Virus Research 318 (2022) 198847

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Genetic diversity of hepatitis C virus and resistance associated substitutions to direct-acting antiviral treatment in Colombia

Maria C. Lopez-Osorio [a], José Aldemar Usme-Ciro [b], José William Martínez [c], Dioselina Peláez-Carvajal [d], Javier Hernández [e], Sergio Hoyos [a,f], Juan Carlos Restrepo [a,f], Maria-Cristina Navas [a,*]

[a] Grupo de Gastrohepatología, Facultad de Medicina, Universidad de Antioquia, UdeA. Calle 70 No. 52-21, Medellín, Colombia
[b] Centro de Investigación en Salud Para el Trópico-CIST, Facultad de Medicina, Universidad Cooperativa de Colombia, Santa Marta 470003, Colombia
[c] Facultad de Medicina, Universidad Tecnológica de Pereira, Cra. 27 #10-02, Pereira, Colombia
[d] Instituto Nacional de Salud, Avenida 26 No. 51-20, Zone 6, Bogota, Colombia
[e] Facultad de Medicina, Universidad del Magdalena, Cra. 32 No. 22 – 08, Santa Marta, Colombia
[f] Hospital Pablo Tobón Uribe, Cl. 78b #69-240 Antioquia, Medellín, Colombia

ARTICLE INFO

Keywords:
Hepatitis C virus
Infection
Epidemiology
Genetic diversity
Evolution

ABSTRACT

Hepatitis C virus (HCV) infection is one of the leading risk factors for end-stage liver disease development worldwide. This RNA virus displays high genetic diversity with 8 genotypes and 96 subgenotypes with heterogeneous geographical distribution around the world. In this study, we carried out an active case finding of individuals with a history of transfusion events before 1996 in three cities in Colombia. Then, the characterization of the HCV genotypes, subgenotypes, and resistance associate substitutions (RAS) was performed in samples positives for antibodies anti-HCV + from this study population. In addition, samples from PWID and patients with end-stage liver disease submitted to liver transplantation were included in the phylogenetic and RAS analysis. The 5′UTR, NS5A, and NS5B regions of the HCV genome were amplified in serum or liver explants samples. After the edition, assembly, and alignment of the sequences, genotyping through phylogenetic analysis was performed using IQTREE V2.0.5 based on the maximum likelihood approach. The identification of RAS was carried out by alignments based on the reference sequence (GenBank NC_004102). Two hundred sixty individuals with blood transfusion events before 1996 were recruited. The seroprevalence of antibodies anti-HCV was 2.69% in this population. The HCV genotypes 1, 2, and 4 and subgenotypes 1a, 1b, 2a, 4a and 4d were characterized in samples of the study populations. Three RAS (Q30R, C316N, and Y93H) were identified in samples obtained from 2 individuals who received blood transfusion before 1996 and without previous antiviral treatment and 6 samples obtained from patients with end-stage liver disease. Among the 20 samples analyzed, the HCV genotype 1, subgenotype 1b, was the most frequent (60%). We report the first characterization of HCV subgenotypes 4a and 4d and the first RAS identification in patients in Colombia.

1. Introduction

The Hepatitis C Virus (HCV) infection is a major public health problem and one of the leading etiologies of end-stage liver disease. According to the World Health Organization (2021a), 58 million people have chronic hepatitis C worldwide

HCV is classified into the family *Flaviviridae,* genus *Hepacivirus,* species *Hepacivirus C* (Walker PJ et al, 2020).The viral genome is a single positive-sense RNA encoding a polyprotein of 3000 amino acids (aa), approximately. This polyprotein is processed by cellular and viral proteases into three structural proteins (Core, E1, and E2) and seven non-structural proteins (P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Manns et al., 2017; Moradpour and Penin, 2013). HCV displays high genetic diversity as a result of its error-prone RNA-dependent RNA

Abbreviations: WHO, World Health Organization; HCV, Hepatitis C Virus; DAA, Direct Acting Antiviral; RAS, resistance associate substitutions; SVR, sustained virologic response; PWID, people who inject drugs; UTR, untranslated region; IFN, interferon.

* Corresponding author.
E-mail address: maria.navas@udea.edu.co (M.-C. Navas).

https://doi.org/10.1016/j.virusres.2022.198847
Received 5 May 2022; Received in revised form 6 June 2022; Accepted 7 June 2022
Available online 10 June 2022

polymerase, NS5B protein, and the turnover of replication complex (Chaiwong and Sistayanarain, 2016). Based on the phylogenetic analysis of the nucleotide sequences, HCV is classified in eight genotypes and 96 subgenotypes that differ from each other by more than 30% and between 15 and 25%, respectively (Hedskog et al., 2019, p. 19; Simmonds et al., 2005; Smith et al., 2014). HCV genotype 1 is the most prevalent worldwide followed by genotypes 3 and 4 (Gower et al., 2014; Manns et al., 2017; Petruzziello et al., 2016).

Several studies demonstrated viral clearance and sustained virologic response (SVR) in > 95% of patients with chronic HCV infection using direct-acting antiviral (DAA) treatment (Piecha et al., 2020). Viral proteins NS5A, and NS5B are the main targets of DAA to block HCV replication and consequently to achieve the viral clearance (Bhattacharjee et al., 2021; Piecha et al., 2020). Indeed, DAA therapy and active case finding are strategies of the WHO plan for the goal of viral hepatitis elimination as a public health problem by 2030 (World Health Organization, 2021b). In Colombia, the DAA therapy is available through the centralized purchase of the Ministry of Health and Social Protection for all patients with a diagnosis of HCV infection (Ministerio de Salud y Protección Social, 2020).

