Hepatitis C virus transmission between eight high-income countries among men who have sex with men: a whole-genome analysis

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Summary

Background Microelimination of the hepatitis C virus (HCV) among men who have sex with men (MSM) could be complicated by continuous external introductions and the emergence of phylogenetic clusters harbouring clinically significant resistance-associated substitutions (RAS). To investigate international clustering and the prevalence and transmission of RAS, we aimed to analyse whole-genome HCV sequences from MSM with a recently acquired infection who participated in a large, international HCV treatment trial.

Methods For this whole-genome analysis, we obtained HCV sequences from 128 MSM who had acquired HCV within the past 12 months and were participating in the REACT trial. The participants from whom sequences were obtained were recruited at 24 sites in eight countries. We inferred maximum-likelihood phylogenies and identified transmission clusters for HCV genotypes separately. We constructed time-scaled phylogenies to estimate cluster introduction dates and used a Bayesian Skygrid approach to estimate the effective population size over the past 50 years. We calculated the prevalence of RAS and the extent of RAS transmission in the study population.

Findings The majority of recent HCV infections were part of international networks that arose in the late 1990s and early 2000s. Sequences obtained in the same country clustered frequently, and in 36% of subclusters since 2015 we found evidence of international transmission. European MSM were more likely than non-European MSM to be in a cluster (odds ratio 11·9 [95% CI 3·6–43·4], p<0·0001). The effective population size decreased rapidly since around 2015 in Europe. RAS associated with substantially diminished cure rates were infrequently detected and transmission of highly resistant viruses was not observed.

Interpretation Despite antiviral treatment becoming widely available, international transmission of HCV among MSM has still occurred over the past 8 years, which could complicate microelimination of the virus in this population. RAS-enriched clusters and widespread RAS transmission are currently not a threat to elimination goals. These findings support an international approach for HCV microelimination among MSM.

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Introduction

Driven by the introduction of highly effective, direct-acting antiviral agents, WHO set ambitious targets in 2016 to eliminate hepatitis C virus (HCV) as a public health threat by 2030.[1] Pursuing microelimination—ie, rapidly reducing HCV in discrete populations by intensive testing and rapid treatment—with the involvement of all relevant stakeholders, facilitates reaching the overall goal of elimination.[2] Key populations at risk of HCV include people who are incarcerated, people who inject drugs, and men who have sex with men (MSM) with HIV.[3] Since the introduction of direct-acting antivirals between 2015 and 2017, HCV incidence among MSM with HIV has decreased to some extent, but reinfection rates remain high[4–7] and sustained efforts are needed to achieve HCV elimination goals. In addition, MSM who use pre-exposure prophylaxis for HIV prevention have recently emerged as a group at risk of HCV infection.[8]

There are potential obstacles to microelimination of HCV in the MSM population. Microelimination among MSM can be complicated by continuous external introductions of new infections from international transmission networks, with potential local onward transmission of imported HCV.[9,10] HCV clusters can overlap internationally among MSM with HIV;[11] however, whether this international transmission has remained the case since direct-acting antiviral therapies became available is not known. Approaches to minimise local HCV transmission could be insufficient in settings where such internationally acquired infections are common. Local approaches are more effective if

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed using the keywords "HCV", "MSM", and "international transmission" or "resistance" for primary research articles published between database inception and April 1, 2022, without language restrictions. Our search resulted in 38 records, from which we identified ten studies that used phylogenetic methods to understand hepatitis C virus (HCV) transmission patterns among men who have sex with men (MSM). These studies showed that transmission between European countries occurred before direct-acting antivirals became widely available between 2015 and 2017. At least two national cohorts also showed evidence of external introductions—again, mainly before the availability of direct-acting antivirals—the proportion of which differed over time and between countries. The countries from which the introductions arose were usually not known. We identified four studies focusing on the transmission of strains harbouring resistance-associated substitutions (RAS) among MSM. These studies found that RAS often occurred at low levels, infections with RAS-carrying viruses were almost always successfully treated using tailored therapeutic schedules, and transmission of variants carrying RAS at several positions in the genome was observed.

Added value of this study

Identifying current threats to HCV microelimination goals requires analysing samples of recently acquired infections from a global cohort. We report that international transmission still occurs among MSM after the introduction of direct-acting antivirals, and is complicating microelimination efforts, particularly in Europe. We found no evidence that RAS-associated clusters have emerged since the introduction of direct-acting antivirals, suggesting that such clusters are currently not a barrier for HCV microelimination among MSM.

Implications of all the available evidence

HCV transmission continues among MSM both locally and in international, particularly European, transmission networks. To achieve and maintain microelimination of HCV in this population, local programmes are essential but international coordination and surveillance might be required. There is currently no evidence that treatment resistance is a barrier to microelimination among MSM, but surveillance remains important to detect the potential emergence of resistance-associated transmission clusters.

international external introductions contribute little to the local epidemic.

Furthermore, if transmission clusters that are highly resistant to current components of direct-acting antiviral treatment either arise or already exist, surveillance of resistance-associated substitutions (RAS) could prove important when pursuing HCV elimination. Although treatment with direct-acting antivirals is currently often successful, most patients who have virological relapse have a single or multiple RAS at baseline or after treatment.[12] With the scale-up of direct-acting antiviral treatments worldwide, the absolute number of patients for whom such therapy is not successful is likely to increase accordingly. Transmission of RAS-harbouring viruses within a particular population could affect local cure rates and therefore complicate microelimination efforts.

