Uncommon Detection of Mixed HCV Genotype Infections in Recently Infected Men Who Have Sex with Men

Thuy Nguyen, Constance Delaugerre, Marc-Antoine Valantin, Corinne Amiel, Emmanuelle Netzer, Thomas L'Yavanc, Michel Ohayon, Gérard Israel, Nadia Valin, Nesrine Day, et al.

Uncommon Detection of Mixed HCV Genotype Infections in Recently Infected Men Who Have Sex with Men

Short title: Mixed HCV Genotype Infections in Men Having Sex with Men

Authors: Thuy NGUYEN[1*], Constance DELAUGERRE[2,3], Marc-Antoine VALANTIN[4], Corinne AMIEL[5], Emmanuelle NETZER[6], Thomas L'YAVANC[7,8], Michel OHAYON[7,8], Gérard ISRAEL[8], Nadia VALIN[9], Nesrine DAY[10], Georges KREPLAK[10], Gilles PIALOUX[8], Vincent CALVEZ[1], Jean-Michel MOLINA[3,11], Anne-Geneviève MARCELIN[1], Eve TODESCO[1]

Affiliations:

[1] Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), AP-HP, Hôpital Pitié-Salpêtrière, Laboratoire de virologie, F-75013 Paris, France ;

[2] AP-HP, Hôpital Saint-Louis, Laboratoire de virologie, Paris, France ;

[3] INSERM UMR 941, Université de Paris Diderot, Sorbonne Paris Cité ;

[4] Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), AP-HP, Hôpital Pitié-Salpêtrière, Services de maladies infectieuses et tropicales, F-75013 Paris, France ;

[5] Sorbonne Université, Centre d'Immunologie et de Maladies Infectieuses (CIMI) UMRS CR7, Persistent Viral Infection (PVI) Team, Inserm U1135, APHP, Groupe Hospitalier Paris Est, Hôpital Tenon, Laboratoire de virologie, F-75020 Paris, France;

[6] INSERM SC10, Villejuif, France ;

[7] Centre de santé sexuelle Le 190, Paris, France;

[8] Sorbonne Université, APHP, Hôpital Tenon, Department of Infectious Diseases, Paris, France;

[9] Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), AP-HP, Hôpital Saint Antoine, Department of Infectious Diseases, F-75012 Paris, France;

[10] Cerballiance Laboratory, Paris, France;

[11] AP-HP, Hôpital Saint-Louis, Department of Infectious Diseases, Paris

***Corresponding author:**

Thuy NGUYEN

Service de virologie, Bât CERVI, Hôpital Pitié-Salpêtrière, 83 Bd de l'Hôpital, 75013 Paris, France

Email: thuthuynguyend07@gmail.com or thuy_nguyen@med.unc.edu

Fax: +33 1 42 17 74 11 ; Phone : +33 1 42 17 58 42

Funding

This work received financial support from the Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) (decision number 2018-139).

ABSTRACT (249 words)

Introduction: Mixed HCV genotype (GT) infections are clinically important as different genotypes have different sensitivities to direct-acting antivirals (DAAs). A high prevalence of mixed GT infections was observed in people who inject drugs due to their multiple HCV exposures. The prevalence of mixed HCV GT infections in men having sex with men (MSM) at high-risk behaviors was investigated by ultra-deep sequencing (UDS).

Methods: NS5B fragment was sequenced from viruses of patients with recent HCV infection: 50 HIV-positive and 18 HIV-negative including 13 from the ANRS Pre-Exposure Prophylaxis (PrEP) IPERGAY study. UDS data were analysed by Geneious (version 10.3.2). Phylogenetic trees were constructed by FastTree (version 2.1).

Results: HCV sequencing showed GT1a (47.1%), GT4d (41.2%), GT3a (8.8%) and GT2k (2.9%). We detected three (4.4%) mixed GT infections: one between predominant GT4d and minority GT1a, one between predominant GT4d and minority GT1b, and one between predominant GT1a and minority GT4d virus. The rates of minority GT viral populations detected in virus of the three above patients were 0.32%, 10.7%, and 1.3%, respectively. The two first patients were HIV co-infected and the other was HIV-negative under PrEP. The anti-HCV treatment was successful in the three patients.

