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Transmission of hepatitis C virus in HIV-positive and PrEP-using MSM in England

Running title: HCV in HIV-positive and PrEP-using MSM

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Conflict of interests

Gail Matthews has received research grants from Gilead and Abbvie. Daniel Bradshaw has received research grants from Gilead and Viiv. Yvonne Gilleece has received honoraria for educational meetings and advisory boards and sponsorship to attend conferences from Gilead and Viiv.

Abstract

Background and Aims

We sought to characterise risk factors and patterns of HCV transmission amongst men who have sex with men (MSM).

Methods

MSM with recently-acquired HCV (AHCV) were prospectively recruited ('clinic cohort') between January and September 2017. Clinical data and risk behaviours were identified and blood obtained for HCV whole genome sequencing. Phylogenetic analyses were performed, using sequences from this cohort and two other AHCV cohorts, to identify transmission clusters.

Results

Sixteen (40.0%) men in the clinic cohort were HIV-negative MSM. HIV-negative MSM were younger than HIV-positive MSM; most (81.3%) had taken HIV PrEP in the preceding year.

Eighteen men (45.0%) reported injection drug use; most (34, 85.0%) reported non-injection drug use in the last year. Most in both groups reported condomless anal sex, fisting and sex in a group environment. Few (7, 17.5%) men thought partners may have had HCV.

There were 52 sequences in the HCV genotype 1a phylogeny, 18 from the clinic cohort and 34 from other AHCV cohorts; 47 (90.4%) clustered with ≥1 other sequence. There were 7 clusters of 2-27 sequences; 6 clusters contained HIV-negative and HIV-positive MSM and 1 cluster only HIV-positive MSM. Four of these clusters were part of larger clusters first described in 2007.

Conclusions

PrEP-using MSM are at risk of HCV, sharing similar risk factors to HIV-positive MSM. Phylogenetics highlights that PrEP-using and HIV-positive MSM are involved in the same HCV transmission networks. Few men demonstrated HCV awareness and risk reduction strategies should be expanded.

Key words

HIV, hepatitis C, pre-exposure prophylaxis, men who have sex with men

Word count: 3489 words

MAIN TEXT

INTRODUCTION

Since the early 2000s, an epidemic of hepatitis C virus (HCV) has been observed amongst HIV-infected men who have sex with men (HIV-positive MSM) in industrialised countries, transmitted through sex or

injection drug use (IDU) [1]. In a meta-analysis, HCV incidence increased from 2.6/1000 person-years (PY) in 2000-2005 to 8.1/1000 PY after 2010 [2], with an alarming incidence of reinfection (73/1000 PY) [3]. However, since 2013-2014, HCV incidence in the Netherlands and UK has reportedly declined in HIV-positive MSM, likely due to unrestricted access to new direct acting antiviral therapies (DAAs) [4,5].

By contrast, HCV prevalence (1.5%) [6] and incidence (0.4/1000PY) [2] in HIV-negative MSM has remained low and similar to that of the general population. However, recent reports from France and the Netherlands identified acute HCV cases in MSM who were using HIV pre-exposure prophylaxis (PrEP), with phylogenetic analysis suggesting genetic similarities to those in HIV-positive MSM [7,8].

PrEP access has been expanding in England since 2016, through the PrEP IMPACT Trial, self-sourced online (www.prepimpacttrial.org.uk) or from private providers. However it is unknown whether similar patterns of HCV transmission between HIV-positive and HIV-negative PrEP-using MSM are occurring in England. The aims of this study were to characterise the risk factors for, and patterns of, HCV transmission amongst MSM in England. Such information is critical to inform appropriate HCV transmission prevention strategies and thereby facilitate attainment of ambitious WHO HCV elimination targets by 2030 [9].

METHODS

Behavioural Study

Recruitment

First, a cohort of MSM with recently-acquired HCV (AHCV) was prospectively recruited and constituted the basis for our behavioural study ('the clinic cohort'). This study was conducted at five English National Health Service (NHS) clinics, three in London, and one each in Southern and Northern England, between 12 January and 5 September 2017. Clinicians working within sexual health, HIV or viral hepatitis settings invited MSM with AHCV to attend for a single study visit. Recent HCV was defined as anti-HCV and/or HCV antigen and/or HCV RNA seroconversion within 12 months; and/or clinical and biochemical criteria (acute hepatitis in individuals without pre-existing liver disease, excluding other infective, metabolic, toxic and drug causes for hepatitis, and a serum alanine aminotransferase (ALT) level ≥ 10 times the upper limit of normal) with positive HCV RNA.

