Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviours

Mark Danta[a], David Brown[a], Sanjay Bhagani[b], Oliver G. Pybus[f], Caroline A. Sabin[c], Mark Nelson[d], Martin Fisher[e], Anne M. Johnson[c] and Geoffrey M. Dusheiko[a] for the HIV and Acute HCV (HAAC) group

Objective: To characterize the mode of hepatitis C virus (HCV) transmission in a recent epidemic of acute HCV in HIV-infected individuals using linked molecular and clinical epidemiological studies.

Design: Individuals diagnosed with acute HCV between 1999 and 2005 at three urban HIV units in the UK were enrolled into a phylogenetic and case–control study. Phylogenetic trees were constructed from the amplified sequences of the E1/E2 region of the HCV genome and were used to compare cases with unrelated sequences. A questionnaire-based, case–control study using matched controls recruited from each HIV unit identified putative transmission factors.

Results: One hundred and eleven HIV-positive men who have sex with men with acute HCV (genotype 1: 84%) were enrolled. Phylogenetic analysis of 93 E1/E2 sequences revealed seven monophyletic clusters signifying multiple independent HCV lineages co-circulating in the HIV-positive population. Permucosal rather than percutaneous transmission factors were associated with case/control status. Cases ($n = 60$) had more sexual partners, increased levels of high-risk sexual behaviour and were more likely to have shared drugs via a nasal or anal route in the preceding year in comparison with controls ($n = 130$). Sex in a group of more than two people was the strongest predictor of case/control status; odds ratios associated with participation in two or at least three types of high-risk sexual behaviour in a group were 9.16 (95% confidence interval, 3.51–23.90) and 23.50 (95% confidence interval, 9.47–58.33), respectively.

Conclusion: The identified co-circulating HCV lineages belong to different subtypes and genotypes, implying that rather than viral change, the epidemic is due to permucosal transmission factors that should be the focus of public health interventions.

AIDS 2007, **21**:983–991

Keywords: HIV, hepatitis C virus, hepatitis C, acute, co-infection, transmission, phylogenetic, epidemiology

Introduction

Hepatitis C virus (HCV) and HIV are two of the most prevalent persistent viral infections, with an estimated 10 million people co-infected worldwide [1]. Following the introduction of highly active antiretroviral therapy (HAART) for HIV and the consequent reduction in HIV-related morbidity and mortality, HCV has emerged as an

From the [a]UCL Institute of Hepatology, the [b]Department of HIV Medicine, the [c]Department of Primary Care and Population Sciences, Royal Free and University College Medical School, the [d]Department of HIV Medicine, Chelsea and Westminster Hospitals, London, the [e]Department of HIV Medicine, Brighton and Sussex University Hospitals Trust, and the [f]Department of Zoology, Oxford University, Oxford, UK.

Correspondence to Dr Mark Danta, UCL Institute of Hepatology, Rowland Hill Street, London, NW3 2PF, UK.

E-mail: m.danta@unsw.edu.au

Received: 5 October 2006; revised: 9 January 2007; accepted: 6 February 2007.

increasingly significant problem in this group. HCV/HIV co-infection is associated with accelerated hepatic fibrosis, resulting in increased hepatic-related morbidity and mortality [2–4]. In the developed world, end-stage liver disease is now a leading cause of death among HIV-infected individuals [5,6].

Acute HCV mono-infection rarely presents clinically [7]. However, since 2000 there has been a reported rise in the diagnosis of acute HCV among HIV-positive men who have sex with men (MSM) in London [8]. Cohorts of acute HCV in HIV-positive MSM have recently been reported from Europe and USA [9–12]. Interestingly, the majority of these cases did not have the usual recognised parenteral transmission risk factors for acute HCV.

The aim of this study was to determine factors associated with the outbreak of HCV among HIV-positive MSM in the UK using a combined molecular and clinical epidemiological approach. Identification of these factors will allow specific strategies to be developed to mitigate the spread of HCV in the HIV-positive population.

Materials and methods

Case definition

The cohort consisted of all HIV-positive patients diagnosed with acute HCV infection at three large urban HIV clinics in south-east England between 1999 and 2005. As previously reported, acute HCV was defined as a documented seroconversion to anti-HCV antibodies and/or clinical and biochemical criteria (acute hepatitis in individuals without pre-existing liver disease, excluding other infective, metabolic, toxic and drug causes, and a serum alanine aminotransferase (ALT) level ≥ 10 times the upper limit of normal (ULN)) and positive HCV-RNA by PCR [13]. ALT is usually screened every 6 months and anti-HCV testing was performed in any individual if their ALT was at least twice the ULN. Anti-HCV testing is also performed routinely at HIV diagnosis and, since 2002, on an annual basis in all patients at the three participating clinics, which all treat parenterally and permucosally acquired HIV: Royal Free Hospital (RFH), London; the Chelsea and Westminster Hospitals (CWH), London; and the Brighton and Sussex General Hospital (BSGH), Brighton. The study was performed with the approval of the local research ethics committee at each unit (LREC references: RFH 6148; CWH 3247; BSGH B03/83) and the Central Office for Research Ethics Committees. Patients provided written informed consent to participate in the study.