Conclusion: This work evidenced uncommon mixed HCV GT infections in MSM at high risk of multiple HCV exposures. Their impact on treatment response has not been established but further studies on more patients are necessary. To prevent treatment failure in this population, regular monitoring of treatment response is needed, particularly when pan-genotypic treatment is not used.

Keywords: mixed HCV genotype infections, deep sequencing, men who have sex with men, recent HCV infection

INTRODUCTION

Although current treatments of HCV infection especially pan-genotypic direct-acting antivirals (DAAs) allow a high rate of sustained virological response (SVR) [1], some failures are still observed, e.g. in case of HCV genotype (GT) 3 infection [2]. Mixed HCV genotype (GT) infections (infection with two or more HCV GTs) [3] are still a clinical concern as HCV of different GTs have different sensitivities to current GT-specific DAAs. The observed prevalence of mixed HCV GT infections ranges from 14% to 39% in people who inject drugs depending on the sensitivity of methods used [3–6]. The prevalence is high in this population mostly due to their high-risk behaviors such as ongoing injection and needle sharing. As well, the prevalence of mixed HCV GT infections in men having sex with men (MSM) at high risk of multiple HCV exposures may probably be high. However, few data about the mixed HCV GT infections are available in this population. To the best of our knowledge, a few documented case reports of superinfection defined as detection of different HCV strains after the persistent infection of primary HCV strains [3] were reported in HIV/HCV co-infected MSM via sexual transmission [7–9]. More profound knowledge about the prevalence of mixed HCV GT infections in this community could help to establish an optimized strategy for surveillance, diagnostics, and treatment regimen. Ultra-deep sequencing (UDS) allows detecting minority viral population down to 1%, which is suitable for an extensive analysis of complex viral populations. In this study, we aimed to investigate by UDS the prevalence of mixed HCV GT infections in a population MSM with high-risk behaviors who were recently diagnosed with HCV infection.

MATERIALS AND METHODS

Study design and patients

Pre-treatment plasma samples within the period defined as recent HCV infection were collected from 55 patients (50 HIV-positive and 5 HIV-negative), followed at the Pitié-Salpêtrière, Saint-Antoine and Tenon hospitals, Paris, France and 13 HIV-negative patients from the ANRS IPERGAY study (Intervention for prevention of HIV acquisition by antiretroviral therapy for PrEP among gay men at high risk of HIV-1 infection) [10,11]. The 55 patients followed at the three hospitals were previously enrolled in the recently published study using Sanger sequencing technique and addressing HCV transmission and associated sexually transmitted infection issues in this population [12]. Overall, six patients were enrolled between July 2012 and December 2013 and 62 between March 2014 and May 2016.

The study was carried out in accordance with the Declaration of Helsinki. This work was a retrospective non-interventional study with no addition to standard care procedures. Reclassification of biological remnants into research material after completion of the ordered virological tests was approved by the local interventional review board of Pitié-Salpêtrière hospital. According to the French Public Health Code (CSP Article L.1121-1.1) such protocols are exempted from individual informed consent.

Recent HCV infection was defined as a positive serology test and/or a positive HCV viral load (VL) associated with a negative HCV serology within the previous 12 months, or a positive HCV VL beyond 24 weeks of a successful treatment or spontaneous clearance with modification of genotype. Furthermore, patients with a positive HCV VL with increase of alanine aminotransferase (ALAT) ≥10 upper limit of normal without any other etiology of hepatitis, or a positive HCV VL beyond 24 weeks of a successful treatment or spontaneous

clearance without modification of genotype were also enrolled and considered as possible recent HCV infections.

Extraction, amplification, and deep-sequencing

HCV RNA were extracted from 1 mL plasma using NucliSENS® easyMAG® (bioMérieux Clinical Diagnostics) and the NS5B fragment of 388 bp (8256 to 8644) was reverse-transcribed and amplified by PCR in a one-step process (Superscript III One-step RT-PCR with platinum Taq kit; Invitrogen, USA) according to the manufacturers' protocol by 2 pan-genotypic primers Forward: 5'-*A*TATGAYACCCGCTGYTTTGACTC-3' and Reverse: 5'-GCNGARTAYCTVGTCATAGCCTC-3'. Multiplexed samples were pooled and subjected to standard Illumina Miseq paired-end sequencing at 2x250 bp.