The duration of HCV infection was calculated according to previously-described criteria, with a maximum duration of 48 weeks [12]. Individuals were excluded if any of the following applied: not MSM, age <16 years, HCV RNA negative, chronic HCV infection or unwilling to provide additional blood samples.

Data collection

Demographic and clinical information was gathered from medical notes review and participant-completed questionnaires, including sexual and drug taking behaviours (See Questionnaire in supplementary information). Route of HCV acquisition was assigned by the clinician. If sexual and injecting risks coexisted within the twelve months preceding the study visit, the injecting route was assigned.

Sampling

Participants provided a 20mL EDTA blood sample for HCV RNA quantification and sequencing. Sexually transmitted infection (STI) testing was performed: HIV serology if appropriate, syphilis serology, and nucleic acid amplification testing of swabbed mucosae for chlamydia, gonorrhoea and, where symptomatic, herpes simplex virus.

Statistical analysis

Demographic and clinical characteristics of HIV-positive vs HIV-negative MSM were compared for individuals from the clinic cohort using the Mann Whitney U test for continuous variables and Fishers Exact or Chi-squared tests for categorical variables. Statistically significant findings were defined as $p<0.05$.

Phylogenetic study

HCV sequencing

To improve the resolution of the phylogenetic tree and to investigate patterns of phylogenetic clustering, subtype 1a HCV sequences from participants in the clinic cohort were combined with sequences from two other UK AHCV cohorts, CHAT[10] and Cohort 1 of TARGET3D[11]. In CHAT, HIV-positive individuals with AHCV genotype 1 co-infection received therapy with telaprevir, pegylated interferon and ribavirin. In Cohort 1 of TARGET3D, HIV-negative or HIV-positive individuals with AHCV genotype 1 received therapy with ritonavir-boosted paritaprevir, ombitasvir and dasabuvir. Figure 1 outlines the overall study schema and sources of data for the behavioural and phylogenetic components of the study.

All blood specimens were separated into plasma and cells. HCV whole genome sequencing (WGS) on plasma was performed using a target enrichment next generation sequencing approach using Nimblegen (Roche) probes as previously described [14].

Alignment

HCV consensus sequences were used for phylogenetic analyses. Sequences from all three cohorts were collated in one dataset. Sequences were aligned using Clustal W software [15] and then manually corrected using MEGA7 [16]. Genotype assignment was determined by WGS and then by using the Oxford HCV Subtyping [17] and NCBI Genotyping [18] tools. Genotype 1a sequences were used for further analyses, forming Dataset1. The final Dataset1 alignment consisted of 52 sequences, 9033 nucleotides long, corresponding to the HCV genotype 1 reference sequence H77 positions 342 - 9374.

Phylogenies and molecular clock analysis

We used RAxML [19] to estimate a Maximum Likelihood (ML) phylogenetic tree from the Dataset1 alignment under a general time-reversible nucleotide substitution model with gamma-distributed rate variation among sites (GTR+G). We ran ML bootstrap analysis with 100 replicates to evaluate statistical support for the clades on the tree.

We used TempEst [20] to evaluate the strength of the molecular clock signal in our data by plotting the root-to-tip genetic distance of each sequence against its sampling date. Since temporal signal was weak, we augmented our dataset using a previously-published alignment of reference sequences (RefSet), which was constructed to estimate reliably a molecular clock for HCV genomes [21]. When combined, Dataset1 and RefSet generate Dataset2, which was used in all further molecular clock analyses. We used RAxML (with the model specifications above) to estimate a ML phylogeny for Dataset2.

We used Cluster Picker software [22] to identify HCV clusters, defined as those with bootstrap support >90%, containing sequences from Dataset1 only (i.e. no RefSet sequences). For all clusters that included >2 sequences, we estimated the association index (AI) statistic using BaTS [23]. The AI statistic assesses whether characteristics (traits) are randomly distributed, or clustered, on a phylogenetic tree. AI ranges from 0 to 1, where 0 represents strong clustering and 1 represents randomly distributed.