However, failure in patients treated with DAA has been described in a low proportion of patients, related to different factors including the viral escape to antivirals due to resistance-associated substitutions (RAS). Nevertheless, the relevance of RAS on reduced efficacy of DAA depends on baseline mutational background, viral genotype and treatment regimens (Aldunate et al., 2018; Kai et al., 2017; Li and Chung, 2019a).

The aims of this study were to carried out an active case finding in three cities in Colombia of individuals with history of transfusion events before 1996 to identify cases of HCV infection and therefore candidates for DAA treatment. Moreover, to characterize the HCV genotypes, subgenotypes, and RAS in samples obtained from individuals with blood transfusion and positives for antibodies anti-HCV +, samples from PWID and from patients with end-stage liver disease.

2. Materials and methods

2.1. Ethical statement

This study was approved by the ethical committee of the Universidad de Antioquia (SIU, UdeA) and was conducted following the Declaration of Helsinki and Colombian legislation (Ministerio de Salud y Protección Social 1993). All participants were informed about the protocol and study objective and then, the informed consent was obtained. The collected biological material was codified to ensure the privacy of the participants.

2.2. Study population

Active case finding of individuals with history of transfusion events before 1996 was conducted from July 2018 to January 2020 in three cities in Colombia: Medellín (the second largest city of the country), Pereira (Andean region), and Santa Marta (Caribbean coast). The case-finding strategy was based on a campaign in printed and digital media, meetings with community leaders and in health centers from each city.

In addition, two individuals who inject drugs (PWID) that were diagnosed by Hepatitis C rapid test in another study in 2019, and a patient with a diagnosis of chronic hepatitis C with IFN treatment failure in 2019 were invited to the study.

Moreover, liver tissues from end-stage liver disease patients submitted to liver transplantation between August 2002 and February 2008 at Hospital Pablo Tobon Uribe were included in the study. None of the transplant donors were from vulnerable population.

2.2.1. Blood collection

Blood samples were collected, centrifugated and sera stored at –70°C. Serum samples were tested in duplicate for HCV antibodies using a commercial kit (Murex anti-HCV version 4.0, DiaSorin). The fourth-generation enzyme immunoassay contains purified antigens corresponding to Core, NS4 and NS5 sequences of HCV genome. All participants were informed of the anti-HCV test results (positive or negative) by email and/or phone.

2.3. HCV RNA extraction and reverse-transcription polymerase chain reaction (RT-PCR)

Viral RNA was isolated from serum/plasma samples (QIAamp Viral RNA Mini. QIAgen, Netherlands) following the manufacturer's instructions. Furthermore, total RNA was extracted from 13 liver tissue samples using TRIzol Reagent (Invitrogen).

The 5' untranslated region (UTR) of the viral genome was detected by RT-nested PCR as described previously by Chan et al. (1992). Briefly, the cDNA synthesis reaction was performed at 37°C for 1 h with M-MLV reverse transcriptase (Invitrogen, USA) using the primer HCV-209 (TCGAGGTGCACGGTCTACGAGACCT nt 299–327). Then, the first PCR was performed using the primers HCV-209 (TCGAGGTGCACGGTCTACGAGACCT nt 299–327) and HCV-939 (CTGTGAGGAACTACTGTCTT nt 23–42). The nested PCR was carried out using the primers HCV- 211 (ACTCTCGAGCACCCTATCAGGCAGT nt 266–292) and HCV-940 (TTCACGCAGAAAGCGTCTAG nt 41–60) as previously described (di Filippo et al., 2012a).

The cDNA synthesis for NS5A region was performed using random primers and conditions as described above. The PCR amplification was carried out using the primers for genotype 1, subgenotypes 1a and 1b, and conditions described by Aldunate et al. (2018) (HCV1a_Fw1 (GACRTYTGGGACTGGATATGCG nt 6276-6297), HCV1a_Rev1 (GCTCRATGTCYTAYWCCTGGAC nt 7600-7621), HCV1b_Fw1 (GGATYAAYGARGACTGYTCYAC nt 6177-6198) and HCV1b_Rev1 (GACCARGAMCCGTCRCTGAGRT nt 7485 - 7506). For nested PCR, primers HCV1a_Fw2 (GATATGCGAGGTGYTGAGCG nt 6290-6310), HCV1a_Rev2 (GAGCARCACACGACRTCYTC nt 7584-7603), HCV1b_Fw2 (GGGAYTGGATATGYACGGT nt 6231-6249) and HCV1b_Rev2 (GGCATGGAGGARWAYGAC nt 7438–7455) were used (Aldunate et al., 2018).

Two sets of primers targeting the NS5B region of HCV genome were used as described by Sulbarán et al. (2010). The cDNA synthesis was carried out using the reverse primer 8645n (GGCGGAATTCCTGGTCATAGCCTCCGTGAA nt 8616-8645). The PCR was performed using the primers 8645n and 8245p (TGGGGATCCCGTATGATACCCGCTGYTTYGA nt 8245-8275). Then, the semi nested PCR was carried out using the primers 8645n and 8276p (CTCCACAGTCACTGAGAGCGACAT nt 8276-8299) (Sulbarán et al., 2010).

The amplified PCR products were analyzed by agarose gel electrophoresis.

2.4. Sequence handling and phylogenetic analysis

Nucleotide sequences of amplicons in both directions were determined by automatic dideoxy-sequencing (Macrogen, Inc. Korea). Sequences were assembled and primer-trimmed through the BioEdit 7.2 and Geneious assembler (software Geneious v.9.1.8) (Hall T, 1999).

The multiple sequence alignment of the different datasets was carried out using the Muscle algorithm implemented in the MEGA 7 software (Hall T, 1999; Kumar et al., 2016).

Phylogenetic analysis of 5' UTR and NS5B regions was carried out to identify the viral genotype and subgenotype. While aa sequence analysis of NS5A and NS5B regions was performed for RAS identification.