Phylogenetic study

HCV RNA was amplified from the first stored HCV RNA positive serum sample. A 495-nucleotide segment of the E1/E2 region was sequenced using previously published methods [14]. Viral epidemic history was assessed by constructing phylogenetic trees of the case sequences plus all available unrelated reference sequences, which were obtained from GenBank (www.ncbi.nlm.nih.gov) and the Los Alamos HCV Sequence Database (www.hcv.lanl.gov). Phylogenies were constructed using maximum likelihood in PAUP* version 4, under the Hasegawa-Kishino-Yano substitution model with gamma distributed among-site rate variation (HKY-Γ) [15]. Statistical robustness of the phylogenies was assessed by bootstrapping with 1000 replicates (bootstrap scores > 70 represent robust clusters). The date of origin of each HCV cluster, which approximates the year of entry of HCV into the cluster, was estimated using a molecular clock approach. An evolutionary rate for our specific E1/E2 gene region was estimated using BEAST v1.3 software and a previous estimate of the E1/E2 nucleotide substitution rate [16]. The inferred nucleotide evolutionary rate for our sequences was 2.13×10^{-3} substitutions per site per year. Dates of origin for each cluster were subsequently calculated using a Bayesian MCMC approach in BEAST v1.3 [17].

Case–control study

All patients from the phylogenetic study were approached for enrollment into a case–control study at their respective clinics. HCV negative controls were identified from each clinic's database matching for: age (± 5 years), length of HIV infection (± 1 year), homosexuality, race, and HAART exposure status (ever/never). When dealing with sensitive sexual and drug behaviours, it has been suggested that self-completion questionnaires may result in more valid reports than with interviewers, so this methodology was used [18]. Questionnaires were completed by individuals in private during clinic visits. The self-administered questionnaire assessed recognized HCV transmission risk factors over the 12 months before diagnosis of HCV. Data were collected on where participants met other men and number of sexual partners. Specific high-risk sexual and drug behaviours were explored, a behaviour being considered high-risk if there was a risk of mucosal trauma and/or exposure to body fluids. This included unprotected anal intercourse (UAI), rimming (anal/oral sex play), fisting (insertion of the hand into the anus), use of sex toys and shared drug implements. Participants were asked about public place and group sex practices. Finally, data were collected on recreational drug use and participation in sexual activity under the influence of alcohol or drugs. Controls were allocated the same 'pseudo-date' of diagnosis as their matched case and were asked to relate their responses to the 12 months before this date.

Statistical analysis

As matching was incomplete, simple chi-squared tests and Fisher's exact tests were used to compare qualitative variables between cases and controls and Mann–Whitney U tests were used to compare quantitative variables.

Table 1. Patient demographics.

	Phylogenetic study		Case–control study			
	All HCV patients	HCV patients not participating in case–control study	All patients	Matched controls	HCV patients participating in case–control study	*P*-value*
Number of patients (%)	111 (100)	51 (46)	190 (100.0)	130 (68.4)	60 (31.6)	
Median (range) age (years)	36 (24–58)	35 (24–55)	36 (22–58)	36 (22–58)	36 (24–58)	0.76
Estimated median (range) duration of HIV (years)	5.3 (0–19.2)	6.2 (0–19.2)	4.5 (0–18.7)	4.9 (0–18.2)	3.7 (0–18.7)	0.44
Number (%) ever exposed to HAART	66 (62.9)	27 (52.9)	131 (68.9)	91 (70.0)	40 (66.7)	0.77
Median (range) CD4 cell count at 'date of interest' (cells/μl)	493 (33–2715)	462 (33–2713)	517 (27–1854)	521 (27–1854)	514 (61–1382)	0.76
Number (%) with HIV RNA < 50 copies/ml at 'date of interest'	56 (56)	27 (52.9)	88 (54.0)	59 (51.8)	29 (59.2)	0.38

*P-values comparing cases and controls from case-control study calculated using chi-squared test or Mann–Whitney U-test as appropriate. HCV, hepatitis C virus; HAART, highly active antiretroviral therapy.

Factors associated with case/control status in univariable analyses ($P < 0.05$) were then entered into a multivariable logistic regression model to identify factors independently associated with acute HCV infection.

As many of these variables were highly correlated, summary variables that provided measures of overall risk were retrospectively created to simplify the analysis following review. For example, a variable (taking values from 0 to 5) was created that measured the number of different types of locations where men had met other men in the preceding 12 months. Similarly, variables were created to record the number of different behaviours undertaken relating to: (1) oral sexual risks; (2) anal sexual risks; (3) fisting or use of sex toys; (4) risks involving semen; (5) risks involving blood; and (6) sexual behaviour undertaken in a public place or as part of a group of more than two people (receptive or insertive anal intercourse, receptive or insertive fisting). Recreational drugs were reclassified into the number of different (1) intranasal route 'club' drugs (metamphetamines, ketamine, gamma hydroxybutyrate, amyl nitrites, cocaine), (2) oral route 'club' drugs (lysergic acid diethylamide, ecstasy), and (3) other drugs (barbituates/benzodiazepines, crack cocaine, amphetamines and heroin).

