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Epidemiological and genetic analyses of Hepatitis C virus transmission among young/short- and long-term injecting drug users from Rio de Janeiro, Brazil

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ABSTRACT

Background: Injecting drug users (IDU) have a key role in Hepatitis C Virus (HCV) epidemiology. Young/short-term IDUs constitute a target group for preventive/harm reduction interventions.
Objectives: To investigate HCV transmission among young/short-term (ST) and long-term (LT) IDUs, from the perspective of epidemiology and molecular biology.
Study design: Cross-sectional study assessing the prevalence of HCV infection/genotypes, as well as risk behaviours/practices among IDUs from Rio de Janeiro. Phylogenetic analyses were performed and the extent of segregation between sequences was quantified by the Association Index.
Results: ST were more likely to engage into needle-sharing ($p = .021$) and LT to attend Needle Exchange Programs ($p = .006$). HCV prevalence was 10.1% vs. 23.4% among initiates and LT, respectively ($p < .001$). Older age vs. imprisonment and longer duration of IDU career were independent predictors for HCV infection among ST and LT, respectively. Among the latter, NEP attendance was inversely associated with viral infection. HCV3a infections were the most prevalent. A moderate extent of phylogenetic segregation between sequences was found, suggestive of transmission between IDU subgroups.
Conclusions: The lower HCV prevalence among young/short-term IDUs cannot be viewed with complacency, due to their frequent engagement into direct/indirect sharing practices and the ongoing transmission between IDU subsets. To avert new infections, preventive/harm reduction policies must be tailored to empirical findings.

1. Introduction

Worldwide, Hepatitis C Virus (HCV) infection is a public health challenge, due to its health and economical impacts.[1] About 60–85% of infected-subjects develop chronic infection, of which major complications are liver cirrhosis and hepatocarcinoma.[2]

In the last decades, HCV epidemiology has been changing in developed countries, due to improved blood transfusion safety and better healthcare conditions. Consequently, injection drug use became the main mode of viral transmission and accounts for more than 60% of prevalent cases in Europe.[3]

Drug injectors are frequently engaged into risky behaviours,[4–6] favouring an extensive HCV spread throughout IDUs networks.[7] Many new infections occur in young/new IDU, among whom incidence rates from 11.6 to 37.3/100 persons-year have been described.[8,9]

HCV is classified into six major genotypes and several subtypes,[10] which present distinct geographical patterns and response to antiviral therapy.[11] Moreover, a relationship between viral variants and mode of transmission has been shown.[12,13] Changing patterns/introduction of new subtypes can ensue relatively fast within IDU networks.[3]

In this study, practices and behaviours and their putative association with HCV infection/genotypes were investigated among young/short- and long-term IDUs. Phylogenetic relationships

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between IDU sequences and the extent of transmission between both subsets were also analyzed. This information is unavailable in Brazil, although critical to avert harmful practices among initiates.

2. Methods

2.1. Population

From 1999 to 2001, 606 IDUs were recruited in the Rio de Janeiro's "drug scenes", as previously described.[14] After signing an informed consent, volunteers were interviewed using a structured questionnaire standardized by the World Health Organization (WHO)[15] and a blood sample was collected.

All interviewees received pre-/post-test counselling and were referred to Health Services, if viral RNA-positive. HBV vaccination was also available. The study was approved by the Oswaldo Cruz Foundation's Human Subjects Protection Committee.

IDUs were grouped into short-(ST) and long-term injectors (LT), according to the number of years of drug injection (equal or less vs. more than 6 years, respectively). The rationale for this cutoff[16] and definitions for other variables are explained elsewhere.[14]

2.2. Laboratory methods

HCV antibodies were detected by an immunoassay (UBI HCV EIA 4.0, Beijing United). Viral RNA was extracted using QIAamp® Viral RNA extraction kit (QIAgen). Detection of 5′NCR HCV-RNA was performed as previously described.[17] For NS5b, one-step PCR was carried out using the SuperScript™III One-Step RT-PCR System (Invitrogen). Termocycling conditions consisted of 1 cycle-42 °C/45 min; followed by 1 cycle-95 °C/4 min; 40 cycles-95 °C/20 s; 54 °C/30 s; 72 °C/1 min and a final extension cycle (72 °C/10 min). For nested PCR, 1 μl of first PCR product was used. Primers and nested-PCR protocol were already published.[18]

PCR products were sequenced in sense and antisense directions (BigDye sequencing kit 3.1, Applied Biosystems) and analyzed with ABI 3730 automated DNA sequencer. 5′ÚTR and NS5b sequences spanned nucleotide positions 49–287 and 8277–8606, respectively[19]. Clustal X was used for DNA alignment.