In this study, we analysed whole-genome HCV sequences from MSM who participated in a large, international HCV treatment randomised controlled trial, known as the REACT study,[13] and had been infected with HCV within 12 months before enrolment. We aimed to investigate the extent of international HCV transmission and the prevalence of RAS transmission. These potential threats to elimination can only be evaluated using samples from recently acquired infections, because intrahost evaluation will have had minimal effect on the viral genomes. This requirement makes samples obtained during the REACT study[13] appropriate for the assessment of recent transmission clusters. We used phylogenetic methods to establish whether recent HCV infections among MSM are part of small, local outbreaks or whether transmission occurred in international networks. We assessed the effective population size trajectory—an abstract quantity reflecting changes in genetic diversity over time—to understand the current and historical dynamics of the HCV epidemic. We also calculated the prevalence of RAS and the extent of RAS transmission, both locally and internationally. The results of this study can inform HCV surveillance, prevention, and education strategies.

Methods

Study design and participants

This study was a secondary analysis of plasma specimens and demographic data collected during the REACT trial.[13] REACT was an open-label, international, multicentre, phase 4 trial that assessed the non-inferiority of short-course (6 weeks) versus standard-course (12 weeks) therapy with sofosbuvir–velpatasvir for recent HCV infection (estimated duration of infection ≤12 months). The trial enrolled 222 participants with and without HIV between March, 2017, and September, 2019 at 24 international sites in Australia (n=5), Canada (n=4), Germany (n=4), the Netherlands (n=1), New Zealand (n=1), Switzerland (n=3), the UK (n=4), and the USA (n=2). Participants with an HCV RNA concentration greater than 10 000 U/L were eligible for inclusion. Full eligibility and inclusion criteria are provided in the published study.[13]

Participants provided written informed consent before study procedures. The study protocol was approved by

local ethics committees at all study sites and was conducted according to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. Further details are outlined in a previous publication.[13]

Next-generation sequencing of viral isolates

Samples were collected during screening or baseline visits before treatment began. Additional samples were collected from all participants with an HCV reinfection during follow-up. For the current study, we defined reinfection if genome analysis confirmed viral sequence heterogenicity (genotype switch or ≥5% difference between genomes) between the follow-up sample and the last available sample from the individual at the baseline visit. We extracted viral RNA and synthesised full-length cDNA using an adaptation to a method previously described[14] (appendix p 2). We generated near full-length HCV nucleotide consensus sequences using next-generation sequencing (appendix p 2). The sequences obtained spanned the HCV genome from nucleotides 119 to 9276 of the H77 reference sequence (GenBank accession number NC_004102) and the final sequence fragments analysed were 9157 (genotype 1a) and 9134 (genotype 4d) base pairs in length (GenBank accession numbers ON630763–ON630900).

Molecular transmission cluster inference

We stratified sequences by genotype and aligned them using MAFFT.[15] After manually curating the alignment, we used IQ-TREE[16] to infer genotype-specific maximum-likelihood phylogenetic trees, under the nucleotide substitution model automatically selected by ModelFindel.[17] Transmission clusters were inferred using Phydelity, a statistically principled and phylogeny-informed framework to infer transmission clusters.[18] Phydelity had previously been validated for HCV sequences.[18] We defined transmission pairs as clusters of two sequences and transmission clusters as a collection of three or more sequences in the same Phydelity-defined cluster.

We identified temporal characteristics of clusters, such as the timing of the most recent common ancestor (MRCA), using time-resolved trees that were inferred using Bayesian phylogenetic analyses in BEAST (v1.10.4).[19] We conducted a BEAST analysis using parameters described in the appendix (p 2). Maximum clade credibility trees were summarised by TreeAnnotator and visualised using ggtree in R.[20] We used the glm function of the stats package in R to calculate the odds ratios (ORs) for belonging to a cluster.[20] To estimate the effective population size over time, a subset with only European sequences was analysed using the Skygrid reconstruction method in BEAST v1.10.4.[21] This effective population size is an abstract number that, when measured over time, provides an estimate of changes in population size, and is calculated on the basis of the rate of change in genetic diversity observed in the study population.[21,22] Results were visualised using Tracer v1.7.2.

Initial results revealed a low molecular clock signal (convergence not reached after 100 million iterations), which could be explained by the short timeframe in which the samples were collected. To improve the clock signal, full-length sequences that were not obtained during this study but were collected between 2002 and 2019 from MSM in the Netherlands were added to the dataset (appendix p 2).