We analysed Dataset2 in BEASTv1.10 [24] to generate a time-scaled tree and to estimate the time of the most recent common ancestor (TMRCA) of each identified cluster. We used a genomic partition model

implemented in BEAST to split our alignment into two partitions: the "NS5B" (corresponding to the NS5B genomic region) and the "genome" partitions (the rest of the genome). We used the evolutionary rate estimated from Iles et al [25] (mean= $9.87x10^{-4}$, stdev=$2.3x10^{-4}$) as an informative prior for the evolutionary rate of the NS5B genomic region. We then used a relative rate parameter to link the "genome" and "NS5B" partitions. We used the Bayesian skyline coalescent model (10 intervals) with a lognormal relaxed molecular clock model, and the GTR+G substitution model with codon site partitions (codon positions 1+2, 3). Markov Chain Monte Carlo (MCMC) analyses were run for 150 million states (with 10% burn-in). Convergence of the MCMC sampler was visually inspected using Tracer [26].

Sensitivity analysis

We conducted a sensitivity analysis to check if the same clusters were still found if we used two larger reference datasets comprising shorter genetic sequences, corresponding to the E1 and NS5B HCV genomic regions (i.e. reference datasets described previously in [27]).

Approvals

All participants provided written, informed consent. The study was conducted according to the Helsinki Declaration and approved by the South Central Oxford A Research Ethics Committee (approval number 18/SC/0208).

RESULTS

Behavioural study

Participant Characteristics

Forty men were recruited, of whom 24 (60.0%) were HIV-positive (Table 1). HIV-negative vs HIV-positive men were younger (34, IQR 29-43 vs 44, IQR 36-50 years, respectively p=0.021) and were recruited exclusively from London clinics (100% vs 70.8%, respectively, p=0.030). Most HIV-negative MSM (81.3%)

reported PrEP use in the preceding year. Most HIV-positive MSM had well-controlled HIV: 22 (91.7%) were aviraemic on antiretrovirals and median CD4 cell count was 663 c/mm^3 (IQR 499-779). Approximately 30% in each group was diagnosed with an STI at enrolment.

Most HCV infections were classified as GT1a (81.3% HIV-negative vs 58.3% HIV-positive men); GT4 was less frequent in HIV-negative (6.3%) vs HIV-positive men (37.5%) p=0.032. In both HIV-negative and HIV-positive groups, a similar proportion (21-25%) of HCV diagnoses represented reinfection. The proportion of cases with a clinician-assigned IDU-acquisition was also similar for both groups (33-38%). Outcomes of AHCV infection were similar: for HIV-negative and HIV-positive MSM, 2 (12.5%) and 4 (16.7%), respectively, cleared HCV spontaneously, whilst all those receiving treatment (n=31) achieved SVR12.

Drug use and sexual behaviours

Eighteen men (45.0%) reported IDU and, of note, 13 men reported being injected by another person, although none reported injecting with a needle used by another individual (Table 2). Thirty-four men (85.0%) reported non-injected drug use in the preceding 12 months. Men reported a median of 16 (IQR 8-39) partners and the majority (92.5%) reported condomless anal sex (CAS) fisting (62.5%) and sex in a group environment (79.5%). Most men (82.5%) thought some, most or all sexual partners were HIV-positive. By contrast, only 17.5% thought that some, most or all partners were HCV-positive.

Upon comparing PrEP-using and non-PrEP using HIV-negative MSM, we found that socio-demographic and behavioural characteristics were broadly similar, although the former group reported more sexual partners (Supplementary Table 1).

Venues

Men commonly met sexual partners via both virtual (mobile phone apps and internet websites) and physical venues (including sex on premises venues (SOPV), bars/clubs) with apps being used most often (Supplementary Table 2). Regarding locations reported for sex, these were most often the individual's own home or another individual's home. For IDU, most HIV-negative and HIV-positive men who had injected recently (<12 months) did so in their own or another private home

Phylogenetics

Considering the clinic cohort, CHAT and TARGET3D cohorts together, our study generated 52 near full-length HCV genotype 1a genomes. These 52 sequences formed Dataset1. Socio-demographic and clinical characteristics of the patients in Dataset1 can be found in Table 3 and details of individual patients are provided in Supplementary Table 3.

Most genotype 1a-infected individuals (47/52, 90.4%) clustered together on the ML phylogeny of Dataset2 (Suppl. Figure). Four clusters were identified (comprising 4, 5, 5 and 27 sequences respectively) plus three pairs. All clusters and 2/3 pairs contained a mixture of HIV-positive and -negative individuals. This was confirmed on the Bayesian phylogeny (Figure 2).