UDS data analysis

UDS data were analyzed by Geneious software (version 10.3.2, http://www.geneious.com) [13]. Paired reads were firstly merged, primer-removed and quality-trimmed. Sequences of good quality were error-corrected by BBNorm from the BBtools package included in Geneious. Corrected reads of each sample were clustered by *de novo* assembly approach at 90% of similarity where almost all reads were assembled. All contigs and unassembled reads were aligned to a reference sequence corresponding to the predominant subtype with maximum mismatches allowed per reads depending on the intra-genotype variability (according to the literature, 17% of maximum mismatches for GT1, 18% for GT2 samples, 20% for GT3 and 16% for GT4) [14]. Sequences unable to map to the reference were put aside and their subtypes were verified by Geno2Pheno (available at https://www.geno2pheno.org/) [15]. When their subtypes were different with the predominant subtype, these sequences were considered either mixed infections or contaminations. Suspected contaminations were detected by building phylogenetic trees using FastTree [16] (General Time Reversible model, available at

http://www.microbesonline.org/fasttree/#Install) with viral sequences of the other samples in the same experiment. If the genetic distance among them was superior to 3%, we considered these sequences as mixed infections. If else, we suspected contaminations.

RESULTS

Sequencing results and patients' characteristics

A median of 2389 sequences (interquartile range (IQR): 1851-2960) per sample was obtained after quality trimming step. The median age of patients was 38.5 years (IQR: 30.5-46.0); the median of HCV viral load was 5.9 log IU/ml (IQR: 5.3-6.6); and the median value of ALAT was 320.0 IU/L (IQR: 146.5-535.5). Most of them were MSM (85.3%) and the others were reported with unknown sexual orientation. HCV genotyping by Sanger sequencing showed GT1a, GT4d, GT3a, and GT2k infection in 47.1%, 41.2%, 8.8%, and 2.9% of patients, respectively. Fifteen patients (22.1%) experienced HCV reinfections and three (4.4%) were possible recent HCV infections. HIV-coinfection was found in 50 patients (75.3%) with a median of 673 CD4 cells/mm^3 (IQR: 531-873, available data on 25 patients). Five among them had a detectable HIV-RNA level (> 50 copies/mL) for reasons of antiretroviral therapy (ART) absence (n=1), loss of follow-up (n=1), resistance to the received tenofovir/emcitritabine/raltegravir (n=1), viral blips (n=1), and no resistance to the received ART but suppression of the replication after treatment intensification (n=1). Sexually transmitted infections were detected in 15 patients (22.1%) $\leq$ 1 month before recent HCV infection diagnosis (seven *Chlamydia trachomatis*, eight *Treponema pallidum*, two *Neisseria gonorrhoeae*). HCV infection mainly occurred in a context of high-risk sexual behaviours (unprotected anal sex) and frequently associated with recreative drug use. Patient characteristics are presented in table 1.

Mixed HCV genotype infections

After eliminating suspected contaminations as described in the method section, three (4.4%) mixed GT infections were detected. All the three patients were infected by HCV for the first time. Two patients were co-infected by HIV and the other was HIV negative and enrolled in the ANRS IPERGAY trial.

In detail, a mixed HCV GT infection between predominant GT4d (at frequency of 99.68%) and minority GT1a (at frequency of 0.32%) was detected in the viral population of one HIV-positive patient. The patient was treated by 6 months of peginterferon alfa-2a/ribavirine in 2013 and obtained undetectable HCV VL after one month. His HCV viral load remains undetectable during the 5 years of follow-up.

In the viral population of the second patient co-infected with HIV, another mixed infection between predominant GT4d (at frequency of 89.3%) and minority GT1b (at frequency of 10.7%) was identified. This patient was treated later by 12 weeks of sofosbuvir and ledipasvir. The HCV viral load was undetectable 9 months after the end of treatment.