Resistance-associated substitution testing

We calculated the prevalence of RAS in this cohort and assessed whether RAS were being transmitted among MSM. We cross-referenced all study sequences with all amino acid mutations defined as RAS, as defined by the European Association for the Study of the Liver treatment recommendations from 2020.[23] These RAS have been shown to confer reduced drug efficacy in vitro, reported in patients for whom treatment with direct-acting antivirals was not successful, or both. We denoted all listed mutations as RAS, regardless of the clinical significance of the substitution. However, genotype-specific substitutions present in wild-type sequences, such as Leu30Arg in genotype 4d, were not defined as RAS as they do not present a treatment barrier for viral clearance with current treatment regimens. To assess transmission dynamics of RAS, we selected all RAS detected more than once per genotype and identified the phylogenetic relationship of all sequences containing that RAS. Because only MSM were part of transmission clusters, we conducted the RAS analysis using the dataset containing only MSM sequences. We used only consensus sequences to cross-reference with known RAS; minority variants were not investigated.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Samples were available for 212 (95%) of 222 screened participants, of whom 179 (84%, appendix p 5) were gay or bisexual men (referred to here as MSM) and were included in further analysis. Table 1 shows the demographic characteristics of the study population in each country (see appendix p 12 for demographics of all 212 participants). Of the 179 participants, 149 (83%) were located in Europe, 16 (9%) in Oceania, and 14 (8%) in North America. Overall, 141 (79%) participants were co-infected with HIV. HCV risk factors differed by global region: the number of participants reporting injection drug use in the month before baseline was lower in Europe (21 [14%]) than in North America (three [21%]) and Oceania (five [31%]). In addition, injection drug use as the most likely route of HCV transmission was less

	Australia (n=15)	Canada (n=7)	Germany (n=55)	Netherlands (n=26)	New Zealand (n=1)*	Switzerland (n=8)	UK (n=60)	USA (n=7)
Sex								
Male	15 (100%)	7 (100%)	55 (100%)	26 (100%)	1 (100%)	8 (100%)	60 (100%)	7 (100%)
Female	0	0	0	0	0	0	0	0
Race								
Asian	3 (20%)	0	1 (2%)	1 (4%)	NS	0	2 (3%)	0
White	11 (73%)	6 (86%)	50 (91%)	22 (85%)	NS	7 (88%)	49 (82%)	5 (71%)
Indian	1 (7%)	0	0	0	NS	0	0	0
Black	0	0	0	0	NS	0	0	1 (14%)
Other	0	1 (14%)	0	0	NS	0	4 (7%)	0
More than one race	0	0	1 (2%)	3 (12%)	NS	0	5 (8%)	0
Unknown or not reported	0	0	3 (6%)	0	NS	1 (13%)	0	1 (14%)
Age, years								
0–19	0	0	0	0	NS	0	0	0
20–29	0	0	8 (15%)	6 (23%)	NS	1 (13%)	3 (5%)	0
30–39	9 (60%)	2 (29%)	13 (24%)	6 (23%)	NS	3 (38%)	12 (20%)	4 (57%)
40–49	2 (13%)	2 (29%)	18 (33%)	8 (31%)	NS	2 (25%)	27 (45%)	1 (14%)
50–59	3 (20%)	2 (29%)	15 (27%)	5 (19%)	NS	1 (13%)	14 (23%)	2 (29%)
≥60	1 (7%)	1 (14%)	1 (2%)	1 (4%)	NS	1 (13%)	4 (7%)	0
Current HCV infection type								
Primary infection	13 (87%)	4 (57%)	36 (66%)	15 (58%)	NS	4 (50%)	41 (68%)	4 (57%)
Reinfection	2 (13%)	3 (43%)	19 (35%)	11 (42%)	NS	4 (50%)	19 (32%)	3 (43%)
HIV infection								
Yes	9 (60%)	4 (57%)	46 (84%)	21 (81%)	NS	7 (88%)	49 (82%)	4 (57%)
No	6 (40%)	3 (43%)	9 (16%)	5 (19%)	NS	1 (13%)	11 (18%)	3 (43%)
PrEP use in past 6 months								
Yes	6 (40%)	3 (43%)	7 (13%)	4 (15%)	NS	0	8 (13%)	3 (43%)
No	0	0	2 (4%)	0	NS	0	2 (3%)	0
Not applicable	9 (60%)	4 (57%)	46 (84%)	21 (81%)	NS	7 (88%)	49 (82%)	4 (57%)
Missing	0	0	0	1 (4%)	NS	1 (13%)	1 (2%)	0
Ever been to prison								
Yes	0	1 (14%)	2 (4%)	0	NS	1 (13%)	2 (3%)	1 (14%)
No	15 (100%)	6 (86%)	52 (95%)	24 (92%)	NS	6 (75%)	50 (83%)	6 (86%)
Missing	0	0	1 (2%)	2 (8%)	NS	1 (13%)	8 (13%)	0
Sexual orientation								
Bisexual	1 (7%)	1 (14%)	1 (2%)	0	NS	1 (13%)	0	2 (29%)
Gay or lesbian	14 (93%)	6 (86%)	54 (98%)	26 (100%)	NS	7 (88%)	60 (100%)	5 (71%)
Self-reported most likely route of transmission								
Use of recreational drugs (injecting)	6 (40%)	1 (14%)	6 (11%)	0	NS	2 (25%)	8 (13%)	3 (43%)
Use of recreational drugs (snorting or inhaling)	0	0	1 (2%)	2 (8%)	NS	1 (13%)	4 (7%)	0
Sexual exposure	9 (60%)	6 (86%)	45 (82%)	22 (85%)	NS	4 (50%)	38 (63%)	4 (57%)
Other	0	0	2 (4%)	0	NS	0	2 (3%)	0
Missing	0	0	1 (2%)	2 (8%)	NS	1 (13%)	8 (13%)	0

(Table 1 continues on next page)

likely at European sites (16 [11%]) than in North America (four [29%]) and Oceania (seven [44%]).