All statistical tests were two-sided and comparisons were considered statistically significant if $P < 0.05$. All statistical analyses were performed using SAS (version 8: SAS Institute, Cary, North Carolina, USA).

Results

Phylogenetic analysis

The phylogenetic analysis enrolled 111 consecutive patients (RFH, $n = 50$; CWH, $n = 50$; and BSGH, $n = 11$). All cases were HIV-positive MSM. The majority (92.8%) were asymptomatic and diagnosed following investigation for abnormal transaminases or screening for HIV/sexually transmitted infections (STIs). Only eight individuals (7.2%) were icteric at diagnosis. Anti-HCV seroconversion or previous negative HCV RNA was demonstrated in 107 (96%), whereas four (4%) had a biochemical hepatitis with detectable HCV RNA. Table 1 gives the characteristics of phylogenetic analysis patients, the sub-group participating in the case–control study and their matched controls. The median age of patients was 36 years (range, 24–58 years). The median CD4 cell count was 493 cells/μl (range, 33–2715 cells/μl) and 66 (63%) individuals had received HAART. The majority of individuals ($n = 93$, 84%) were infected with HCV genotype 1; median HCV RNA and peak ALT levels were 6.1 log IU/ml (range, 3.0–7.6 log IU/ml) and 285 IU/ml (range, 22–5104 IU/ml), respectively.

The E1/E2 sequences were amplified from 93 (84%) patients (80 genotype 1a; seven genotype 1b; six genotype 3a). Of the 18 samples that could not be amplified, six were genotype 1, six were non-genotype 1, and six could not be typed. None of the genotype 4 HCV ($n = 5$) identified by the EndoLIPA assay was amplified using the E1/E2 primers which probably relates to the primers used. The case sequences were compared to 330 unrelated E1/E2 reference sequences (96 genotype 1a, 183 genotype 1b, and 51 genotype 3a).

The genotype 1a phylogeny revealed five monophyletic clusters (> 2 sequences), which included 73 (78%) of the sequences obtained (Fig. 1). The largest cluster comprised 43 (46%) patient sequences. The bootstrap values (76–100%) demonstrate the robustness of these clusters. Seven individual sequences did not group significantly with any cluster, suggesting that they were unrelated. The genotype 1b phylogeny revealed one monophyletic cluster of seven cohort sequences with a bootstrap value