All clusters and 2/3 pairs contained individuals from at least two recruiting sites; two clusters contained individuals from London and non-London sites. Cluster 2 (n=27) included most (9/10) of the CHAT individuals, in addition to those from the clinic cohort and TARGET3D. Four of seven clusters contained a mix of individuals with IDU and sexually-acquired HCV. Three clusters contained at least one individual who had been re-infected with HCV. Most clusters were supported by sub-analyses of the phylogeny using E1 (see Supplementary Text for details).

To identify if these clusters were part of the previously identified HCV clades circulating in the country as reported by Danta et al [28], we aligned the sequences used here to those reported in [28], using the same approach as for the alignment to RefSet described above. Clusters 1, 2, 3 and 7 were part of the clusters reported in Danta et al, corresponding to previously-described clusters 1, 2, 5 and 3 respectively.

Molecular clock analysis

The TMRCA of Dataset2, which corresponds to the origin of G1a, was 1929 (95% Highest Posterior Density credible intervals (HPD CIs) = 1919-1935) (Fig. 2). Most identified clusters originated in the 1990s; their TMRCAs ranged from 1973 (95% HPD CI= 1967-1980) for cluster 3 to 2001 (95% HPD CI= 1996 – 2006) for cluster 6. These TMRCAs were consistent with those reported for the corresponding clusters in Danta et al., except for Cluster 2, for which our estimates are younger than previously reported.

BaTS analysis

We performed AI analysis in BaTS for the 4 clusters with >2 sequences. There was no significant phylogenetic structure within clusters by age, country of birth or HCV transmission route (Supplementary Table 4). Sequences were significantly clustered within cluster 4 by HIV status and age ($p<0.0001$), i.e. neighbouring tips in the cluster 4 phylogeny were more likely to share these traits than would be expected by chance; this was not seen for clusters 1-3.

DISCUSSION

The HCV epidemic amongst HIV-positive MSM has grown, driven by high rates of infection and reinfection through both sexual and IDU routes, with seroprevalence in the UK Collaborative HIV cohort rising from 7.3% in 2004 to 9.9% in 2011 [1-3,29]. More recently, a decline in incidence was observed in one large UK HIV-positive MSM cohort following DAA roll out, from 17.1/1000 PYFU in 2015 to 4.6/1000 PYFY in 2018. These data, in conjunction with modelling studies, suggest that HCV seroprevalence may stabilise by 2025 and the prevalence of RNA positivity will likely decline [5,29]. Although the epidemic has largely spared HIV-negative MSM, recent reports of PrEP-using cohorts suggest possible bridging of HCV into this community [7,30,31]. In our study, sixteen of 40 (40%) men with AHCV in the clinic cohort were HIV-negative, and most were using PrEP. Men in both HIV-negative and -positive groups shared similar high risk sexual and drug-taking behaviours including CAS, traumatic sexual practices and permucosal drug use, with almost half also reporting IDU. Around 2/3 of men in both groups likely acquired HCV sexually and, for 21-25%, the HCV episode was a reinfection. Of particular note, most individuals were in clusters involving both HIV-positive and HIV-negative MSM, suggesting involvement in the same HCV transmission networks.

Of those reporting recent IDU, there was inconsistency in the safety of injecting practices. Most recent injectors reported IDU <1x/week; none reported using a needle that another person had used. However, approximately a third of men in both groups had been injected by a partner. These findings suggest that, although some risk-mitigating strategies are being practised, increased promotion of safer practices is needed, such as avoidance of administering injections to partners. Methamphetamine was reported most often as the last drug injected, a finding consistent with other cohorts [28].

Sexual practices were consistent with those reported previously, with a majority in both groups reporting high risk behaviours [7,8,35]. Interestingly, for both groups, most men (81-83%) thought that some, most or all partners might be HIV-positive, whilst few (13-25%) considered this possibility for HCV. This suggests either a lack of awareness of HCV transmission routes, and/or lack of concern due to knowledge of high cure rates. Previous reports have identified inconsistent levels of HCV awareness amongst MSM [36-38]. In addition, PrEP use may result in less HIV serosorting amongst HIV-negative MSM, a strategy reported in the pre-PrEP era, as well as higher levels of CAS [39,40]. Similarly, increasing awareness of the absence of HIV transmission in the context of a suppressed HIV viral load ('Undetectable = Untransmittable' (U=U)) may be driving increases in CAS with HIV-discordant partners [41]. As HCV prevalence is up 10-fold higher amongst HIV-positive than -negative MSM , reductions in HIV serosorting by HIV-negative MSM may increase HCV exposure [1].