We collected 196 samples from the 179 participants (179 obtained at baseline or screening; 17 reinfections during follow-up). The distribution of HCV genotypes differed by country (appendix p 4); genotypes 1a (n=132) and 4d (n=39) were most prevalent. We excluded missing genotypes (n=1) and infrequent genotypes (genotype 2 [n=2] and genotype 3 [n=22]) from the analysis. There were not enough samples of genotype 3 to conduct meaningful clustering analysis; additionally, the amplification of these samples was less successful than that of genotypes 1 and 4, reducing the numbers further. We therefore focused on genotypes 1 and 4 as the most prevalent genotypes of HCV among MSM. We obtained whole-genome sequences for 97 (73%) of

	Australia (n=15)	Canada (n=7)	Germany (n=55)	Netherlands (n=26)	New Zealand (n=1)*	Switzerland (n=8)	UK (n=60)	USA (n=7)
(Continued from previous page)								
Use of injecting recreational drugs								
Yes	9 (60%)	1 (14%)	16 (29%)	7 (27%)	NS	4 (50%)	25 (42%)	4 (57%)
No	6 (40%)	6 (86%)	38 (69%)	17 (65%)	NS	3 (38%)	27 (45%)	3 (43%)
Missing	0 (0%)	0	1 (2%)	2 (8%)	NS	1 (13%)	8 (13%)	0
Genotype at screening								
1	6 (40%)	2 (29%)	34 (62%)	23 (89%)	NS	5 (63%)	46 (77%)	5 (71%)
2	0 (0%)	0	0	1 (4%)	NS	1 (13%)	0	0
3	8 (53%)	1 (14%)	2 (4%)	0	NS	2 (25%)	4 (7%)	2 (29%)
4	1 (7%)	4 (57%)	19 (35%)	2 (8%)	NS	0	9 (15%)	0
Missing	0 (0%)	0	0	0	NS	0	1 (2%)	0

Data are n (%). HCV=hepatitis C virus. PrEP=pre-exposure prophylaxis. NS=not shown. *Data for the participant from New Zealand not shown to protect identity. Data were obtained by self-reported questionnaire. Genotyping was conducted during the primary analysis.

Table 1: **Demographic characteristics**

132 genotype 1a samples and 31 (79%) of 39 genotype 4d samples (appendix p 5) and constructed maximum-likelihood phylogenetic trees for both genotypes separately to assess phylogenetic clustering of sequences. Overall, 104 (81%) of 128 sequences were in 12 clusters (size range 3–22, denoted A–G, I, K–N) and two pairs (denoted H and J; figure 1).

We first looked at country distributions within clusters. Country mixing was detected in most clusters (11 of 12 clusters and one of two pairs). However, cluster B contained only sequences from the UK and one pair, H, consisted of two Australian sequences. Ten of 11 clusters with country mixing contained only European sequences, whereas cluster K contained three Canadian and six German sequences (figure 1B). The largest cluster, G, (n=22) contained 21 sequences from the UK and one sequence from the Netherlands (table 2). European MSM were more likely than non-European MSM to be in a cluster (OR 11·9 [95% CI 3·6–43·4], p<0·0001).

For the majority (nine of 14) of clusters and pairs, the most common self-reported risk factor for HCV transmission was sexual exposure, whereas injecting (n=3) or snorting or inhaling (n=1) drugs was the most common for four of 14 (table 2). For cluster I, sexual exposure and injection drug use were each reported as the most likely risk factors in 50% of cases. In seven of 14 clusters, both drug use and sexual exposure were stated as risk factors; however, notably, in some clusters and pairs the only risk factor was sexual exposure (cluster D and pair J) or injection drug use (cluster E and pair H). In five of 14 clusters and pairs, 100% of cluster members were living with HIV; for the remaining nine clusters this value was 50% or more (table 2). Reinfections during follow-up occurred both within the same transmission cluster and in other transmission clusters (appendix p 6). Sequences from non-MSM (n=10, genotype 1a) did not cluster with any sequences obtained from MSM, nor with each other (appendix p 7).

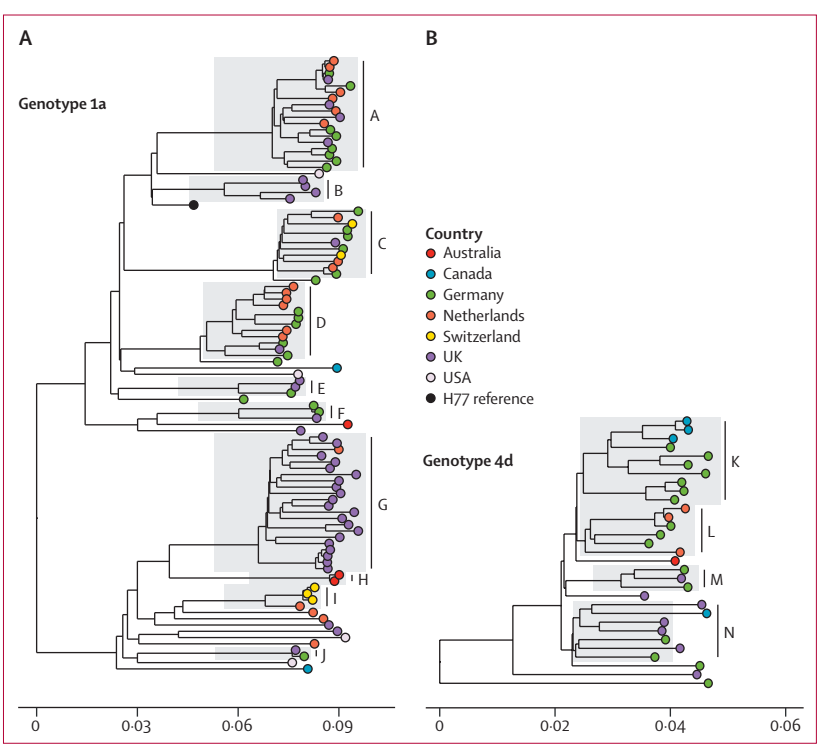

Figure 1: **Phylogenetic clustering**
Maximum-likelihood trees of all genotype 1a (A) and genotype 4d (B) study sequences. Coloured tips denote country of sample collection. The x axis represents genetic distance. Grey areas annotated with a letter indicate transmission clusters.