2.3. Phylogenetic and molecular evolutionary analyses

Analyses were performed using MEGA 4.0.[20] Phylogenetic trees were constructed using the Neighbor-Joining method,[21] with bootstrap re-sampling (1000 replicates).[22] Evolutionary distances were computed using the Kimura 2-parameter method.[23] Standard error estimates were obtained by a bootstrap procedure (1000 replicates).

2.4. Assessment of genetic isolation between ST and LT IDU sequences

The degree to which viruses circulating among ST IDUs were phylogenetically distinct from those found among LT IDUs was measured by the Association Index (AI), which quantifies the degree of phylogenetic segregation between groups of sequences.[24,25,26] Briefly, sequences were labelled according to their subgroup (ST vs. LT) and used to construct 2 subsets of phylogenetic trees: "observed" and "control". The first was used to infer phylogenetic segregation while the second determined how often segregation may occur by chance. AI expresses the ratio of the median association values of the "observed" against the "control" trees. Values close to 0 represent complete segregation, whereas values ≥ 1 correspond to complete phylogenetic mixing. Analyses were performed using the Simmonic 2005 v.1.6 software.[27]

2.5. Statistical analyses

Contingence table statistics (Chi-square or Fisher's exact test, and t-tests for means) were employed to assess associations between socio-demographic/behavioural variables and HCV infection/viral genotypes. All variables of epidemiological relevance with $p < 0.10$ in bivariate analyses were entered into multivariable logistic regression. The Wilcoxon test was used for comparison between observed and control association values.

3. Results

3.1. Epidemiological findings

Socio-demographic features, drug use and sexual behaviours and HCV infection/viral genotypes prevalence among short-and long-term injectors are shown in Table 1. Two-hundred ninety nine and 307 interviewees were grouped as short- and long-term injectors, with a mean duration of injection of 2.2 vs.16.1 years ($p < .001$) and a median year of first injection of 1997 vs. 1986 ($p < .001$), respectively. ST were significantly younger and more likely to be single, compared to their counterparts. About half of the interviewees had a low education background and most interviewees reported informal activities as their main source of income.

Detention was mentioned by roughly half of the sample. LT were more likely than ST to share injection equipment while imprisoned.

For both groups, cocaine was the drug of choice and non-injection use the main mode of drug intake. Higher injection frequencies were found among LT IDUs, whereas short-term injectors reported a significantly higher frequency of syringe/needle-sharing ($p = .021$). Indirect sharing practices were reported by both subgroups under similar frequencies. Unprotected sex was common, irrespectively of the nature of partnership or sexual orientation.

LT were less likely than ST IDUs to have been treated for drug abuse ($p = .003$). In contrast, they reported a higher attendance of Needle Exchange Programs (NEPs) ($p = .006$).

The overall prevalence of HCV infections was 16.9% and LT IDUs were 2.7 times more likely to be infected with HCV than new injectors ($p < .001$) (Table 1). In bivariate analyses, variables that were significantly associated with viral infection were: "sexual intercourse with casual partners from the opposite sex in the last 6 months" ($p = .024$), "male-to-male sex" ($p = .025$), "older age" ($p < .001$) and "more years of full education" ($p = .005$) among short-term IDU, vs. "daily frequency of injection" ($p = .001$), "ever been in prison" ($p = .001$); "injection with an HIV-infected IDU" ($p = .002$), "older age" ($p < .001$), "younger age of first injection" ($p = .002$) and "duration of injection drug use" ($p < .001$) among long-term drug injectors. Finally, "attendance in Needle Exchange Programs in the last 6 months" was found to be a protective factor for HCV infection (OR = 0.2; $p = .015$). Independent predictors for HCV infection among ST and LT IDUs are shown in Table 2.

3.2. Molecular findings

Viral RNA (5′UTR) was detected in 74.5% (76/102) of anti-HCV positive samples, from which 74 (97.4%) had their HCV subtype determined. Two samples were not sequenced due to a weak PCR product signal. NS5b nested-PCR was carried out in 76 samples, from which only 47 (61.8%) showed reactivity; even after retesting since RNA extraction. A similar distribution of viral genotypes was observed in both IDU subgroups (Table 1). Finally, none association was found between viral variants and epidemiological data.