The third mixed infection between predominant GT1a (at frequency of 98.7%) and minority GT4d (at frequency of 1.3%) was detected in the viral population of a HIV-negative patient under PrEP. Interestingly, a switch of virus from GT1a to GT4d was observed by Sanger sequencing in this patient two years later. The comparison among anterior minority GT4d sequences obtained from UDS with posterior GT4d sequence obtained from Sanger sequencing showed a 2% of minimum genetic distance among these sequences. At the time of HCV GT4d infection diagnosis, the patient was treated by 12 weeks of sofosbuvir and ledipasvir and obtained an undetectable HCV viral load after 2 months. However, the patient did not continue his follow-up in the hospital so we could not obtain more details about the SVR post-treatment.

An example of phylogenetic tree constructed from viral sequences of a mixed infection between predominant GT4d and minority GT1b virus is shown in figure 1 (the second patient). This patient was possibly infected with multiple minority transmitted GT1b viruses.

DISCUSSION

In our study, a low prevalence (4.4%) of mixed HCV GT infections was observed in a population of MSM with high-risk behaviors who were recently diagnosed with HCV infection. The prevalence of mixed HCV GT infections varies depending on the study population and the technique sensitivity. Indeed, a study using UDS showed the low prevalence of mixed HCV GT infection at 1.7% in 76 seronegative, HCV-RNA positive blood donors while a higher prevalence ranging from 14%-39% of mixed HCV GT infections was reported in people who inject drugs with both chronic and acute hepatitis C [4,17]. In our study, the prevalence of mixed HCV GT infections was investigated by UDS in a population of patients at high risk of multiple HCV exposures, HIV+ and HIV- MSM at high risk of HIV acquisition. Among 68 patients enrolled, only three (4.4%) were infected with HCV of mixed GTs involving GT4 and GT1 with frequencies of minority viral populations ranging from 0.32% to 10.7%. Interestingly, a switch from GT1a to GT4d virus (based on Sanger sequencing) after 2 years was observed in a patient previously infected by predominant GT1a and minority GT4d virus (based on UDS). However, the actual 2% minimum genetic distance among the previous minority GT4d sequences obtained from UDS and the later GT4d sequence from Sanger sequencing could not distinguish if the same virus emerged, or a different virus was contracted.

Of note, two patients were HIV co-infected and the other was included in a PrEP program (the IPERGAY trial). A concurrent mixed HCV GT infection is associated with faster immunological progression and faster clinical progression in patients co-infected with HIV if they are not treated effectively with antiretrovirals [5]. Moreover, mixed HCV GT infections

possibly impact the treatment outcome of GT-specific DAAs [18,19]. However, in this study, the three patients with mixed infections obtained virological success under anti-HCV treatment. It is not surprising because the minority GT and the predominant GT viral populations involving GT1 (GT1a and GT1b) and GT4 virus have equivalent susceptibility to anti-HCV treatment (either by sofosbuvir/ledipasvir or peginterféron alfa-2a/ribavirine). Indeed, a study on 335 patients co-infected with HIV-1 and HCV GT1 (GT1a and GT1b) or GT4 who received sofosbuvir/ledipasvir showed similar SVRs across different HCV GTs [20]. It is probably the reason why no deleterious impact on treatment response has been expressed in the three cases of mixed GT infections in this study.

In this study, a strict cut-off of 3% of genetic distance was used to eliminate contamination from PCR or sequencing steps. Only sequences of a sample with genetic distance greater than 3% compared to sequences of other samples in the same experiment were considered as mixed infections. This cut-off is quite strict, which may underestimate the mixed GT infection rate in our study. Indeed, another mixed GT infection between predominant GT3a and minority GT1a virus was detected if using a cut-off less strict at 1% of genetic distance. In this study, we identified only mixed infections of different GT viruses while mixed infections of different subtype viruses in the same GT are possible. Therefore, further studies using different analysis approaches will be interesting to address this question.

In conclusion, we observed a low prevalence of 4.4% of mixed HCV GT infections in a population of MSM at high-risk behaviors with recent HCV infection. Determining HCV genotype becomes less clinically significant with the introduction of pan-genotypic DAAs. However, these treatments are still not globally available and affordable, especially in resource-limited countries. The impact of mixed HCV genotype infections has not been established in this study. It should be noted that the study population involved a small group of MSM in a specific area (Paris) and treatment success of patients with mixed HCV GT infections was

limited to only three patients. From a public health perspective, the MSM population engaging in high-risk behaviors still requires special attention in terms of mixed infections as pointed above compared to the general HCV-infected population with a regular monitoring of anti-HCV treatment response particularly when pan-genotypic treatment is not used.