Next, we aimed to understand the temporal characteristics of these transmission dynamics. We conducted a Bayesian analysis of the sequences to infer a rooted, time-resolved phylogeny for each genotype. Owing to the short period of sample collection (around 2·5 years), there was insufficient temporal signal for a well-supported, time-resolved phylogeny in this dataset (no convergence of Markov chain Monte Carlo chains).

	A (n=18)	B (n=4)	C (n=11)	D (n=12)	E (n=3)	F (n=3)	G (n=22)	H (n=2)	I (n=4)	J (n=2)	K (n=10)	L (n=6)	M (n=3)	N (n=4)
Mean age, years (SD)	42·4 (10·5)	47·5 (10·0)	48·1 (9·7)	42·3 (8·3)	49·5 (7·1)	49·1 (3·3)	44·9 (8·4)	35·6 (4·3)	38·8 (10·9)	52·7 (18·4)	48·9 (10·1)	48·8 (14·0)	43·2 (10·5)	52·1 (9·4)
HIV	12 (67%)	12 (75%)	9 (82%)	12 (100%)	3 (100%)	3 (100%)	21 (96%)	1 (50%)	4 (100%)	1 (50%)	7 (70%)	5 (83%)	2 (67%)	4 (100%)
Genotype	1a	1a	1a	1a	1a	1a	1a	1a	1a	1a	4d	4d	4d	4d
Country														
Australia	0	0	0	0	0	0	0	2 (100%)	0	0	0	0	0	0
Canada	0	0	0	0	0	0	0	0	0	0	3 (30%)	0	0	0
Germany	8 (44%)	0	5 (45%)	5 (42%)	1 (33%)	2 (66%)	0	0	0	1 (50%)	7 (70%)	3 (50%)	2 (67%)	2 (50%)
Netherlands	6 (33%)	0	3 (27%)	6 (50%)	0	0	1 (5%)	0	1 (25%)	0	0	3 (50%)	0	0
New Zealand	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Switzerland	0	0	2 (18%)	0	0	0	0	0	3 (75%)	0	0	0	0	0
UK	4 (22%)	4 (100%)	1 (9%)	1 (8%)	2 (66%)	1 (33%)	21 (95%)	0	0	1 (50%)	0	0	1 (33%)	2 (50%)
USA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total countries	3	1	4	3	2	2	2	1	2	2	2	2	2	2
Risk factor														
Drug use (snorting or inhaling)	1 (6%)	2 (50%)	0	0	0	0	0	0	0	0	0	0	0	0
Drug use (injecting)	2 (11%)	1 (25%)	1 (9%)	0	3 (100%)	2 (67%)	2 (9%)	2 (100%)	2 (50%)	0	1 (10%)	0	0	0
Sexual exposure*	17 (78%)	1 (25%)	10 (91%)	12 (100%)	0	1 (33%)	13 (59%)	0	2 (50%)	2 (100%)	9 (90%)	4 (67%)	2 (67%)	3 (75%)
Other†	1 (6%)	0	0	0	0	0	7 (31%)	0	0	0	0	2 (33%)	1 (33%)	1 (25%)
MRCA date (range)	March, 2000 (Aug, 1998–Oct, 2001)	Dec, 1997 (Jan, 1993–July, 2002)	Nov, 2000 (Jan, 1999–Sept, 2002)	Feb, 1996 (May, 1993–Sept, 1998)	Feb, 2000 (Aug, 1997–Sept, 2002)	April, 1998 (June, 1994–Feb, 2002)	May, 1998 (March, 1995–April, 2000)	Aug, 2015 (Sept, 2014–June, 2016)	Oct, 2005 (May, 2004–March, 2007)	July, 2016 (Aug, 2015–April, 2017)	Feb, 2005 (Feb, 2001–July, 2008)	Aug, 2005 (Nov, 2001–Dec, 2008)	Dec, 2009 (Dec, 2006–July, 2012)	Oct, 2003 (Aug, 1999–Oct, 2007)

Data are n (%) unless otherwise stated. MRCA=most recent common ancestor. *Self-reported sexual exposure to people of known or unknown hepatitis C status of the same sex. †Self-reported and self-defined risk-factors other than drug use (injection, snorting, or inhaling) or sexual exposure.

Table 2: **Cluster characteristics**

The temporal signal was improved by the addition of background sequences from the Netherlands (appendix p 8) and convergence was achieved after a chain of 100 million.

We estimated when clusters and pairs were introduced by estimating the date of their MRCAs (table 2). Most clusters were introduced between the late 1990s and early 2000s; the MRCAs of the two pairs were in the mid-2010s (table 2, appendix p 18).