Table 1
Socio-demographic characteristics, drug use, sexual behaviours and viral hepatitis infections among 299 short- and 307 long-term injecting drug users. WHO multicity study Phase II, Rio de Janeiro, 1999/2001.

Variables	N (%)[a]	Short-term IDU, N (%)[b]	Long-term IDU, N (%)[c]	p-value[d]
Socio-demographic characteristics				
Gender (male, %)	554 (91.4)	274 (91.9)	280 (90.9)	.378
Source of income (%)[e]				.494
Informal, benefits, student financial aid, someone else's income	497 (86.0)	235 (84.2)	262 (87.6)	
Permanent job	65 (11.2)	35 (12.5)	30 (10.0)	
Illegal activities	16 (2.8)	9 (3.2)	7 (2.3)	
Marital status (%)[e]				.005
Single	390 (64.4)	209 (70.1)	181 (58.8)	
Legally married/living as married	156 (25.7)	69 (23.2)	87 (28.2)	
Widowed/legally separated/divorced	60 (9.9)	20 (6.7)	40 (13.0)	
Residence[e]				.006
House of parents, relatives, friends, shelters	413 (68.2)	218 (73.2)	195 (63.3)	
Owned or rented house, flat or apartment	193 (31.8)	80 (26.8)	113 (36.7)	
Ever been in prison	250 (45.2)	130 (43.6)	120 (47.1)	.235
Injected while in prison[f]	66 (24.5)	22 (18.2)	44 (29.7)	.020
Shared injection equipment while imprisoned[f]	45 (70.3)	11 (52.4)	34 (79.1)	.030
Drug use behaviours				
Main mode of drug use[e]				.087
Non-injecting	516 (89.0)	258 (91.5)	258 (86.6)	
Injecting	38 (6.6)	12 (4.3)	26 (8.7)	
Both	26 (4.5)	12 (4.3)	14 (4.7)	
Main drugs used[e]				
Cocaine	264 (97.4)	110 (97.3)	153 (97.5)	.618
Amphetamines	14 (5.1)	2 (1.8)	12 (7.5)	.028
Heroine	8 (2.9)	3 (2.7)	5 (3.1)	.558
Number of lifetime injections				<.001
2–9	129 (21.3)	104 (34.9)	25 (8.1)	
10–99	232 (38.3)	116 (38.9)	116 (37.7)	
>100	245 (40.4)	78 (26.2)	167 (54.2)	
Frequency of injection[e,g]				.253
1–3 times/month	115 (42.3)	82 (45.0)	63 (39.6)	
1–3 times/week	118 (43.6)	50 (44.2)	68 (42.2)	
>3 times/week	32 (11.8)	9 (8.0)	23 (14.5)	
Needle-sharing[e,g]	100 (36.8)	50 (42.2)	50 (31.4)	.021
Indirect sharing practices[e,g]				
Injection with a pre-filled syringe	86 (31.7)	40 (35.4)	46 (29.1)	.275
Front/backloading	44 (16.2)	15 (13.3)	29 (18.2)	.275
Sharing of injection paraphernalia	130 (47.8)	56 (49.6)	74 (46.5)	.225
Got new/clean needles in the last 6 months[e,g]	256 (92.4)	105 (89.0)	151 (95.0)	.050
Pharmacist	193 (75.4)	71 (67.6)	122 (80.8)	.012
Exchange Program	79 (30.9)	37 (24.5)	42 (40.0)	.006
Ever received treatment for drug use	189 (31.2)	109 (36.6)	80 (26.6)	.003
Sexual behaviours				
Never used condom with principal partners of the opposite sex (last 6 months)	216 (62.8)	110 (59.5)	106 (66.7)	.102
Never used condom with casual partners of the opposite sex in the last 6 months	254 (68.3)	139 (76.4)	115 (60.5)	.001
Never used condom with clients of the opposite sex in the last 6 months	27 (4.5)	11 (3.7)	16 (5.2)	.240
Sexual intercourse with another man in the last 5 years[h]	190 (34.4)	81 (29.6)	109 (38.9)	.013
Never used condom, men who have sex with men in the last 6 months	49 (48.5)	24 (57.1)	25 (42.4)	.103
HCV infection[i]	102 (16.9)	30 (10.1)	72 (23.4)	<.001
HCV genotypes				.800
1	44 (57.9)	15 (60.0)	29 (56.9)	
3a	32 (42.1)	10 (40.0)	22 (43.1)	