Phylogenetic trees based on the different datasets were constructed with Bayesian probability model using BEAST 1.8.4. After running of 50 million generations, a value that allowed reaching an Effective Sample

Size (ESS) above 200, following the ESS value in the Tracer v1.6 program.

A first dataset of 151 sequences representative of each HCV genotype obtained from GenBank was used for phylogenetic analysis (Supplementary tables 1 and 2). Sequences were selected based on worldwide representation of each genotype with special focus on the American continent. The HCV sequences selected belonged to Colombia, Argentina, Uruguay, Chile, Venezuela, Mexico, Brazil, United States, Canada, Turkey, Spain, Germany, France, England, Portugal, Cyprus, Russia Japan, India, China, Egypt among other countries. There were no genotype 4 sequences from Colombia available in public databases at the moment of the analysis.

For RAS analysis, wild-type amino acid sequence was defined according to reference sequence NC_004102 (GenBank accession number). The NS5A and NS5B sequences were aligned, and the RAS identification and frequency analysis were performed using BioEdit and MEGA 7 bioinformatic tools.

2.5. Data availability

The nucleotide sequences reported in this study were submitted to GenBank under the accession numbers: 5' UTR (ON093986 - ON094004), NS5A (ON123730 - ON123736) and NS5B (ON123710 - ON123729) (GenBank accession numbers).

3. Results

The active case finding population included 260 individuals with a history of blood transfusion before 1996 enrolled in three cities in Colombia, Medellin ($n = 159$), Pereira ($n = 43$), and Santa Marta ($n = 58$). Majority of participants were female (69.4%) and the median age was 56 years (range of 24–99 years). The more frequent indication for blood transfusion in men were surgery (22.5%), car accident (8.77%) and firearm injury (7.25%) while in women were postpartum hemorrhage (23.6%), surgery, including cesarean, (22.5%) and anemia (9.54%).

From total of samples obtained from individuals with history of blood transfusion before 1996, 7/260 (2,69%) were positive for anti-HCV antibodies (Table 1). The HCV genome was amplified in 4 of these anti-HCV positive samples. The four individuals were informed of the viral genome detection and genotype assays results by phone. Furthermore, the cases were notified to the Public Surveillance system (SIVIGILA) of the Colombian Ministry of Health and Social Protection.

Moreover, the HCV genome was amplified in 13 liver explant samples (Supplementary Table 3) and in 3 serum samples obtained from PWID and from the patient with chronic hepatitis C and with IFN treatment failure.

The phylogenetic analysis was performed using 151 HCV sequences, including 101 sequences from American countries. The phylogenetic trees generated for NS5B sequences showed the presence of HCV genotype 1 in 17 samples (85%): among them 12 corresponded to subgenotype 1b (02-155, 02-156, 04-006, 02-005, TH1b, TH9, TH14, TH15, TH16, TH17, TH29 y TH67) and 5 to subgenotype 1a (PID-02-007, PID-02-008, TH2, TH58, TH86). HCV genotype 2 (5%), subgenotype 2a, was identified in one sample (TH3) and HCV genotype 4 (10%), subgenotypes 4a and 4d, in samples TH7 and 03-041, respectively (Table 2, Figs. 1 and 2).

Finally, we identified RAS in 8 HCV sequences corresponding to 2 samples obtained from individuals with transfusion history and 6 samples from end-liver disease patients. Fig. 3a shows the amino acid alignment of HCV NS5A based on the reference sequence NC_004102 with the presence of the Q30R RAS in samples 02-156 and 04-006 from individuals with a transfusion history and in samples TH15, TH16 and TH17 from patients with a diagnosis of end-stage liver disease, and the Y93H RAS in sample TH16 (patient with a diagnosis of end-stage liver disease). Fig. 3b shows the amino acid alignment of HCV NS5B based on the reference sequence NC_004102 with the C316N RAS in samples 02-156, TH9, TH14, TH16 and TH17 (Table 3).

4. Discussion

After an active case finding in 3 cities in Colombia, the prevalence of anti-HCV described in individuals with a history of blood transfusion before 1996 was 2,69% (7/260). This frequency is lower than expected compared to the frequency of the HCV serological marker described previously in two studies carried out in Colombia in individuals with transfusion events before 1994 (seroprevalence of anti-HCV 6,6%) (Arroyave et al., 2014) and in multi-transfused individuals (seroprevalence of anti-HCV 9%) (Beltrân et al., 2005).

The low frequency of HCV infection in the study population described in this study (2,69%) could be related to the time frame after the transfusion and therefore the loss of antibodies two or three decades after acquiring the infection. Regarding the natural history of the

Table 2
HCV genotypes and subgenotypes identified in individuals with history blood transfusion, PWID and patients with end-stage liver disease in Colombia.

Sample code	Study Population	Genotype/ subgenotype
02-155	Individual transfused before 1996	1b
02-156		1b
03-041		4d
04-006		1b
02-055	Patient with chronic hepatitis C and failure of IFN treatment	1b
PID-02-007 PID-	PWID	1a
02-008		1a
TH1b	End-stage liver disease patient	1b
TH2		2a
TH3		1a
TH7		4a
TH9		1b
TH14		1b
TH15		1b
TH16		1b
TH17		1b
TH29		1b
TH58		1a
TH67		1b
TH86		1a

Table 1
Characteristics of individuals identified during the active case finding in population with history of blood transfusion before 1996 in 3 Colombian cities and markers of HCV infection.