Men were likely to meet sexual partners through a variety of virtual and physical venues, with mobile phone apps being the most commonly reported. A UK study of HIV-positive MSM with AHCV in 2007 identified that participants met partners via private parties, SOPV and internet sites, but not phone apps, which first became widely available after 2008[28]. Other risk behaviours, including reports of group sex and IDU, were broadly similar when comparing these two cohorts.

For the clinic cohort, whilst HIV-positive men were recruited from both London and non-London sites, HIV-negative MSM were recruited exclusively in London. Although this may reflect low total numbers of recruits from outside London , it is plausible that London has seen earlier transmission of HCV from HIV-positive to HIV-negative populations due to a greater prevalence of HIV in MSM in London (134/1000) compared to that of the rest of England (63/1000) [42], and that increasing transmissions to HIV-negative MSM will be observed later in regional populations.

When comparing the two groups, HIV-positive MSM were more likely to be anti-HCV negative (13.6% vs 7.1%) and to have a higher HCV RNA (5.1 vs 3.7 log IU/mL) at diagnosis, consistent with previous cohorts [43,44], although these findings were not statistically significant, perhaps due to low overall numbers. Therapy with DAAs was highly effective, irrespective of HIV status. The high proportion with an STI was also consistent with previous studies [27], with associated mucosal inflammation potentially promoting HCV acquisition.

Almost all individuals with GT1a infection belonged to phylogenetic clusters of varying size. Importantly, men with differing risk characteristics were found within the same clusters, including HIV-positive/HIV-

negative men and those with IDU/sexually-acquired HCV. Studies of HCV clustering in MSM have been reported over the past 15 years, but most included only HIV-positive individuals [27,28,45]. The results of our study, in conjunction with recent findings from France and the Netherlands, confirm that mixing of HIV-positive and PrEP-using MSM engaging in high risk behaviours within shared networks may be driving ongoing HCV transmissions [7,8].

The finding of clusters containing individuals from London/Brighton and London/Leeds suggests that transmissions may be extending from London to regional cities. Four clusters are part of larger MSM clusters described previously [46,47], highlighting that the current HCV outbreak involves virus lineages present in the epidemic first described in the early 2000s, which have persisted and extended to include PrEP-users.

Although we found an association between virus phylogeny and the traits of HIV-seropositivity and age within cluster 4, this was not observed for the other three clusters. The overall lack of structure for HIV status within the cluster phylogenies supports the notion that behavioural factors may be more critical than biological factors for driving the HCV epidemic amongst MSM, as modelling studies have predicted [48]. For HIV-negative individuals, it seems implausible that PrEP itself should increase HCV susceptibility. More likely, PrEP use is a marker of high risk behaviours. Consistent with this theory, in a Dutch analysis, HCV prevalence at the baseline PrEP visit was high (4.8%)[7]. In the pre-PrEP era HIV was more likely to be acquired before HCV through the sexual route, given the increased efficiency by which HIV establishes mucosal infection. In the era of PrEP and 'U=U', loss of susceptibility and reduction in exposure to HIV, but continued exposure to HCV, increases the likelihood of HCV preceding HIV acquisition.

Conclusions

These results identify shared transmission networks involving HIV-positive and PrEP-using MSM, confirming that the HCV epidemic amongst MSM in England may be similar to other European cohorts [7,49]. In light of these findings, measures should be intensified to prevent a large scale epidemic in PrEP-using MSM.

First, awareness of HCV transmission routes and the consequences of infection should be increased. Potential opportunities include PrEP clinics, online PrEP sites, social networking apps and SOPV. Second, HCV testing strategies should be optimised. In particular, UK guidelines recommend at least three monthly testing with anti-HCV in PrEP-using MSM [50]. However, HCV seroconversion may take three months or longer, with the possibility of false negative anti-HCV and delayed HCV diagnosis in

asymptomatic individuals with high partner numbers, leading to considerable onward transmission. Indeed, in the current study, 7.1% of HIV-negative MSM were anti-HCV negative and only 2/16 (12.5%) presented with jaundice. Guidelines should therefore be strengthened with the recommendation for the additional use of HCV antigen, PCR or ALT to reduce the diagnostic window period. In view of the high proportion of individuals who met sexual or drug taking partners in private homes, and who may be accessing PrEP online, scaling up of HCV home testing may also be important including online accessed HCV testing kits. Third, given the number of men reporting IDU, including in the chemsex context, widespread access to needle and syringe kits should be prioritised through PrEP and HIV services. Finally, MSM with acute HCV should be linked to DAA therapies early, with no restrictions on treatment of reinfections, to benefit from the 'treatment as prevention' effect [4]. Nonetheless, scaling up of treatment may not be sufficient to achieve HCV elimination in MSM, unless accompanied by behavioural change [51,52].