Continuous variables	Mean (±SD)		
Age (years)	27.4 (8.8)	36.7 (8.3)	<.001
Years of full education	9.1 (3.7)	9.3 (4.4)	.639
Age of first injection	20.6 (6.3)	18.5 (4.0)	<.001
Duration of injection drug use	2.2 (1.9)	16.1 (7.7)	<.001
Median year of first drug injection (range)	1997 (1992–2001)	1986 (1953–1994)	<.001

Short-term IDUs were defined as those who inject illicit drugs for less than 6 years.

[a] Total = 606 subjects.

[b] N = 299.

[c] N = 307.

[d] Significance was considered when p-value < .005.

[e] Last 6 months.

[f] Among 66 interviewees who shared needles/syringes while imprisoned who shared needles/syringes in prison.

[g] Among 272 active IDUs, who injected drugs in the last 6 months (short-term, 113 and long-terms, 159).

[h] Among 554 men (short-term-274; long-term-280).

[i] HCV [OR = 2.7 (1.7–4.3)].

Table 2
Independent predictors for HCV infection among 299 short- and 308 long-term injecting drug users. WHO multicity study Phase II, Rio de Janeiro, 1999/2001.

Variables	AdjOR (95%CI)	*p*-value
Short-term IDU[a]		
Older age	1.14 (1.09–1.20)	<.001
Long-term IDU[b]		
Duration of injection drug use	1.12 (1.07–1.18)	<.001
Ever been in prison	3.19 (1.23–8.24)	.017
Attendance in needle exchange program[c]	0.18 (0.04–0.88)	.034

Short-term IDUs are defined as those who inject drugs for less than 6 years.
[a] Final model: $\chi^2 = 40.0$; $p < .001$.
[b] Final model $\chi^2 = 49.4$; $p < .001$.
[c] In the last 6 months.

Some facts speak in favour of dissimilar results between ST and LT, including: i) the different behavioural profiles of ST and LT may lead to different phylogenetically-related groups; ii) time of first injection was markedly different for ST and LT (consequently, changes in the background genotype distribution over time).

However, analyses of mean pairwise evolutionary distances showed similar results for ST and LT NS5b sequences. Within HCV subtypes 1a, 1b and 3a, the mean evolutionary distances (standard error) were 0.027 (0.005), 0.085 (0.012) and 0.054 (0.009) vs. 0.038 (0.006), 0.082 (0.011) and 0.048 (0.007) among short- and long-term IDUs, respectively. Thus, a more detailed assessment of HCV phylogenetic profile of the two subgroups was performed.

Phylogenetic relationships between IDU sequences are shown in Fig. 1. Except for two related pairs (one for subtype 1a and another for subtype 3a), with high bootstrap values, sequences from both subsets were dispersed along the reconstructed trees, corroborating previous findings.

The extent of segregation between ST and LT IDU sequences, measured in terms of AI, is shown in Table 3. HCV1a and 3a sequences showed slightly lower AI values, compared to HCV1b. Indeed, in agreement with evolutionary distance data, a considerable phylogenetic mixing between IDU sequences was observed, what may be explained by frequent (but not free) transmission within IDU strata. Moreover, since AI values were < 1, there is some segregation between IDU subsets, with the rejection of the null hypothesis (no difference between observed and control AIs) ($p < .001$ for HCV1a and 3a and $p = .013$ for HCV1b)

4. Discussion

In this study, epidemiological and molecular patterns of HCV transmission among short- and long-term IDUs were assessed. These are pivotal information to provide the basis for sound preventive policies, management and care of such population.

Table 3
Association indices and association values in short- and long-term injecting drug users sequences, according to HCV subtype and genome region.

Sequences compared	Median association values		Association index
	Observed	Controls	
Short- and long-term IDUs—5′UTR[a]			
HCV 1a	1.33	2.09	0.63
HCV 1b	1.33	1.62	0.70
HCV 3a	1.32	2.03	0.65
Short- and long-term IDUs—NS5b*			
HCV 1a	0.42	0.74	0.56
HCV 1b	0.48	0.67	0.72
HCV 3a	0.50	0.73	0.69

[a] For all tested samples. Association values and AI were calculated using the program Simmonic 2005 v.1.6[27].