Sample code	City	Age	Gender	Transfusion Indication	Transfusion Event Date	Antibodies anti-VHC	HCV genome
02-155	Medellín	66	F	Ectopic pregnancy	1986	+	+
02-156	Medellín	63	M	Non available	1968	+	+
02-157	Medellín	48	F	Surgery	1986	+	-
03-007	Pereira	53	F	Surgery	1990	+	-
03-012	Pereira	62	F	Postpartum hemorrhage	1986	+	-
03-041	Pereira	67	M	Car accident	1970	+	+
04-006	Santa Marta	59	F	Postpartum hemorrhage	1986	+	+

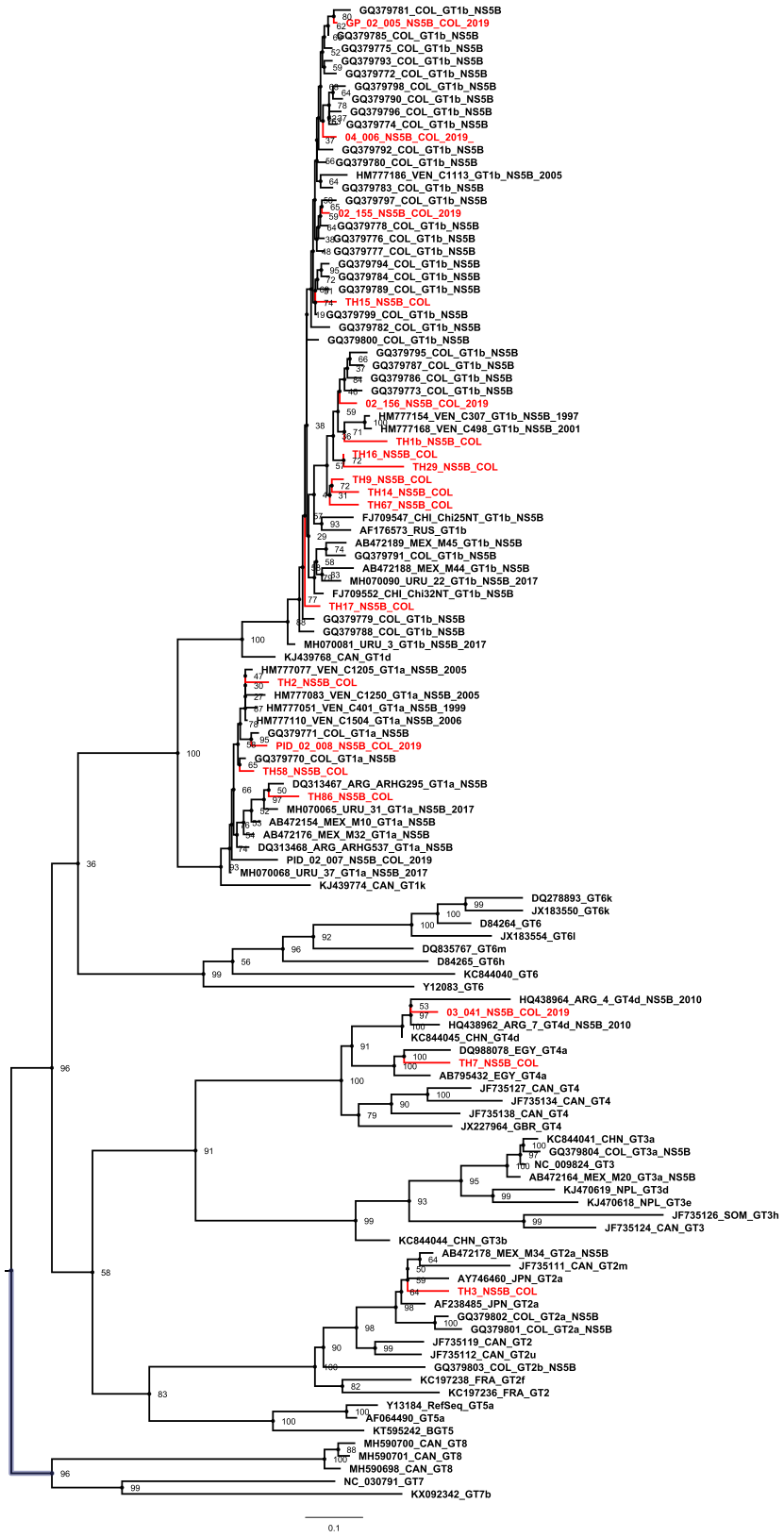

Fig. 1. Phylogenetic tree using the HCV NS5B region
Construction by maximum likelihood analysis in IQ-TREE version 2.1.2 using the F81+I+G nucleotide substitution model. The HCV sequences of this study appear in red. The sequences of HCV NS5B region from Colombia available in GenBank were included in the analysis (COL).