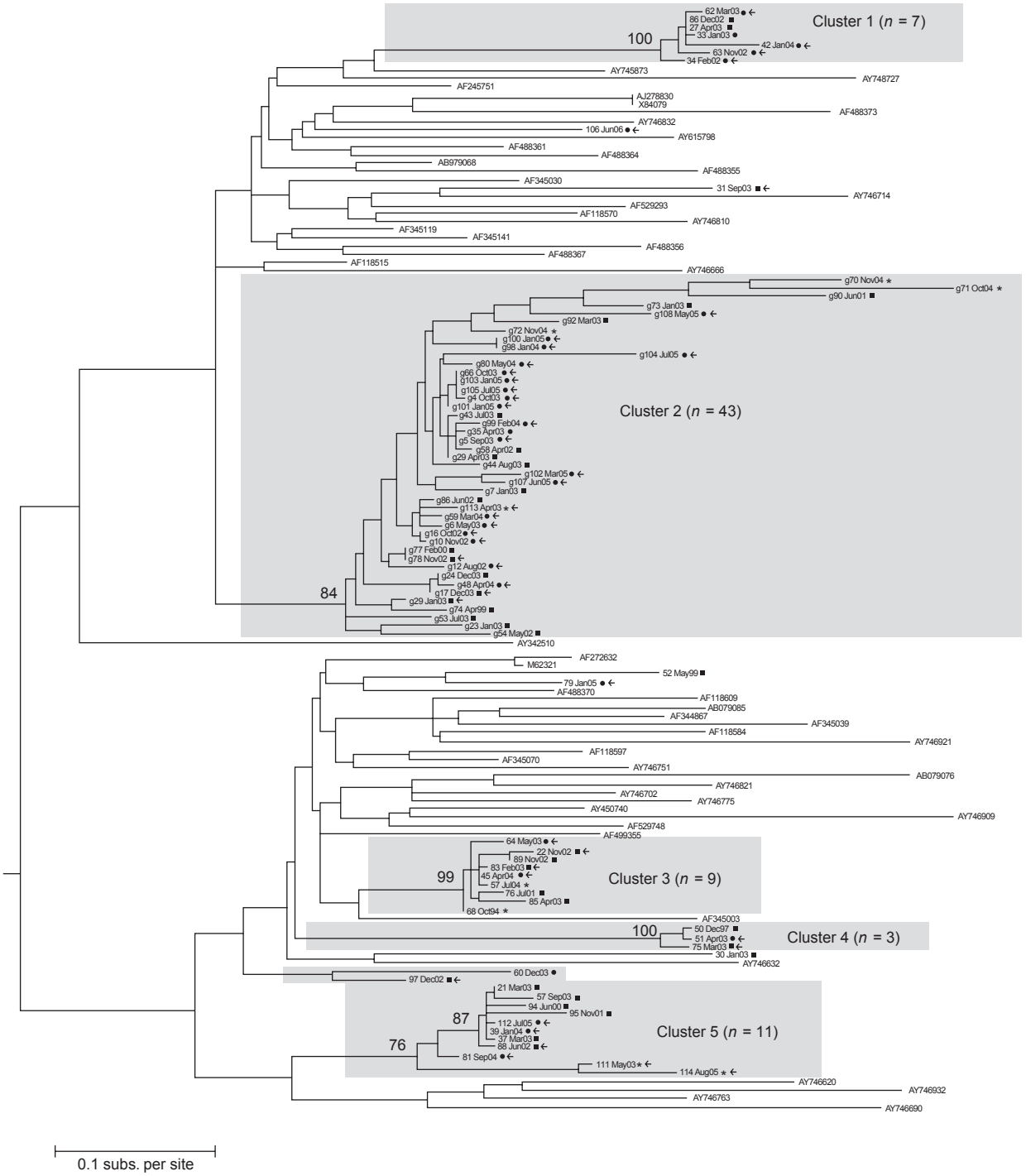

Fig. 1. Genotype 1a maximum likelihood phylogenetic tree. Maximum likelihood phylogeny of the subtype 1a sequences. Case sequences are highlighted. Each hepatitis C virus (HCV) cluster among HIV-positive men who have sex with men (MSM) is identified and a bootstrap value is given at the root of each cluster. Each sequence's centre of origin is given: ● = Royal Free Hospital (RFH), London; ■ = Chelsea and Westminster Hospitals (CWH), London; ⋆ = Brighton and Sussex General Hospital (BSGH), Brighton.

of 100% [Supplemental Figure 1 (available online)]. The genotype 3a phylogeny revealed one monophyletic cluster consisting of four sequences with a bootstrap value of 74% [Supplemental Figure 2 (available online)] and two ungrouped sequences.

Four of the seven clusters have origins in the mid-1990s (Table 2), implying the onset of transmission of these lineages around this time. The majority (64%) of all lineage divergences have occurred since 1995, indicating increased transmission from this time.

Table 2. Date of origin for each identified hepatitis C virus (HCV) cluster.

Cluster	HCV subtype	Number of sequences	Year of origin (CI)*	Number (%) of divergence events since 1995
1	1a	7	1994 (1988–1998)	5 (83)
2	1a	43	1962 (1946–1972)	21 (50)
3	1a	9	1995 (1991–1998)	6 (75)
4	1a	3	1995 (1990–1998)	1 (50)
5	1a	11	1983 (1974–1989)	8 (80)
6	1b	7	1999 (1996–2001)	6 (100)
7	3a	4	1982 (1971–1989)	2 (66)

*Estimated using a Bayesian MCMC approach in BEAST v1.3. CI, confidence interval.

Case–control study

Sixty (54%) patients from the phylogenetic study participated in the case–control study (RFH, $n=42$; CWH, $n=13$; and BSGH, $n=5$). The reasons for non-participation were: refusal ($n=6$), questionnaire not returned ($n=42$), lost to follow-up ($n=3$). There were no significant demographic differences between phylogenetic study patients who did or did not participate in the case–control study (Table 1). The sequences from those participating in the case–control study were representative of the overall phylogenetic tree structures.

Cases were matched with 130 controls (RFH, 122 and CWH, 8); 87% had at least one (up to four) matched control(s), and controls could not be found for the remaining 13% of cases. The number of controls per case was as follows: 13 had one control; 35 had two controls; 13 had three controls; and two had four controls. Whereas the controls were identified for all cases, these controls were not recruited because of refusal or clinic non-attendance. There was a trend towards a higher proportion of cases than controls reporting injection drug use in the preceding 12 months (17.2 versus 6.6%; $P=0.08$). Importantly, the majority of cases (82.8%) did not have a parenteral risk factor. A greater proportion of cases compared to controls had a history of percutaneous piercing (70 versus 52.3%; $P=0.03$) (Table 3).

Sexual risk factors

Cases had significantly more sexual partners than controls, with a median (range) of 30 (0–1000) versus 10 (1–800) partners over the preceding 12 months, $P<0.001$. Over the 12 months, cases were more likely than controls to have met other men at private parties (83.3 versus 65.1%; $P=0.02$), recognised sex venues such as clubs/bath-houses/saunas (75.0 versus 53.5%; $P=0.008$), and internet sites (81.7 versus 58.9%; $P=0.004$). The internet was the most frequently used location by cases to meet men, cases using the internet a median of 50 times versus seven times for controls, $P=0.003$.

Table 3. HCV risk factors among cases and controls participating in the case–control study: parenteral risk factors.