To assess whether international transmission also occurred after the introduction of direct-acting antivirals, we identified all modelled subclusters with MRCAs after Jan 1, 2015 within detected clusters and assessed whether country mixing was present. From 13 identified subclusters, country mixing was present in four (appendix p 10), indicating international transmission after 2015. Transmission within the same country was detected in 11 of 13 subclusters. In this cohort, we detected recent country mixing only among European countries. The bifurcation separating a German and Canadian subcluster, the only inter-continental cluster, was dated to approximately 2008, and the MRCA of the Canadian sequences was dated to approximately 2012 (appendix p 11).

The introduction of clusters (between the late 1990s and early 2000s) coincided with, and resulted in, an increase in effective population size between 2000 and 2010 (figure 2). Between 2010 and 2015, the effective population size was fairly stable with some fluctuations. Notably, a marked decrease is apparent after 2015, coinciding with increased uptake of direct-acting antiviral treatment. Owing to the absence of samples from before 2000, changes in effective population size before 2000 have a higher uncertainty than those after; however, a trend of increasing population size is seen after 1970 until approximately 1990, after which the population declines again until the early 2000s.

We detected 65 sequences (18 of 31 of genotype 4d and 47 of 93 of genotype 1a) with RAS in the MSM study population, at 13 different positions in the genome (figure 3). We did not detect transmission of clinically relevant RAS in the HCV proteins NS3, NS5A, and NS5B that are associated with diminished efficacy of the direct-acting antivirals currently recommended as treatment for HCV. Gln80Lys (NS3, 33%) and Met28Val (NS5A, 11%) were the most prevalent RAS detected for genotype 1a and Thr58Pro (NS5A, 52%) was most prevalent for genotype 4d. Transmission of viruses harbouring these RAS was detected; however, the introduction of viruses with these substitutions into the population was dated to well before the introduction of direct-acting antivirals. As such, these substitutions are most likely due to naturally selected polymorphisms or a so-called founder effect, rather than resistance induced by direct-acting antiviral treatment (appendix pp 13–18). Transmission of clinically relevant RAS—those that impart high resistance to current direct-acting

antivirals—was not identified, although singletons were found. For example, Tyr93His in NS5A was present in one genotype 1a sequence, but onwards transmission was not observed. Further details of the detected RAS are outlined in the appendix (pp 13–18).

Discussion

In this study we sequenced full-length viral genomes from a large cohort of MSM from eight countries who had recently been infected with HCV. We showed that international transmission occurs and could therefore hamper successful microelimination of HCV among MSM, particularly in Europe.

The majority of recent HCV infections in our study population were part of international networks that arose in the late 1990s and early 2000s. Our findings that a large proportion of European MSM with HCV are infected with a strain that is co-circulating in other European countries is congruent with the results of previous work,[11] which was conducted before the introduction of direct-acting antivirals. Cross-border transmission was identified in 30% of networks that were active after 2015 and was observed exclusively between European countries, which are therefore susceptible to continuing external introductions of HCV into the key populations at risk of acquiring HCV. This finding is in line with those from Switzerland and the Netherlands, where a substantial proportion of incident HCV infections were international.[9,24] Such international transmission can potentially hamper local microelimination efforts: after successful elimination of local transmission networks, by early diagnosis and expedient treatment of new HCV infections, such networks can be reintroduced if they remain active abroad. Microelimination is more likely to be achieved when the population at risk is not exposed to continuing external introductions. Real-world data show that, in such a setting, microelimination is achievable: among an HCV-antibody-positive population with HIV in Australia, HCV RNA prevalence decreased from 82% to 8% in 3 years.[25] For countries where external introductions remain common, both a strong local surveillance programme and collaborative, coordinated, international surveillance is needed. At a local level, externally introduced infections should be diagnosed and treated rapidly to prevent further transmission. Internationally,

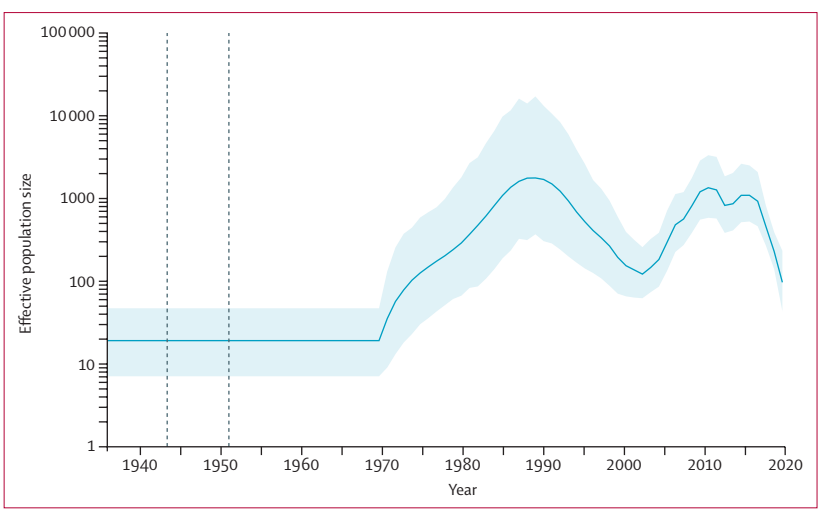

Figure 2: **Skygrid reconstruction**
Bayesian Skygrid estimations of changes in effective population size over time for genotype 1a sequences collected in European countries. The bold line represents median effective population size and the shading indicates the 95% highest posterior density credible intervals. The dashed vertical lines represent the median date and upper highest posterior density credible interval of the most recent common ancestor of all genotype 1a sequences.