References

[1] Pol S, Parlati L. Treatment of hepatitis C: the use of the new pangenotypic direct-acting antivirals in “special populations.” Liver Int Off J Int Assoc Study Liver 2018;38 Suppl 1:28–33. doi:10.1111/liv.13626.

[2] Foster GR, Afdhal N, Roberts SK, Bräu N, Gane EJ, Pianko S, et al. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. N Engl J Med 2015;373:2608–17. doi:10.1056/NEJMoa1512612.

[3] Pham ST, Bull RA, Bennett JM, Rawlinson WD, Dore GJ, Lloyd AR, et al. Frequent multiple hepatitis C virus infections among injection drug users in a prison setting. Hepatol Baltim Md 2010;52:1564–72. doi:10.1002/hep.23885.

[4] Cunningham EB, Applegate TL, Lloyd AR, Dore GJ, Grebely J. Mixed HCV infection and reinfection in people who inject drugs--impact on therapy. Nat Rev Gastroenterol Hepatol 2015;12:218–30. doi:10.1038/nrgastro.2015.36.

[5] van Asten L, Prins M. Infection with concurrent multiple hepatitis C virus genotypes is associated with faster HIV disease progression. AIDS Lond Engl 2004;18:2319–24.

[6] van de Laar TJW, Molenkamp R, van den Berg C, Schinkel J, Beld MGHM, Prins M, et al. Frequent HCV reinfection and superinfection in a cohort of injecting drug users in Amsterdam. J Hepatol 2009;51:667–74. doi:10.1016/j.jhep.2009.05.027.

[7] Ghosn J, Thibault V, Delaugerre C, Fontaine H, Lortholary O, Rouzioux C, et al. Sexually transmitted hepatitis C virus superinfection in HIV/hepatitis C virus co-infected men who have sex with men. AIDS Lond Engl 2008;22:658–61. doi:10.1097/QAD.0b013e3282f4e86f.

[8] Chung E, Ferns RB, He M, Rigatti R, Grant P, McCormick A, et al. Ultra-deep sequencing provides insights into the virology of hepatitis C super-infections in a case of three sequential infections with different genotypes. J Clin Virol Off Publ Pan Am Soc Clin Virol 2015;70:63–6. doi:10.1016/j.jcv.2015.06.105.

[9] Loulergue P, Mir O, Sogni P. Super-infection with genotype 4 hepatitis C virus in a patient treated for genotype 3 acute hepatitis C. AIDS Lond Engl 2012;26:655–6. doi:10.1097/QAD.0b013e3283519397.

[10] Molina J-M, Capitant C, Spire B, Pialoux G, Cotte L, Charreau I, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. N Engl J Med 2015;373:2237–46. doi:10.1056/NEJMoa1506273.

[11] Molina J-M, Charreau I, Spire B, Cotte L, Chas J, Capitant C, et al. Efficacy, safety, and effect on sexual behaviour of on-demand pre-exposure prophylaxis for HIV in men who have sex with men: an observational cohort study. Lancet HIV 2017;4:e402–10. doi:10.1016/S2352-3018(17)30089-9.

[12] Todesco E, Day N, Amiel C, Elaerts S, Schneider V, Roudiere L, et al. High Clustering of Acute HCV Infections and High Rate of Associated STIs Among Parisian HIV-Positive Male Patients. Int J Antimicrob Agents 2019. doi:10.1016/j.ijantimicag.2019.02.002.

[13] Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinforma Oxf Engl 2012;28:1647–9. doi:10.1093/bioinformatics/bts199.

[14] Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatol Baltim Md 2014;59:318–27. doi:10.1002/hep.26744.