	Number participating (%)		
	Controls (%)	Cases (%)	*P*-value*
Injection drug use in prior 12 months (%)	6 (6.6)	10 (17.2)	0.08
Tattoo in lifetime (%)	57 (43.9)	36 (60)	0.06
Blood product transfusion in lifetime (%)	10 (7.7)	10 (16.7)	0.11
Percutaneous piercing in lifetime (%)	68 (52.3)	42 (70)	0.03

Overall, cases reported more high-risk sexual behaviour than controls in the preceding 12 months (Table 4). Although there was no difference in participation in protected (using condom) anal intercourse (AI) between cases and controls, cases were more likely than controls to have had receptive and insertive UAI without ejaculation and with ejaculation. The median number of partners for each of these practices was 10 versus 1, $P=0.0001$; 10 versus 0, $P=0.0001$, 3 versus 0, $P=0.0001$; 1 versus 0, $P=0.0003$, respectively. This pattern of a significantly higher median numbers of partners in cases was consistent in all practices where cases participated more than controls. Insertive and receptive fisting and the use of sex toys were also significantly associated with case/control status. Cases were also more likely to have had a STI in their lifetime than controls, specifically syphilis (41.7 versus 18.5%; $P=0.001$), gonorrhoea (73.3 versus 47.7%; $P=0.002$), and non-specific urethritis (55 versus 33.1%; $P=0.007$).

Group sex participation was significantly more common in cases (Table 4). Cases involved in group sex were more likely to participate in four specific practices: receptive and insertive UAI and receptive and insertive fisting when compared with controls. Participation in these specific practices in a group was the only independent predictor of case/control status in the multivariable analysis (analysis not shown). The odds ratios for HCV associated with participation in two or at least three of these specific sexual practices in a group were 9.16 (95% confidence interval, 3.51–23.90) and 23.50 (95% confidence interval, 9.47–58.33), respectively.

Drug use

Significantly more cases than controls had participated in sex while under the influence of recreational drugs

Table 4. Hepatitis C virus risk factors among cases and controls participating in the case–control study: sexual practices over preceding 12 months.

	Number participating (%)		
	Controls (%)	Cases (%)	*P*-value*
Sexual practice:			
Active oral sex (no ejaculation)	112 (94.1)	57 (96.6)	0.73
Active oral sex (ejaculation)	56 (47.1)	37 (62.7)	0.07
Active oral sex with condoms (safe)	12 (10.1)	5 (8.5)	0.94
Passive oral sex (no ejaculation)	109 (91.6)	52 (89.7)	0.89
Passive oral sex (ejaculation)	42 (35.3)	30 (50.9)	0.07
Passive oral sex with condoms (safe)	11 (9.2)	2 (3.4)	0.22
Receptive UAI (no ejaculation)	60 (50.4)	53 (89.8)	0.0001
Receptive UAI (ejaculation)	42 (35.3)	46 (78.0)	0.0001
Receptive AI with condoms (safe)	83 (69.8)	44 (74.6)	0.62
Insertive UAI (no ejaculation)	57 (47.9)	49 (83.1)	0.0001
Insertive UAI (ejaculation)	39 (32.8)	34 (57.6)	0.003
Insertive AI with condoms (safe)	82 (68.9)	40 (69.0)	1.00
Passive rimming	92 (77.3)	58 (98.3)	0.0007
Active rimming	92 (77.3)	54 (91.5)	0.03
Insertive fisting	31 (26.3)	44 (74.6)	0.0001
Receptive fisting	15 (12.6)	34 (57.6)	0.0001
Use of sex toys	51 (42.9)	46 (78.0)	0.0001
Lifetime sexually transmitted infection (%)	78 (78)	51 (92)	0.005
Group sex participation (group of >2 individuals):	63 (52.5)	52 (88.1)	0.0001
Group sex practices			
Receptive UAI	26 (41.3)	49 (94.2)	0.0001
Insertive UAI	30 (47.6)	44 (84.6)	0.0001
Receptive fisting	9 (14.3)	29 (55.8)	0.0001
Insertive fisting	10 (15.9)	35 (67.3)	0.0001
Group sex by number of sex practices			
0–1	94 (78.3)	11 (18.6)	
2	14 (11.7)	15 (25.4)	
3–4	12 (10.0)	33 (55.9)	0.0001

*The proportions who have ever had each type of sex were compared using chi-squared tests (or Fisher's exact test if appropriate). anal intercourse (AI)Unprotected anal intercourse (UAI);

(91.7 versus 61.5%; $P<0.001$). There was no difference in sex under the influence of alcohol between the groups (60 versus 67.7%, $P=0.38$). Recreational drug use was more common in cases than controls (Table 5). Recreational drugs more frequently used by cases were methamphetamines (crystal meth), ketamine, gamma hyroxybutyrate (GHB), amyl nitrites (poppers), ecstasy [3,4-methylnedioxymethamphetamine (MDMA)], and lysergic acid diethylamide (LSD). In addition, cases were more likely than controls to have shared the implements for the intranasal and rectal routes of administration. After adjusting for group sex in a multivariable logistic

Table 5. HCV risk factors among cases and controls participating in the case–control study: drug risk factors in preceding 12 months.

