompromised individuals with CD4 counts below 200 cells/mm³, HCV RNA testing may be appropriate.⁴⁴ If no action is taken, there is a risk that the current HCV epidemic might bridge into the HIV-negative MSM population. **Table 4.**

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Conflicts of interest

The authors disclose no conflicts.

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