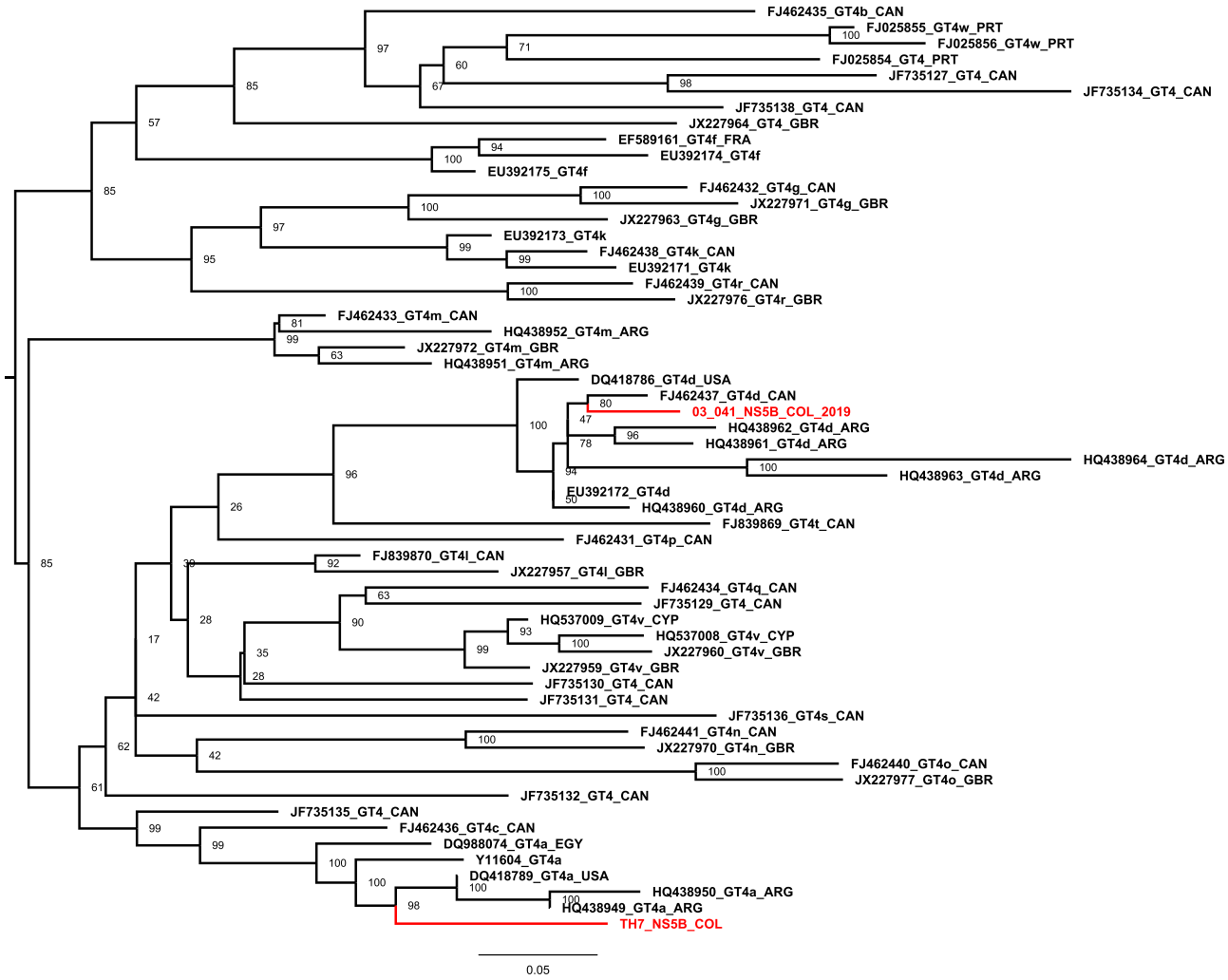

Fig. 2. Phylogenetic tree using the HCV NS5B region for genotype 4 subgenotypes
Construction by maximum likelihood analysis in IQ-TREE version 2.1.2 using the F81+I+G nucleotide substitution model. This tree was constructed using the genotype 4 reference sequences reported in the ICTV for classification of subgenotypes. The HCV sequences of this study appear in red corresponding to the sample from an individual with blood transfusion, subgenotype 4d, and to a liver explant sample from a patient undergoing transplants, genotype 4a.

infection, Wiese et al reported a slow progression of liver disease in people negative for anti-HCV antibodies, 20 years after acquiring the infection (Wiese et al., 2000).

Furthermore, the rate of viral genome detection (54.2%) on anti HCV + samples of this study population is low compared to the results of HCV genome detection frequency reported by Sookoian and Castaño (75.5%) in patients anti HCV + attended a Liver Unit and by Kermani et al (71.7%) in patients referred to a Blood transfusion research center (Sookoian and Castaño., 2002; Kermani et al., 2015).

However, Gómez-Escolar et al. (2018), Sirin et al. (2019) described frequencies similar to the present study (43.3% and 44.8% viral genome detection) in samples from adult general population and in samples from individuals with indications of HCV testing such as abnormal liver test, long-term hospitalization, surgeries, etc., respectively. One of the factors that determine the rate of the viral genome amplification is the limit of HCV RNA detection. Unfortunately, the lower limit of the 5' UTR RT-nested PCR is not available in the present study.

On the other hand, blood screening for transfusion-transmissible infections was implemented in Colombia since 1993. However, the 100% of blood screening in the blood banks of the country was achieved until 1995 (Ministerio de Salud y Protección Social, 1993; Wiese et al., 2000). Indeed, the study in multi-transfused patients demonstrated an important reduction of HCV prevalence in transfused patients between 1993 and 1995 (OR= 2,72, IC 95%) compared with patients transfused before 1993 (OR = 13,68 95% IC 6,20–0,86). Moreover, the reduction of HCV prevalence in patients transfused after 1995 was > 90%, in agreement with the 100% screening coverage of blood units at that time (Beltrán et al., 2005). However, according to the Colombian Minister of Health and Social Protection, HCV was transmitted through blood transfusion in the most of patients with known risk factors treated with DAA in 2019 (Ministerio de Salud y Protección Social, 2019). Although, in the cohort of treated patients in 2020, blood transfusion was the second most important risk factor after sexual transmission among the patients with known risk factors (Ministerio de Salud y Protección Social, 2020).

We report in total 12 HCV sequences in genotype 1, subgenotype 1b (02-155, 02-156, 04-006, 02-005, TH1b, TH9, TH14, TH15, TH16, TH17, TH29, and TH67) that clustered with sequences from Colombia and Mexico isolated from blood donors and collected between 2003–2007 and 2006–2007, respectively; these sequences were also closely related to sequences from Venezuela and Uruguay corresponding to anti-HCV positive samples collected between 1995–2004 and 2015–2017, respectively; and with sequences from Chilean patients with chronic hepatic diseases collected between 1997 and 2006.