	Controls (%)	Cases (%)	*P*-value*
Recreational drug use over 12 months:	90 (70.9)	58 (96.7)	0.0001
Marijuana	67 (70.5)	40 (69.0)	0.98
Methamphetamines (crystal meth)	23 (24.2)	33 (56.9)	0.0001
Ketamine	46 (48.4)	48 (82.8)	0.0001
Gamma hydroxybutyrate (GHB)	24 (25.3)	30 (51.7)	0.002
Amyl nitrites (poppers)	64 (67.4)	52 (89.7)	0.003
Cocaine	61 (64.2)	46 (79.3)	0.07
Lysergic acid diethylamide (LSD)	13 (13.7)	20 (34.5)	0.005
Ecstasy (MDMA)	57 (60.0)	48 (82.5)	0.006
Downers (barbiturates, benzodiazepines)	28 (29.5)	27 (46.6)	0.05
Crack cocaine	4 (4.2)	3 (5.2)	1.00
Other amphetamines (speed)	10 (10.5)	6 (10.3)	1.00
Heroin	8 (8.4)	4 (6.9)	0.98
Shared routes of drug use:			
Oral route	15 (16.5)	12 (20.7)	0.67
Nasal route	45 (48.9)	46 (79.3)	0.0004
Injection route	0 (–)	1 (1.7)	n/a
Other route (rectal)	5 (5.4)	12 (21.4)	0.007

*The proportions who have ever had used each type of drug were compared using chi-squared tests (or Fisher's exact test if appropriate).

regression model, recreational drug use did not remain significantly associated with case/control status.

Discussion

This study confirms that transmission of HCV is occurring in HIV-positive MSM. It revealed that there have been multiple independent introductions of HCV into the HIV-positive MSM population, which subsequently led to a sustained chain of transmission in this risk group over a period of years. The phylogenies identified robust HCV transmission clusters, demonstrating that at least seven genetically distinct HCV variants are co-circulating within the HIV-positive population of London and Brighton. Importantly, this implies that the recent increase in HCV transmission is not due to viral change but rather to patient and/or environmental factors. The clusters comprised 84 (76%) of cases, with the largest cluster containing almost half of all case sequences. In contrast to the reported European cohorts, the UK sequences are predominantly genotype 1. Interestingly, every cluster except cluster 7 (subtype 3a) contained sequences from at least two of the three participating HIV units, indicating little geographical clustering of transmission. This has important implications for preventative strategies.

Although the clinics treat and screen both permucosally and parenterally acquired HIV patients, all cases identified were MSM, suggesting that factors specific to this group contribute to HCV transmission. In contrast to the usual pattern of HCV transmission, parenteral risk factors did not explain the vast majority (82.8%) of transmissions [19]. However, our study identified a number of putative permucosal transmission factors. For permucosal transmission of HCV to occur there must be disruption of mucosal integrity with exposure to infected body fluids, which could arise through mucosally traumatic sexual practices or instrumentation used when sharing drugs.

High-risk mucosally traumatic sexual practices were significantly associated with case/control status, consistent with evidence that high-risk sexual behaviour has increased in MSM since the introduction of HAART [20–23]. The dates of origin and sequence divergence of the transmission clusters provide strong evidence of increased HCV transmission since the mid to late 1990s, coinciding with the introduction of HAART. The reasons for an increase in high-risk sexual behaviour since the introduction of HAART are complex. A recent meta-analysis suggested a belief that HAART reduces HIV transmission or less concern about HIV given the availability of HAART were both associated with high-risk sexual behaviour [24]. It had been assumed until recently that individuals did not purposely seek to have unprotected sex, however, HIV seroconcordant sexual partnering is now considered a 'harm reduction' strategy by reducing HIV transmission in those having UAI. However, this places these men at increased risk of HIV super-infection and STIs such as lymphogranuloma venereum, which has been associated with acute HCV in HIV-positive MSM [10]. Intentional unprotected intercourse ('barebacking') has been associated with higher levels of sexual compulsivity, lower responsibility for safer sex and increased recreational drug use [25]. Finally, the internet is a new environment that may foment high-risk sexual behaviour, with internet sex seekers reporting higher rates of STIs, more partners and more AI [26]. In our study, the internet was the most frequently used location where the cases met other men.

Recreational drug use has been linked with high-risk sexual practices [27,28]. Many of the drugs taken by cases in our study are taken by intra-nasal and rectal routes, the implements of which were shared more often by cases than controls. Although contaminated implements could transmit HCV, intranasal transmission of HCV is rare [29]. Rectal transmission associated with shared implements could potentially occur in the context of mucosal trauma associated with sex. It is more likely, however, that drug effects lead to higher-risk sexual behaviours due to disinhibition and sexual arousal. Thus, the use of any of these drugs represents a marker of potential risk behaviour [30]. Similarly, the lifetime risk of STIs was significantly higher in cases compared with controls, which is probably a marker of high-risk sexual behaviour, however, STIs could potentiate HCV transmission through mucosal lesions.

The interaction between sexual and drug behaviours is complex. Many of these factors are highly correlated and their effects are difficult to disentangle in multivariable analysis. The final multivariable model identified group sex as the only independent factor associated with acute HCV infection. While this behaviour displays the strongest association with HCV infection, it is not, in itself, a specific mechanism for transmission of HCV. Instead, it is more likely that it represents a context within which other risk factor(s), such as mucosally traumatic sex or sharing of drug implements, may play a role. Qualitative research among HIV-positive MSM participating in group sex parties has found that individuals used these parties as a means of serosorting with very high rates of UAI [31].