[15] Kalaghatgi P, Sikorski AM, Knops E, Rupp D, Sierra S, Heger E, et al. Geno2pheno[HCV] - A Web-based Interpretation System to Support Hepatitis C

Treatment Decisions in the Era of Direct-Acting Antiviral Agents. PloS One 2016;11:e0155869. doi:10.1371/journal.pone.0155869.
[16] Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. PloS One 2010;5:e9490. doi:10.1371/journal.pone.0009490.
[17] Janiak M, Caraballo Cortés K, Perlejewski K, Kubicka-Russel D, Grabarczyk P, Demkow U, et al. Next-Generation Sequencing of Hepatitis C Virus (HCV) Mixed-Genotype Infections in Anti-HCV-Negative Blood Donors. Adv Exp Med Biol 2018. doi:10.1007/5584_2018_190.
[18] Abdelrahman T, Hughes J, Main J, McLauchlan J, Thursz M, Thomson E. Next-generation sequencing sheds light on the natural history of hepatitis C infection in patients who fail treatment. Hepatol Baltim Md 2015;61:88–97. doi:10.1002/hep.27192.
[19] Del Campo JA, Parra-Sánchez M, Figueruela B, García-Rey S, Quer J, Gregori J, et al. Hepatitis C virus deep sequencing for sub-genotype identification in mixed infections: A real-life experience. Int J Infect Dis IJID Off Publ Int Soc Infect Dis 2018;67:114–7. doi:10.1016/j.ijid.2017.12.016.
[20] Naggie S, Cooper C, Saag M, Workowski K, Ruane P, Towner WJ, et al. Ledipasvir and Sofosbuvir for HCV in Patients Coinfected with HIV-1. N Engl J Med 2015;373:705–13. doi:10.1056/NEJMoa1501315.

CONFLICT OF INTEREST

All authors do not have any conflicts of interest to declare.

ACKNOWLEDGEMENTS

We would like to thank all the patients who agreed to participate in the study, all the participant doctors who followed the patients, Drs. L.ROUDIERE, JY.LIOTIER, D.GOSSET, B.CARDON, JP.GRIVOIS, M.KIRSTETTER, F.LAYLAVOIX, J.BOTTERO, L.WORMSER and Pr. C.KATLAMA and Dr. V.SCHNEIDER, virologist.

We thank the INSERM SC10 and the Trial Scientific Committee for the IPERGAY trial.

We thank ANRS AC43 Next Generation Sequencing and STIs working groups for their support.

311 **Table 1:** Patient characteristics

Characteristics	**Total (n=68)**	**HIV-positive patients (n=50)**	**HIV-negative patients (n=18)**
Age (years), median (IQR)	38.5 (30.5-46.0)	42.5 (34.5-46.0)	32.0 (27.5-35.8)
Men having sex with men, n (%)	58 (85.3)	43 (86.0)	15 (83.3)
Unknown sexual orientation, n (%)	10 (14.7)	7 (14.0)	3 (16.7)
HCV viral load, log IU/ml, median (IQR)	5.9 (5.3-6.7)	5.9 (5.3-6.9)	5.5 (5.3-5.6)
HCV genotype			
➢ Genotype 1a, n (%)	32 (47.1)	24 (48.0)	8 (44.4)
➢ Genotype 4d, n (%)	28 (41.2)	20 (40.0)	8 (44.4)
➢ Genotype 3a, n (%)	6 (8.8)	5 (10.0)	1 (5.6)
➢ Genotype 2k, n (%)	2 (2.9)	1 (2.0)	1 (5.6)
ALAT (IU/L), median (IQR)	320.0 (146.5-535.5)	315.0 (144.8-480.8)	467.0 (234.0-647.0)
HIV co-infection (%)	50 (73.5)	50 (100.0)	0 (0.0)
Number of patients with detectable HIV-RNA, n (%)	N/A	5 (10.0%)	N/A
CD4 count (cells/mm^3), median (IQR)	N/A	673.0 (531.0-873.0)	N/A
Number of patients with STIs*, n (%)	15 (22.1)	10 (18.2)	5 (27.8)
HCV reinfection (%)	15 (22.1)	14 (28.0)	1 (5.6)

312 IQR: Interquartile range, ALAT: ALanine AminoTransferase, *: sexually transmitted infections
313 detected less than 1 month before recent hepatitis C diagnosis, N/A: not applicable