Limitations

This study has limitations. First, although clustering was identified between HIV-positive and HIV-negative men, overall numbers were low and longer term follow up of a larger cohort is required to confirm this finding, as well to evaluate the issue of reinfection. Additional recruits from non-London centres would improve our understanding of the evolution of the HCV epidemic MSM across England. Second, the TMRCA of many of the clusters was often >20 years prior to the study period, suggesting that although MSM within each cluster may be involved in loosely-defined transmission networks, direct transmissions between them may be less likely. The TMRCAs may also have been impacted by low sampling density. However, an earlier (1962) TMRCA has been reported in a cluster from the HCV genotype 1a phylogeny of a cohort of UK HIV-positive MSM [28]. Third, as is common with all questionnaire-based studies, data may be biased by issues with recall or non-disclosure due to stigma.

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Table 1. Clinical and demographics characteristics of HIV-negative vs HIV-positive men with recently-acquired HCV

	HIV-negative n=16	**HIV-positive n=24**	**All n=40**	**P value**
Median age, years	34 (29-43)	44 (36-50)	39 (33-49)	0.021*
UK-born	9 (56.3)	18 (75.0)	27 (67.5)	0.215
Median ISCED score	5 (4.5-5.0)	5 (4.0-5.0)	5 (4.0-5.0)	0.549
History of incarceration	0	0	0	1.000
City of recruitment				
London	16 (100)	17 (70.8)	33 (82.5)	0.030*
Jaundice	2 (12.5)	2 (8.3)	4 (10.0)	1.000
Median peak ALT, U/L	648 (288-1618)	448 (276-783)	491 (279-1007)	0.345
HCV results				
HCV antibody result	14 (87.5)	22 (91.7)	36 (90.0)	1.000
HCV antibody negative	1 (7.1)	3 (13.6)	4 (15.4)	1.000
Median HCV RNA, log IU/mL	3.7 (3.2-5.7)	5.1 (4.3-6.3)	4.8 (3.4-6.2)	0.279
HCV genotype[ǂ]				
1a	13 (81.3)	14 (58.3)	27 (67.5)	0.177
3a	2 (12.5)	1 (4.2)	3 (7.5)	0.553
4	1 (6.3)	9 (37.5)	10 (25.0)	0.032*
Median duration HCV infection, months	4.1 (3.3-8.3)	4.9 (2.3-8.0)	4.8 (2.7-8.2)	0.687

Prior HCV episode(s)	4 (25.0)	5 (20.8)	9 (22.5)	1.000
Clinician-assigned IDU acquisition route	6 (37.5)	8 (33.3)	14 (35.0)	0.787
Outcome of HCV infection				
Spontaneous HCV clearance	2 (12.5)	4 (16.7)	6 (15.0)	1.000
Treated	13 (81.3)	18 (75.0)	31 (77.5)	1.000
SVR12 achieved	13 (81.3)	18 (75.0)	31 (77.5)	1.000
Other (Lost to follow up, declined therapy)	1 (6.3)	2 (8.3)	3 (7.5)	1.000
STI at HCV diagnosis‡	5 (31.3)	7 (29.2)	12 (30.0)	0.888
Infectious syphilis	1 (6.3)	5 (20.8)	6 (15.0)	0.373

Brackets denote % or IQR. *<0.05 by chi-squared, Fishers exact or Mann Whitney U test

ISCED denotes International Standard Classification of Education

ŧ. Genotype distribution: 1a (n=27), 3a (n=3), 4d (n=9), 4 no subtype (n=1)

‡. HIV-negative group: 1 participant each with syphilis, chlamydia and gonorrhoea, LGV and gonorrhoea and two with gonorrhoea

HIV-positive group: 4 participants with syphilis, one with syphilis and HSV, two with chlamydia

Table 2. Drug use and sexual behaviours for HIV-negative vs HIV-positive men with recently-acquired HCV