In spite of the limited number samples of the present study, the phylogenetic analysis, including all HCV sequences from Colombia

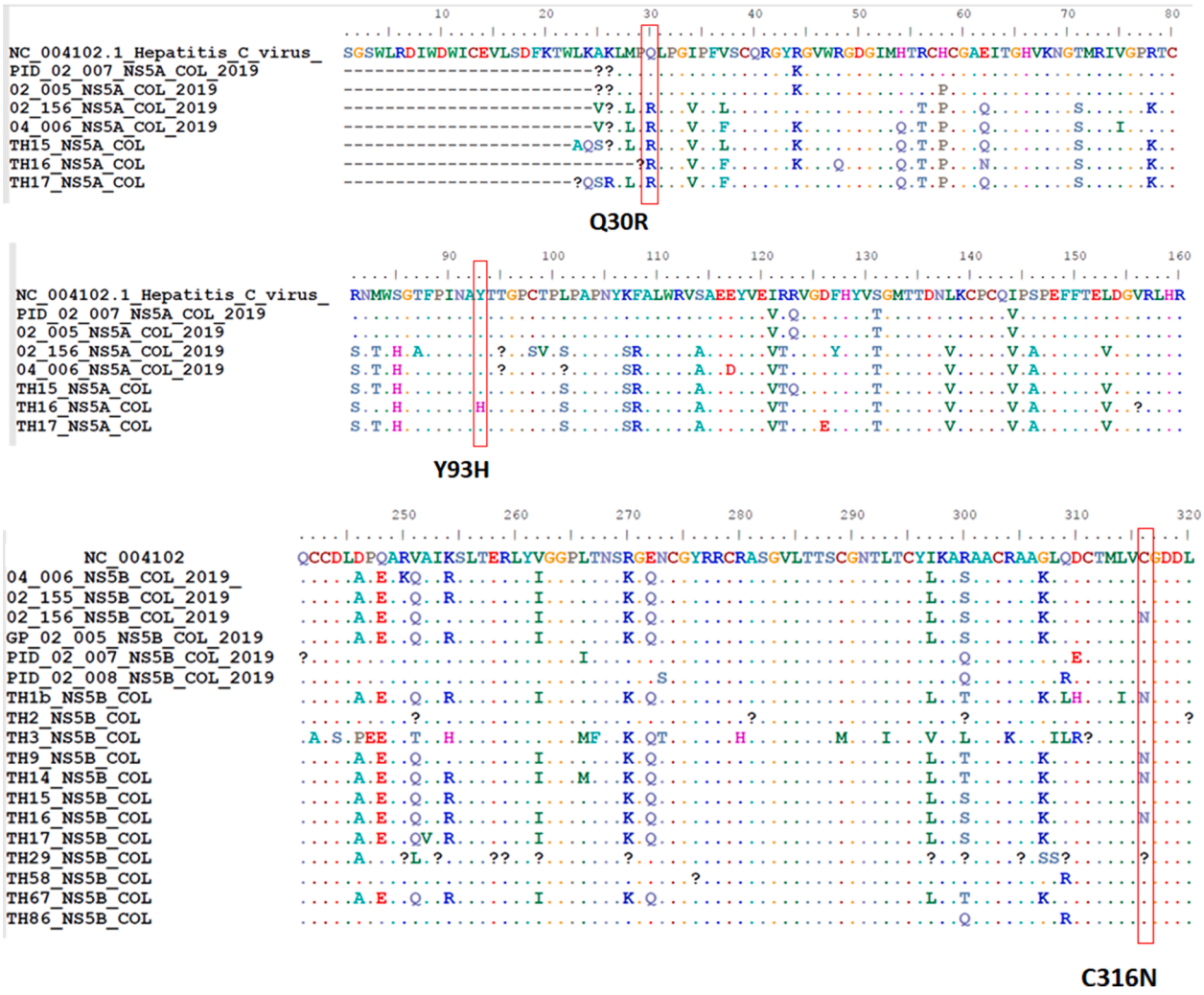

Fig. 3. Amino acid sequence analysis for the identification of RAS in the HCV genome
The alignment of the amino acid sequences of the HCV NS5A and NS5B proteins is observed. The RAS were delimited in the red boxes based on the substitutions reported in the literature. 3a. Alignment of amino acids of the NS5A protein. 3b. Alignment of amino acids of the NS5B protein.

Table 3
HCV Resistance Associated Substitutions (RAS) identified in the study populations in Colombia.

Sample code	Study group	RAS	Genotype/ subgenotype
02-156	Individual transfused before 1996	Q30R C316N	1b
04-006	Individual transfused before 1996	Q30R	1b
TH9	End-stage liver disease patient	C316N	1b
TH14	End-stage liver disease patient	C316N	1b
TH15	End-stage liver disease patient	Q30R	1b
TH16	End-stage liver disease patient	Q30R Y93H C316N	1b
TH17	End-stage liver disease patient	Q30R C316N	1b

available in databases, showed the prevalence of HCV genotype 1, subgenotype 1b, in the country and in the present study (60%). Indeed, the high frequency of this viral genotype had already described in Colombian individuals with blood transfusion history (Mora et al., 2010; Arroyave et al., 2014; di Filippo et al., 2012a) as well as other populations in American countries (Kershenobich et al., 2011; Manns et al., 2017; Santos et al., 2017; Zein et al., 1996, 2000).

In addition, the report of the cohort of Colombian patients under DAA treatment on 2019 described the predominance of the HCV genotype 1, subgenotype 1b (64.3%) among the cases with genotype characterization (Ministerio de Salud y Protección Social, 2019). However, the report of 2020 showed a decline on HCV genotype 1, subgenotype 1b, on the incident case of this cohort (22%) (Ministerio de Salud y Protección Social, 2020).