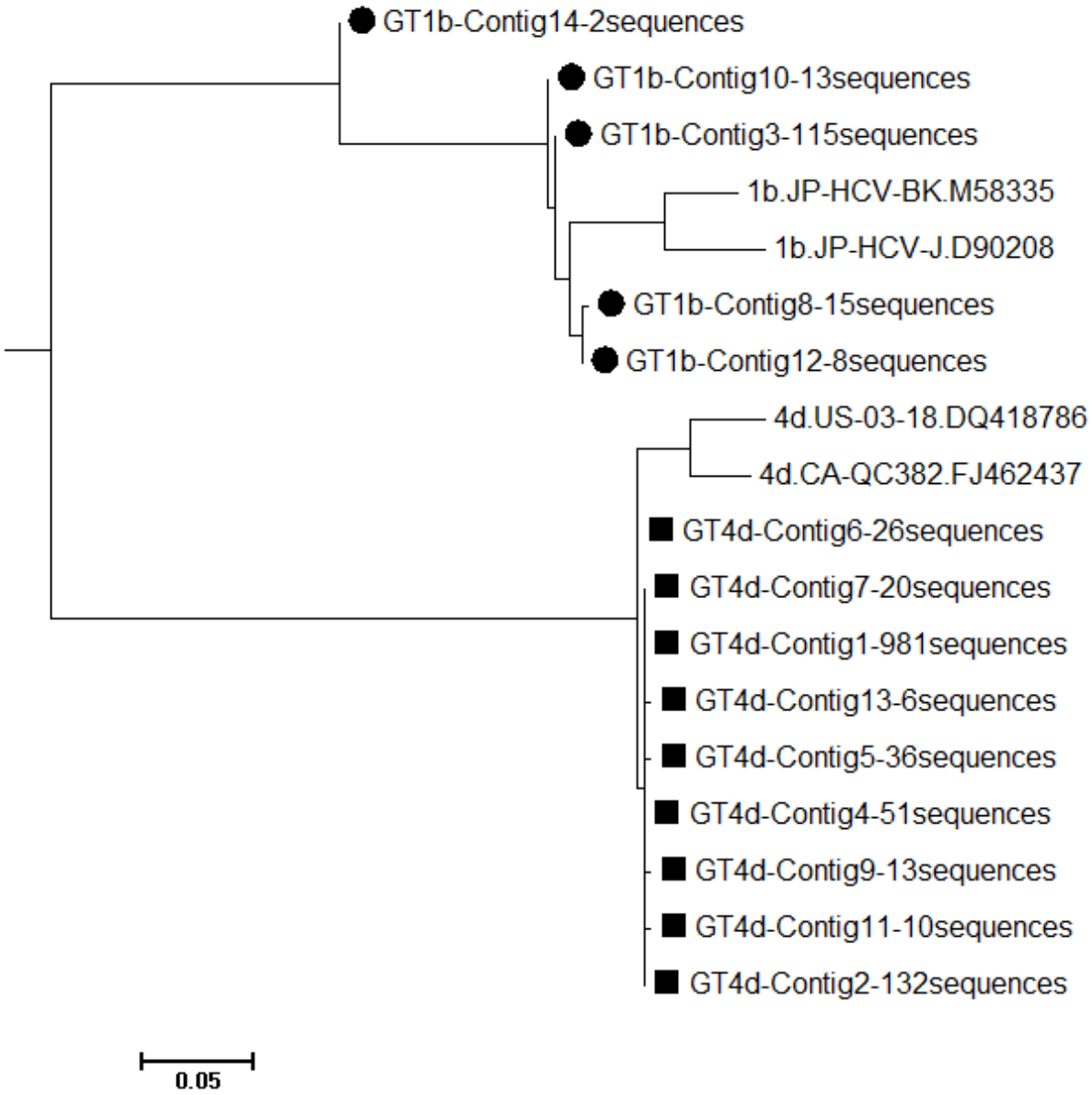

Figure 1: Phylogenetic tree constructed from UDS contig sequences of individual with mixed HCV genotype (GT) infection between predominant GT4d and minority GT1b and reference sequences of GT4d and GT1b virus from Los Alamos HCV database (accession number in their names). Viral sequences of patients are marked with shape (black square for GT4d and black circle for GT1b virus). Number of sequences assembled in each contig is also presented in the taxon's name.