Despite the efforts to obtain a representative sample of Rio de Janeiro's drug scenes, findings cannot be generalized to the IDU population. Moreover, as prevalence of HCV infection was significantly lower than expected, for some variables, a tiny sample remained after stratification, precluding the assessment of some putative associations.

According to the international literature,[28,29] short-term injectors reported significantly higher frequencies of syringe/needle-sharing than long-term IDUs. Furthermore, a high frequency of indirect sharing practices has been also noticed. This is a key problem to be tackled, since both direct and indirect sharing practices are associated with extensive HCV transmission within IDU networks.[5,30]

The prevalence of HCV infection was significantly lower than previously found in the same population and setting.[31] Possible reasons for this scenario are discussed elsewhere.[32] Besides this declining trend, drug injection started at an older age among initiates in recent years, calling for timely intervention.

Imprisonment and longer duration of injection drug use were independent predictors of HCV infection. Association between imprisonment and HCV infection is common among IDUs[33,34] and the second is a major determinant for HCV infection worldwide.[35] Among short-term IDU from Vancouver, for each two additional years of drug injection, the risk for HCV infection had doubled.[8]

LT IDUs who attended NEPs were significantly less likely to be HCV-infected. Declining rates of HCV infection as a benefit of NEP attendance have been described.[36] However, young IDUs have been pointed as particularly susceptible to HIV infection because of inexperience in obtaining clean injection equipment.[37] Our observations seem to corroborate these remarks.

Regardless of sexual orientation/kind of partnership, unprotected sex was frequent, as usually reported among cocaine users.[38] HCV is not efficiently transmitted by sexual intercourse and the literature on this issue remains controversial. However, HIV infection seems to favour the sexual transmission of HCV.[39] Higher odds for HCV infection were observed among ST IDUs reporting unprotected sex with both men and women engaged with casual partners. Although these associations did not remain significant after controlling for possible confounders, we cannot rule out the possibility of sexual transmission among these subjects.

Interestingly, a considerable lower rate of HCV-RNA amplification for NS5b region was obtained among IDU samples from Rio de Janeiro, when compared to IDU samples from São Paulo (62% vs. 90%, respectively; data not shown). Nevertheless, samples were positive for 5′ UTR HCV-RNA and remained NS5b negative, even after retesting. One of the possible explanations for the low amplification frequency of NS5b fragments could be the mismatch of primers, due to heterologous sequences. Sequencing of other fragments/regions could be helpful to elucidate these findings.

The distribution of HCV genotypes corroborates previous findings.[40,31] However, it differed substantially from those observed among chronic patients from Rio de Janeiro, where HCV1b infections are more prevalent.[41] The extent to which HCV circulating among new injectors was phylogenetically distant from those circulating within long-term IDU was measured in terms of AI. These analyses suggest a moderate level of virus exchange between IDU subgroups. Although not significantly different, segregation was higher among HCV1a, whereas a higher phylogenetic mixing was found among HCV1b sequences. If we use the year of first injection as a proxy of the year IDUs became infected, HCV1b infections seems to be introduced earlier, as the most prevalent genotype in the 1960 s, whereas HCV1a infections seems to have emerged in the early 1970 s. Thus, we may speculate that the higher segregation of HCV1a sequences could be partially attributed to a later entrance and dissemination within our IDU sample. Indeed, a study on the epidemic history of HCV estimated that subtypes 1a and