	HIV-negative n=16	HIV-positive n=24	All n=40
IDU			
History of IDU ever	7 (43.8)	11 (45.8)	18 (45.0)
Median age of first IDU, years	36.5 (29.0-47.8)	38.5 (32.0-42.8)	38.5 (31.5-43.5)
Ever been injected by another individual	5 (31.3)	8 (33.3)	13 (32.5)
IDU within last 12 months	6 (37.5)	7 (29.2)	13 (32.5)
Frequency IDU < 1x / week	5 (71.4)	6 (54.5)	11 (61.1)
Used needle after someone else has used it	0	0	0
Methamphetamine use, last drug injected	7 (100.0)	10 (90.9)	17 (94.4)
Permucosal drug use in last 12 months			
Any permucosal drug use	15 (93.8)	19 (79.2)	34 (85.0)
Nasal	14 (87.5)	17 (70.8)	31 (77.5)
Oral	11 (68.8)	11 (45.8)	22 (55.0)
Rectal	8 (50.0)	9 (37.5)	17 (42.5)
Sharing of equipment [all, most or some occasions]	10 (66.7)	12 (50.0)	22 (64.7)
Methamphetamine	9 (56.3)	12 (50.0)	21 (52.5)
GHB/GBL	12 (75.0)	9 (37.5)	21 (52.5)
MDMA/mephedrone	13 (81.3)	14 (58.3)	27 (67.5)
Cocaine	8 (50.0)	10 (41.7)	18 (45.0)
Alcohol [always, often or sometimes]	5 (31.3)	8 (33.3)	13 (37.5)
Sexual history in last 12 months			
Median no. of sex partners	36 (16-50)	16 (4-16)	16 (8-39)
History of sex in group environment	14 (87.5)	17 (70.8)	31 (79.5)
Fisting	12 (75.0)	13 (62.5)	25 (62.5)
Condomless anal sex	16 (100.0)	21 (87.5)	37 (92.5)
IDU during sex	6 (37.5)	9 (37.5)	15 (37.5)
History of rectal bleeding	7 (43.8)	8 (33.3)	15 (37.5)

Anorectal problem	4 (25.0)	6 (25.0)	10 (25.0)
Shared douche equipment	6 (37.5)	6 (25.0)	12 (30.0)
Shared lube	11 (68.8)	16 (66.7)	27 (67.5)
Used sex toys	10 (62.5)	10 (41.7)	20 (50.0)
Infection status of partners in last 12 months[1]			
No. reporting HIV-positive partners	13 (81.3)	20 (83.3)	33 (82.5)
No. reporting HCV-positive partners	4 (25.0)	3 (12.5)	7 (17.5)
Other HCV risk factors			
Tattoo	1 (6.3)	0	1 (2.5)
Piercing	1 (6.3)	0	1 (2.5)
Endoscopy or surgery	0	4 (16.7)	4 (10.0)
Mosaic risk assessment			
Score ≥ 2	13 (81.3)	20 (83.3)	33 (82.5)

Brackets denote % or IQR.

[1.] Responses indicate where participants reported 'all, most or some' partners

Table 3. A comparison of demographic and clinical characteristics of participants from the clinic cohort, CHAT and TARGET3D cohorts included in the phylogenetic analysis

	Clinic Cohort	**CHAT**	**TARGET3D**	**All**
Number of participants	18	10	24	52
Median age, years	36 (33-48)	37 (33-45)	38 (30-48)	37 (32 - 46)
UK born	11 (61.1)	4 (40.0)	10 (41.7)	25 (48.1)
MSM	18 (100)	10 (100)	24 (100)	52 (100)
HIV positive	9 (50.0)	10 (100)	21 (87.5)	40 (76.9)
Duration of HCV infection, months	4.9 (3.3 - 6.8)	4.0 (4.0 - 4.5)	6.4 (4.2-7.9)	5.8 (4.0 - 7.5)
History of IDU	13 (72.2)	3 (30.0)	12 (50.0)	28 (53.8)
STI identified at HCV diagnosis	8 (44.4)	5 (50.0)	NA	13 (46.4)*
Sexual route of HCV acquisition	7 (38.9)	7 (70.0)	20 (83.3)	34 (65.4)

Prior HCV episode(s)	3 (16.7)	3 (30.0)	2 (8.3)	8 (15.4)