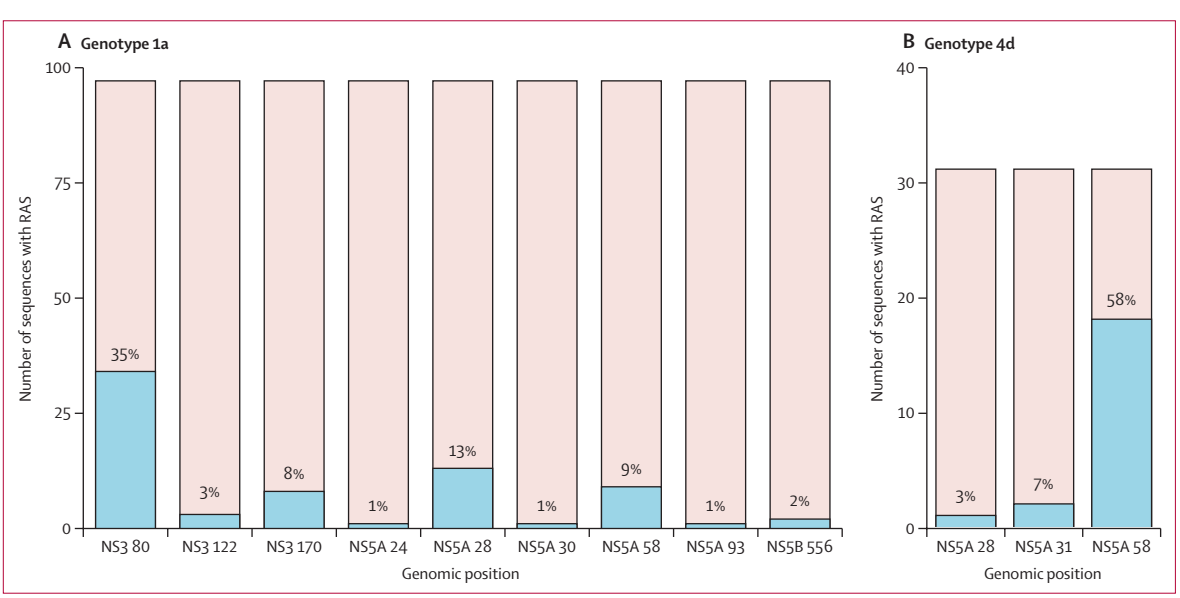

Figure 3: **Prevalence of RAS**
The prevalence of RAS in genotype 1a (A) and genotype 4d (B) sequences. The height of the solid blue bars indicates the absolute number of sequences with RAS at the respective position of the hepatitis C virus genome. Selected positions are highlighted in the appendix (pp 13–18). RAS=resistance-associated substitutions.

monitoring of international networks is important for tailored prevention interventions. Our results suggest that an international surveillance approach is especially applicable to European countries, given that several international transmissions were identified between European countries in the era of direct-acting antivirals. Local efforts to prevent HCV transmission remain essential, as evidenced by large clusters—such as cluster G, with most sequences from the UK—and the many recent subclusters with multiple sequences from the same country. The dynamics of local versus international transmission could change over time and should therefore be monitored closely.

We provide an estimate of the HCV effective population size over time in the studied European countries. Because regions other than Europe were undersampled, we restricted this analysis to European genotype 1a sequences. The effective number of infections is a measure of population size based on virus genetic diversity over time and provides an estimate of historical population dynamics.[21] The increase in the effective population size between the early 2000s and the mid-2010s coincides with the introduction of most of the observed clusters and also with the steep increase in HCV incidence among MSM in Europe at the time. Subsequently, an acute drop in effective population size coincided with the introduction and roll-out of direct-acting antivirals. One explanation could be that high uptake of direct-acting antivirals has resulted in a considerable decrease in HCV prevalence in this population,[4,25–27] which is reflected by the decline in effective population size. HCV emerged as a public health threat among MSM in the late 1990s and early 2000s, but our phylogenetic analyses also provide some information on the characteristics of the epidemic before that. Between the 1970s and the late 1990s, people who inject drugs were one of the key populations at risk of HCV infection. The observed increase and subsequent decrease in HCV infections between 1970 and the late 1990s roughly coincides with the increase and decrease in HCV incidence among people who inject drugs.[28–30] This finding could suggest that ancestral strains from circulating viruses detected in this study were circulating among people who inject drugs between the 1970s and the 1990s.

The prevalence of RAS that could decrease the effectiveness of current treatment was low and the transmission of highly resistant viruses was not observed. However, an argument can be made for international surveillance of RAS transmission, because we detected multiple clusters in which RAS were transmitted, albeit with little consequence for treatment effectiveness. Most RAS-containing clusters were introduced before the roll-out of direct-acting antivirals, and are therefore due to a founder effect rather than selective pressure from treatment with these agents. We observed one event involving the transmission of NS3 Ile170Val in 2018, after the introduction of direct-acting antivirals. Although this substitution has little clinical significance (some reduced sensitivity to voxilaprevir in combination with RAS at additional positions[31]), this finding does show the added benefit of monitoring: only by phylogenetically monitoring new infections can we establish whether RAS-containing subclusters will emerge and grow. More generally, the type and availability of direct-acting-antiviral regimens might differ between countries, which translates into different selective pressures on viral populations. Resistance to direct-acting antivirals could jeopardise micro-elimination efforts if cure rates are affected by RAS transmission within a population. The detection of NS5A Tyr93His in one treatment-naive participant is potentially concerning, because this substitution has been shown to reduce rates of sustained virological response at 12 weeks after treatment with various combinations of direct-acting antivirals. International monitoring will aid the early detection of the emergence and transmission of RAS.