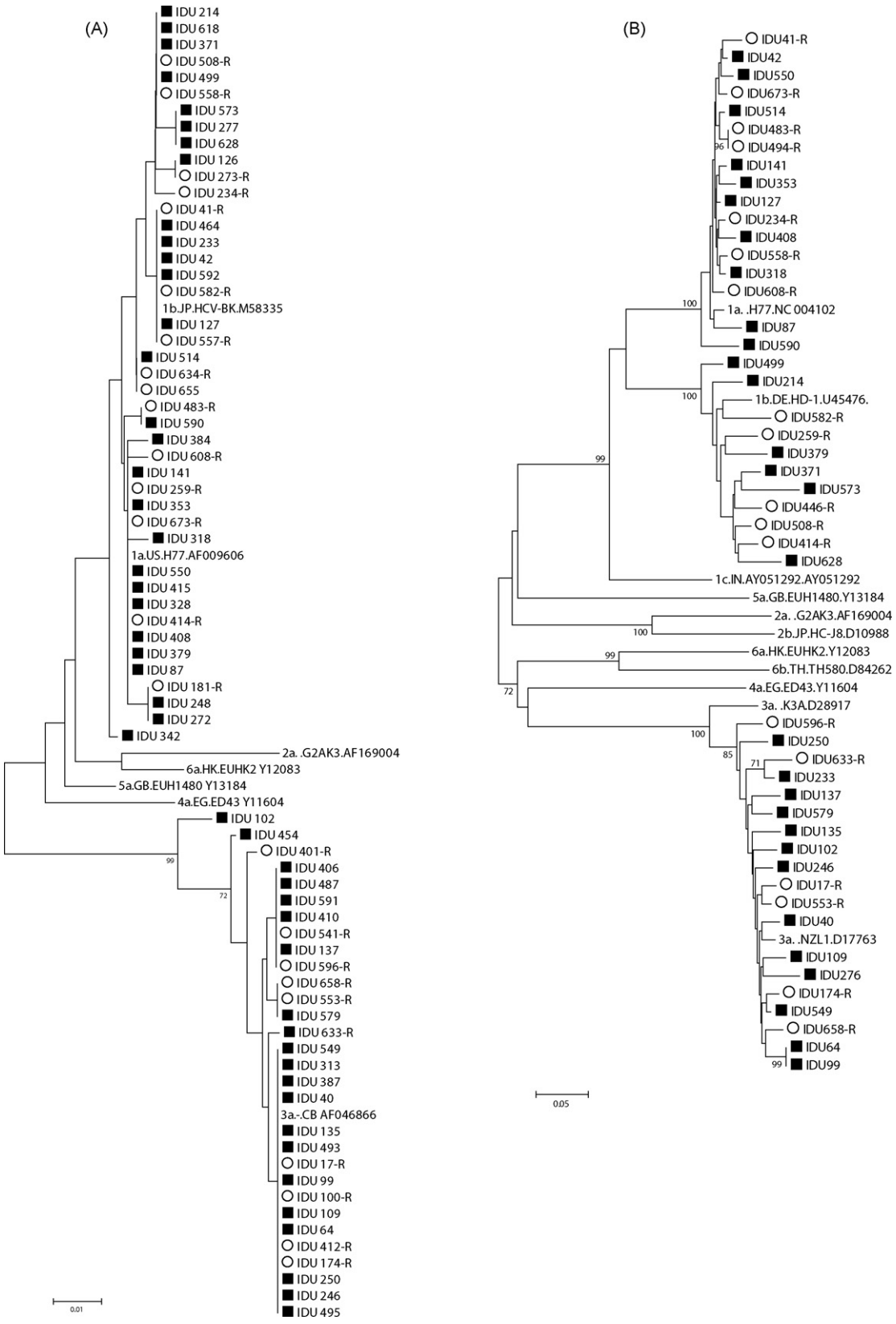

Fig. 1. The evolutionary history of HCV sequences from short-term (○) and long-term ■) injecting drug users for 5′UTR (A) and NS5b (B) regions was inferred using the Neighbor-Joining method.[21] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.[22] The evolutionary distances were computed using the Kimura 2-parameter[23] and are expressed in the units of the number of base substitutions per site. The accession numbers of reference and IDU sequences used in this study were as follows: 1a (AF009606); 1b (M58335); 1c (AY051292); 2a (AF169004); 2b (AF238486); 2c (D50409); 3a (AF046866); 3b (D49374); 4a (Y11604); 5a (Y13184); 6a (Y12083) and 6b (D84262) and IDUs EU678672 to EU678744 for 5′ UTR region and 1a (AF009606); 1b (U45476); 1c (AY051292); 2a (AF169004); 2b (AF238486); 3a (D28917); 3b (D49374); 4a (Y11604); 5a (Y13184); 6a (Y12083) and 6b (D84262) and IDUs EU747741 to EU747787 for NS5b region, respectively.

1b were introduced in Brazil about 1940–1950 and 1820–1920, respectively.[42] Finally, segregation could also be attributed to distinct risk behaviour profiles among IDU subgroups, which could eventually favour the transmission of non-1a subtypes in a larger extent.

Altogether, these findings speak in favour of interventions and management strategies tailored to the specificities of young/new injectors. Declining rates of HCV infection should be viewed as a chance for effective and prompt intervention.

Conflict of interest

None.

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