*for 28 participants

Brackets denote % or IQR

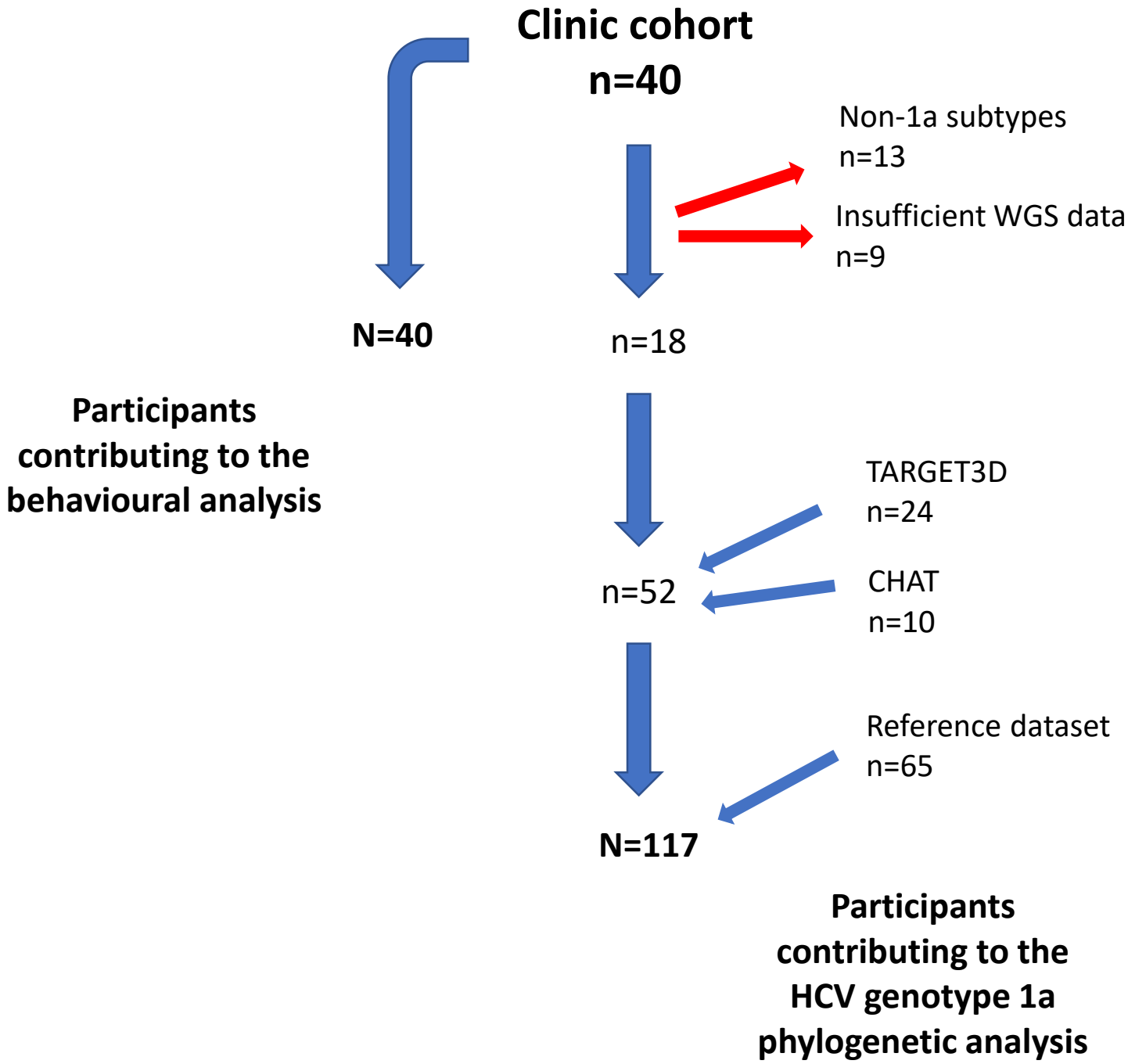
Figure 1 Legend

Study schema

Figure 2 Legend

HCV genotype 1a transmission clusters on the Bayesian phylogenetic tree.

Notes. IDU denotes history of injection drug use. TMRCA denotes time to most recent common ancestor.


Clinic cohort
n=40
Non-1a subtypes
n=13
Insufficient WGS data
n=9
N=40
n=18
Participants contributing to the behavioural analysis
TARGET3D
n=24
n=52
CHAT
n=10
Reference dataset
n=65
N=117
Participants contributing to the HCV genotype 1a phylogenetic analysis

Dataset1

Reference

HIV+ IDU LONDON

Cluster 1

Cluster 2

Cluster 6

Cluster 4

Cluster 7

Cluster 3

Cluster 5

Time (years)

1915 1925 1935 1945 1955 1965 1975 1985 1995 2005 2015

TMRCA Density plots

0.2

0