As for all case–control studies, the retrospective design makes it difficult to imply causation. The response rate for the case–control study was 54% for cases from the phylogenetic study. The majority of non-participants failed to return completed questionnaires, the self-completion methodology probably contributing to this low response rate. Importantly, while there was no significant demographic difference between those participating and those refusing to participate in the case–control study, if more of the higher-risk individuals with HCV participated this would have increased the apparent difference between the

cases and controls. The use of 'pseudo-date' for the matching of the self-completed questionnaire was an attempt to remove temporal behavioural changes as a confounder and to minimize recall bias as far as possible by ensuring that cases and controls referred to the same calendar time period. However, this may not have fully removed recall bias as cases may have had a better recollection of their behaviours for the period preceding the acquisition of HCV. Finally, data collected at the event-level would have provided a more detailed insight into the pattern and temporal relationships of the specific sexual and drug practices of this group.

The molecular clock analysis indicates that the HCV entered the largest cluster (2) in the 1960s. Initially this could have been due to parenteral (intravenous drug use or transfusion) or permucosal (sexual) factors, however we have demonstrated that the recent transmission pattern relates to predominantly permucosal transmission. While this is a new phenomenon, these high-risk behaviours were certainly present at the beginning of the HIV epidemic in the 1970/1980s. We speculate that HIV co-infection may be an important factor in the recent increase in HCV transmission through impaired immunological control of HCV, increased serum and semen HCV loads and high-risk sexual behaviours between seroconcordant couples [32–34]. Unfortunately HIV was a matched variable in our study and therefore could not be analysed.

While the importance of sexual transmission of HCV in the general population remains controversial, this study has identified permucosal HCV transmission related to high-risk mucosally traumatic sexual and drug practices as the putative factors in an ongoing epidemic of acute HCV. This study adds weight to the growing evidence of increased risk behaviour within the MSM community, providing impetus for improved public health messages about the risks of unsafe sexual and drug practices. We recommend enhanced surveillance of individuals participating in these high-risk behaviours through anti-HCV screening. Education-based public health interventions should focus on reducing traumatic sex and drug practices through promotion of safe sex practices (particularly during group sex), and minimization of recreational drug use and sharing of drug implements.

Acknowledgements

HIV and Acute HCV (HAAC) group

M. Nelson, Y. Gileece, M. Aitkins, D. Asboe, R. Browne, E. Low, Department of HIV Medicine, Chelsea and Westminster Hospitals.

S. Bhagani, M. Johnson, G. Mulhoney, T. Fernandez, Department of HIV Medicine.

AM Geretti, Department of Virology.

M. Danta, D. Brown, G. Dusheiko, M. Jacobs, UCL Institute of Hepatology.

C. Sabin, A. Johnson, Department of Primary Care and Population Sciences, Royal Free and Hampstead Trust/ Royal Free and University College Medical School.

M. Fisher, N. Perry, A. Phillips, Department of HIV Medicine, Brighton and Sussex University Hospitals Trust.

O. Pybus, Department of Zoology, Oxford University.

As corresponding author, M.D. had full access to all the data and takes responsibility for the integrity of the data and accuracy of the data analysis.

No author has any financial disclosures.

Sponsorship: This study was supported by the Special Trustees of Royal Free & University College Medical School Fellowship; the Peter Samuel Fellowship; Fellowship of the Royal Pathological Society.

References

1. World Health Organisation regional office for Europe. Health evidence network, HIV/HCV co-infection (http://www.euro.who.int/HEN/Syntheses/hepatitisC/20050411_7). Accessed: 2 March 2007.
2. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, *et al*. **Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.** *N Engl J Med* 1998; **338**:853–860.
3. Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, *et al*. **Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group.** *Hepatology* 1999; **30**:1054–1058.
4. Sulkowski MS, Thomas DL. **Hepatitis C in the HIV-infected person.** *Ann Intern Med* 2003; **138**:197–207.
5. Bica I, McGovern B, Dhar R, Stone D, McGowan K, Scheib R, Snydman DR. **Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection.** *Clin Infect Dis* 2001; **32**:492–497.
6. Puoti M, Spinetti A, Ghezzi A, Donato F, Zaltron S, Putzolu V, *et al*. **Mortality for liver disease in patients with HIV infection: a cohort study.** *J Acquir Immune Defic Syndr* 2000; **24**:211–217.
7. Hoofnagle JH. **Hepatitis C: the clinical spectrum of disease.** *Hepatology* 1997; **26**:15S–20S.
8. Browne R, Asboe D, Gilleece Y, Atkins M, Mandalia S, Gazzard B, Nelson M. **Increased numbers of acute hepatitis C infections in HIV positive homosexual men; is sexual transmission feeding the increase?** *Sex Transm Infect* 2004; **80**:326–327.
9. Gambotti L, Batisse D, Colin-de-Verdiere N, Delaroque-Astagneau E, Desenclos JC, Dominguez S, *et al*. **Acute hepatitis C infection in HIV positive men who have sex with men in Paris, France, 2001–2004.** *Euro Surveill* 2005; **10**:115–117.
10. 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**:969–974.
11. Luetkemeyer A, Hare CB, Stansell J, Tien PC, Charlesbois E, Lum P, *et al*. **Clinical presentation and course of acute hepatitis C infection in HIV-infected patients.** *J Acquir Immune Defic Syndr* 2006; **41**:31–36.