The HCV sequences obtained from PWID (PID-02-007 and PID-02-008) and end-liver disease patients (TH2, TH58 and TH86) were clustered with sequences from Colombian blood donors collected between 2003 and 2007, belonging to the HCV genotype 1, subgenotype 1a, and with sequences from Venezuela and Uruguay corresponding to anti-HCV positive samples collected between 1995–2004 and 2015–2017, respectively.

Regarding the HCV genotype 4, this is the first report of subgenotypes 4a and 4d in Colombia. Samples 03-041 and TH7 grouped with sequences from the United States of America, Canada, Argentina, China, and England. However, only a subset of these previously reported sequences provided metadata and corresponded to samples obtained from African immigrants residing in Quebec, Canada, collected between 2002 and 2007 (Cooper et al., 2020).

Santos et al. (2017), described the circulation of HCV genotype 4 in Colombia for the first time, in a retrospective study of serum samples obtained between 2003 and 2017 and forwarded to three reference

laboratories. The HCV genotype 4 was identified in 62/1538 samples (4%) using a commercial kit with specific probes for each genotype. However, demographic and epidemiologic data, as well as nucleotide sequences were not generated in the study. Interestingly, the prevalence of HCV genotype 4 has increased over time with a frequency of 31.9% in 2019 and 50% in among the cases with genotype characterization as reported by the cohort of patients under DAA treatment in the country (Ministerio de Salud y Protección Social, 2019; Ministerio de Salud y Protección Social, 2020).

Although, HCV genotype 4 was originally described in Egypt, its circulation is currently reported in North America, specifically in Canada (Cooper et al., 2020; Smith et al., 2014) and in Argentina, with circulation of subgenotypes 4a (22%), 4d (67%) y 4m (11%) since 2011 (Bolcic et al., 2011).

We identified the RAS Q30R and Y93H in the NS5A and C316N in the NS5B sequences. The Q30R mutation is located between the N-terminal region and the domain I of the NS5A protein, while the Y93H mutation is in the middle of domain I. These mutations on NS5A protein are related to decreased affinity of DAA (Bartenschlager et al., 2013; Li et al., 2019a). Li et al, have demonstrated that these mutations cause a high *in vitro* resistance (100–1000 more) compared to wild type virus (Li and Chung, 2019a). Some studies described baseline mutations before the DAA treatment, which rapidly increase in frequency once antiviral treatment is started; demonstrating that the selection pressure imposed by treatment on the HCV quasispecies allows the minor variants associated with resistance becoming dominants in the viral populations, and being responsible for the treatment failure (Kai et al., 2017; Sarrazin et al., 2016; Yamashita et al., 2020).

The mutation C316N was identified in NS5B genome region in the active site of the viral polymerase generating an allosteric inhibition for DAA binding (Li and Chung, 2019a). This mutation has been described at baseline and after DAA treatment with a high prevalence in infection cases with HCV genotype 1, subgenotype 1b, as found in this study (Ef et al., 2015; Li et al., 2019a). Although the presence of RAS was demonstrated by direct sequencing (Nishiya et al., 2014), it has been shown that next-generation sequencing could be a better approach to identifify the minor variants (Kai et al., 2017; Li and Chung, 2019b; Wyles, 2017).

HCV, like other RNA viruses, presents quasispecies structure in an infected individual, that is, a heterogeneous population of closely related variants of greater and lesser frequency according to their relative viral fitness. The presence of RAS that can interfere with DAA treatment has demonstrated the need for genomic surveillance of the intra-host viral populations to make decisions on treatment to ensure sustained virological response (Kai et al., 2017; Piecha et al., 2020; Wyles, 2017).

In conclusion, despite some limitations (the sample size, the lack of the lower limit of the 5' UTR RT-nested PCR, the sequencing strategy and the absence of information of some HCV sequences previously characterized in Colombia) this study was the first to described the HCV subgenotypes 4a and 4d and the acid substitutions (Q30R, Y93H, and C316N) associated with resistance to DAAs in Colombia.

The active case finding of individuals with history of transfusion events before 1996 showed a low seroprevalence of anti-HCV antibodies (2,69%); however, this strategy is a fundamental tool to identify individuals with chronic infection who could receive the AAD treatment to achieve viral clearance and therefore to reduce the risk of end-stage liver disease. Further studies are necessary to continue the phylogenetical analysis and evolution of HCV in the country and the active finding cases of chronic infection in order to achieve the viral hepatitis elimination plans in Colombia.

Funding

The study was financed by Minciencias (Ministerio de Ciencia, Tecnología e Innovación, Gobierno de Colombia) (Cod. 111577757692) and Universidad de Antioquia. These institutions did not participate in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2022.198847.

References

Aldunate, F., Echeverría, N., Chiodi, D., López, P., Sánchez-Cicerón, A., Fajardo, Á., Soñora, M., Cristina, J., Hernández, N., Moreno, P., 2018. Pretreatment Hepatitis C Virus NS5A/NS5B resistance-associated substitutions in genotype 1 uruguayan infected patients. Dis Markers 14, 2514901. https://doi.org/10.1155/2018/2514901, 2018.

Arroyave, J., Álvarez, C., Correa, G., Balcázar, N., Arbelaez, M.P., Navas, M.C., Cristina, M., 2014. Hepatitis C infections among individuals who received transfusions prior to 1994 in antioquia. Colombia. Rev. Colomb. Gastroenterol. 29, 383–389.

Bartenschlager, R., Lohmann, V., Penin, F., 2013. The molecular and structural basis of advanced antiviral therapy for hepatitis C virus infection. Nat. Rev. Microbiol. 11, 482–496. https://doi.org/10.1038/nrmicro3046.