We used data collected over a relatively short time period (around 2·5 years), resulting in some limitations when estimating historical virus-transmission events. Many clusters were introduced more than 10 years before the earliest collection date, which means that all infections in those years are unsampled. This lack of samples prevents us from analysing phylogenetic branch movements to estimate phylogeographic movements over time. Furthermore, it decreases the certainty of the estimates of cluster introduction dates, as shown by the wider higher posterior density intervals (especially for genotype 4d sequences). We improved the time estimates by adding viral sequences that had previously been obtained from Dutch MSM. In addition, owing to few sequences from elsewhere, we could only estimate the effective population size dynamics within Europe for genotype 1a; non-European countries could therefore have different dynamics that were not observed in this study. Increased sampling and sequencing are needed for such analyses. However, despite these limitations, this study is valuable because it includes a large number of whole-genome sequences from recent HCV infections among MSM across three continents.

In conclusion, several factors threaten the ambitious goals to eliminate HCV as a public health threat by 2030. We show that, mainly in Europe, continuing international imports could complicate elimination efforts, but highly resistant strains are currently not a barrier to the elimination of HCV in MSM. Our study underlines the importance of considering both international transmission and local transmission patterns when pursuing the microelimination of HCV in this population. Even in the era of direct-acting antivirals, international transmission occurs in Europe, and local preventative measures alone might not be sufficient to achieve elimination, supporting a regional public health

approach. Internationally coordinated, genomic surveillance of HCV—combined with strong local programmes—might be required to achieve and maintain microelimination within a country.

Contributors

MvdV, JS, GM, RS-D, and GJD conceived the project. JK curated the data, conducted formal analyses, created the figures, wrote the analysis scripts, and was responsible for project administration. JK, MvdV, and JS developed the methods. SR conducted laboratory analyses. MvdV and JS acquired funding and GM, RS-D, and GJD contributed resources. JK wrote the original draft of the manuscript and all authors contributed to the review and editing. All authors have seen and approved the final version of the manuscript. JS and MvdV accessed and verified all the data reported in this study. Author contributions for the original REACT study have been published previously.[13]

Declaration of interests

GM reports grants from Gilead Sciences and AbbVie, outside of the submitted work. SB reports grants from Gilead Sciences and personal fees for advisory board membership, lectures, and presentations from Gilead Sciences, all outside the submitted work. MvdV reports grants and personal fees from AbbVie, Gilead Sciences, Johnson & Johnson, MSD, and ViiV, outside the submitted work. JR reports personal fees from Gilead Sciences, Janssen, Merck, Theratechnologies, and ViiV, outside the submitted work. JF reports grants and personal fees from Gilead Sciences, Enanta, and AbbVie; personal fees from GSK, Arubutus, and Roche; and grants from Janssen and Eiger, all outside the submitted work. AR is a member of advisory boards of MSD and Gilead Sciences; has received travel grants from Gilead Sciences, Pfizer, and AbbVie; and has received an investigator-initiated trial grant from Gilead Sciences; all remuneration was to his home institution (Bern University Hospital, Bern, Switzerland) and not to AR personally. JB reports grants from the National Institutes of Health during the conduct of the study; personal fees from AbbVie; grants and personal fees from Gilead Sciences; and grants from the Canadian Institutes of Health Research, Fonds de Recherche—Québec, Substance Use and Abuse program, and Health Canada, outside the submitted work. MH reports grants from Gilead Sciences and AbbVie, outside the submitted work. PI reports grants and personal fees from Gilead Sciences and personal fees from AbbVie and ViiV, outside the submitted work. JG reports grants and personal fees from AbbVie, Gilead Sciences, Merck, and Cepheid; grants from Hologic and Indivior; payment or honoraria and travel support from AbbVie, Gilead Sciences, and Cepheid; receipt of testing equipment and cartridges from Cepheid; and receipt of testing reagents from Hologic; outside the submitted work. GJD reports grants, personal fees, and non-financial support from Gilead Sciences, AbbVie, and Merck and grants from Bristol-Myers Squibb, outside the submitted work. JS's institution (Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, Netherlands) has received research support and consultancy fees from Gilead Sciences (NoCO grant) and a speaker's fee from Janssen Pharmaceuticals, outside the submitted work. CB reports honoraria for lectures and/or consultancies from AbbVie, Gilead Sciences, Janssen, MSD, and ViiV and funding from Dt. Leberstiftung, DZIF, Hector Stiftung, and NEAT ID. JK, SR, RS-D, MM, and TLA declare no competing interests.

Data sharing

Owing to the sensitive nature of some of the data, including those related to injection drug use, data included in this manuscript have not been placed in an open access database. However, data are available to be shared on request to the protocol steering committee. De-identified sequence data are available from GenBank (accession number ON630763-ON630900).

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