12. Serpaggi J, Chaix ML, Batisse D, Dupont C, Vallet-Pichard A, Fontaine H, *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**:233–240.
13. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, *et al*. **Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance.** *Gastroenterology* 2003; **125**:80–88.
14. Farci P, Shimoda A, Coiana A, Diaz G, Peddis G, Melpolder JC, *et al*. **The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies.** *Science* 2000; **288**:339–344.
15. Swofford DL. *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods),* version 4. Sunderland, MA: Sinauer and Associates; 2003.
16. Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JW, Gojobori T, Alter HJ. **Inaugural article: a comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades.** *Proc Natl Acad Sci U S A* 2002; **99**:15584–15589.
17. Drummond A, Rambaut A. *BEAST v1.0.* Available from http://evolve.zoo.ox.ac.uk/beast/; 2003.
18. Catania JA, Binson D, Van der Straten A. **Methodological research on sexual behaviour in the AIDS era.** *Annual Review of Sex Research* 1995; **6**:77–125.
19. Alter MJ. **Epidemiology of hepatitis C in the West.** *Semin Liver Dis* 1995; **15**:5–14.
20. Macdonald N, Dougan S, McGarrigle CA, Baster K, Rice BD, Evans BG, Fenton KA. **Recent trends in diagnoses of HIV and other sexually transmitted infections in England and Wales among men who have sex with men.** *Sex Transm Infect* 2004; **80**:492–497.
21. Simms I, Fenton KA, Ashton M, Turner KM, Crawley-Boevey EE, *et al*. **The re-emergence of syphilis in the United Kingdom: the new epidemic phases.** *Sex Transm Dis* 2005; **32**:220–226.
22. Dodds JP, Mercey DE, Parry JV, Johnson AM. **Increasing risk behaviour and high levels of undiagnosed HIV infection in a community sample of homosexual men.** *Sex Transm Infect* 2004; **80**:236–240.
23. Dodds JP, Nardone A, Mercey DE, Johnson AM. **Increase in high risk sexual behaviour among homosexual men, London 1996–8: cross sectional, questionnaire study.** *BMJ* 2000; **320**:1510–1511.
24. Crepaz N, Hart TA, Marks G. **Highly active antiretroviral therapy and sexual risk behavior: a meta-analytic review.** *JAMA* 2004; **292**:224–236.
25. Halkitis PN, Wilton L, Wolitski RJ, Parsons JT, Hoff CC, Bimbi DS. **Barebacking identity among HIV-positive gay and bisexual men: demographic, psychological, and behavioral correlates.** *AIDS* 2005; **19**:S27–S35.
26. McFarlane M, Bull SS, Rietmeijer CA. **The Internet as a newly emerging risk environment for sexually transmitted diseases.** *JAMA* 2000; **284**:443–446.
27. Stall RD, Hays RB, Waldo CR, Ekstrand M, McFarland W. **The Gay '90s: a review of research in the 1990s on sexual behavior and HIV risk among men who have sex with men.** *AIDS* 2000; **14 (suppl 3)**:S101–S114.
28. Colfax G, Vittinghoff E, Husnik MJ, McKirnan D, Buchbinder S, Koblin B, *et al*. **Substance use and sexual risk: a participant- and episode-level analysis among a cohort of men who have sex with men.** *Am J Epidemiol* 2004; **159**:1002–1012.
29. Centers for Disease Control and Prevention. **Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. Centers for Disease Control and Prevention**. *MMWR Recomm Rep* 1998; **47**:1–39.
30. Purcell DW, Moss S, Remien RH, Woods WJ, Parsons JT. **Illicit substance use, sexual risk, and HIV-positive gay and bisexual men: differences by serostatus of casual partners.** *AIDS* 2005; **19 (suppl 1)**:S37–S47.
31. Clatts MC, Goldsamt LA, Yi H. **An emerging HIV risk environment: a preliminary epidemiological profile of an MSM POZ Party in New York City.** *Sex Transm Infect* 2005; **81**:373–376.
32. Kim AY, Lauer GM, Ouchi K, Addo MM, Lucas M, Schulze Zur Wiesch J, *et al*. **The magnitude and breadth of hepatitis C virus-specific CD8+ T cells depend on absolute CD4+ T-cell count in individuals coinfected with HIV-1.** *Blood* 2005; **105**:1170–1178.
33. Harcourt G, Gomperts E, Donfield S, Klenerman P. **Diminished frequency of hepatitis C virus specific interferon {gamma} secreting CD4+ T cells in human immunodeficiency virus/hepatitis C virus coinfected patients.** *Gut* 2006; **55**:1484–1487.
34. Briat A, Dulioust E, Galimand J, Fontaine H, Chaix M-L, Letur-Könirsch H, *et al*. **Hepatitis C virus in the semen of men co-infected with HIV-1: prevalence and origin.** *AIDS* 2005; **19**:1827–1835.