Beltrân, M., Navas, M.-C., De la Hoz, F., Mercedes Muñoz, M., Jaramillo, S., Estrada, C., Del Pilar Cortés, L., Arbelâez, M.P., Donado, J., Barco, G., Luna, M., Uribe, G.A., de Maldonado, A., Restrepo, J.C., Correa, G., Borda, P., Rey, G., de Neira, M., Estrada, A., Yepes, S., Beltrân, O., Pacheco, J., Villegas, I., Boshell, J., 2005. Hepatitis C virus seroprevalence in multi-transfused patients in Colombia. J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 34 (2), S33–S38. Suppl.

Bhattacharjee, C., Singh, M., Das, D., Chaudhuri, S., Mukhopadhyay, A., 2021. Current therapeutics against HCV. Virusdisease. https://doi.org/10.1007/s13337-021-00697-0, 1–16.

Bolcic, F., Jones, L.R., Laufer, N., Quarleri, J., 2011. Molecular characterization of hepatitis C virus genotype 4 sequences in HIV-coinfected patients from Argentina. J. Med. Virol. 83, 935–940. https://doi.org/10.1002/jmv.22068.

Chaiwong, S., Sistayanarain, A., 2016. Characterization of partial NS5B region among hepatitis C virus genotype 6 subgenotypes isolated from Thai blood donors: characterization of NS5B among hepatitis C Virus genotype 6. J. Med. Virol. 88, 1785–1790. https://doi.org/10.1002/jmv.24536.

Chan, S.W., McOmish, F., Holmes, E.C., Dow, B., Peutherer, J.F., Follett, E., Yap, P.L., Simmonds, P., 1992. Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. J. Gen. Virol. 73 (Pt 5), 1131–1141. https://doi.org/10.1099/0022-1317-73-5-1131.

Cooper, C.L., Read, D., Vachon, M.-L., Conway, B., Wong, A., Ramji, A., Borgia, S., Tam, E., Barrett, L., Smyth, D., Feld, J.J., Lee, S., CANUHC, C., 2020. Hepatitis C virus infection characteristics and treatment outcomes in Canadian immigrants. BMC Public Health 20, 1345. https://doi.org/10.1186/s12889-020-09464-0.

di Filippo, D., Cortes-Mancera, F., Beltran, M., Arbelaez, M.P., Jaramillo, S., Restrepo, J. C., Correa, G., Navas, M.-C., 2012a. Molecular characterization of hepatitis c virus in multi-transfused Colombian patients. Virol. J. 9, 242. https://doi.org/10.1186/1743-422X-9-242.

Ef, D., Pr, H., Jj, O., Lk, N., 2015. Clinical evidence and bioinformatics characterization of potential hepatitis C virus resistance pathways for sofosbuvir. Hepatol. Baltim. 61 https://doi.org/10.1002/hep.27375.

Gómez-Escolar, L., García Herola, A., Sáez Lloret, I., Sánchez Ruano, F., Clemente Paulino, I., Quílez Ivorra, C., Almenta Saavedra, I., Martínez Pérez, D., Valverde de la Osa, J., 2018 Sep. Screening of hepatitis C virus infection in adult general population in Spain. Eur. J. Gastroenterol. Hepatol. 30 (9), 1077–1081. https://doi.org/10.1097/MEG.0000000000001190.

Gower, E., Estes, C., Blach, S., Razavi-Shearer, K., Razavi, H., 2014. Global epidemiology and genotype distribution of the hepatitis C virus infection. J. Hepatol. 61, S45–S57. https://doi.org/10.1016/j.jhep.2014.07.027.

Hall, T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

Hedskog, C., Parhy, B., Chang, S., Zeuzem, S., Moreno, C., Shafran, S.D., Borgia, S.M., Asselah, T., Alric, L., Abergel, A., Chen, J.-J., Collier, J., Kapoor, D., Hyland, R.H., Simmonds, P., Mo, H., Svarovskaia, E.S., 2019. Identification of 19 novel hepatitis c virus subgenotypes-further expanding HCV classification. Open Forum Infect. Dis. 6, ofz076. https://doi.org/10.1093/ofid/ofz076.

Hepatitis C. World Health Organization, 2021. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c.

Kai, Y., Hikita, H., Morishita, N., Murai, K., Nakabori, T., Iio, S., Hagiwara, H., Imai, Y., Tamura, S., Tsutsui, S., Naito, M., Nishiuchi, M., Kondo, Y., Kato, T., Suemizu, H., Yamada, R., Oze, T., Yakushijin, T., Hiramatsu, N., Sakamori, R., Tatsumi, T.,

Takehara, T., 2017. Baseline quasispecies selection and novel mutations contribute to emerging resistance-associated substitutions in hepatitis C virus after direct-acting antiviral treatment. Sci. Rep. 7 https://doi.org/10.1038/srep41660.

Kermani, F.R., Sharifi, Z., Ferdowsian, F., Paz, Z., Tavassoli, F., 2015. The usefulness of anti-HCV signal to cut-off ratio in predicting viremia in anti-HCV in patients with hepatitis C virus infection. Jundishapur J. Microbiol. 8 (4), e17841. https://doi.org/10.5812/jjm.8(4)2015.17841. Apr 18.

Kershenobich, D., Razavi, H.A., Sánchez-Avila, J.F., Bessone, F., Coelho, H.S., Dagher, L., Gonçales, F.L., Quiroz, J.F., Rodriguez-Perez, F., Rosado, B., Wallace, C., Negro, F., Silva, M., 2011. Trends and projections of hepatitis C virus epidemiology in Latin America. Liver Int. Off. J. Int. Assoc. Study Liver 31 (2), 18–29. https://doi.org/10.1111/j.1478-3231.2011.02538.x. Suppl.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Li, D.K., Chung, R.T., Law, M., 2019a. Overview of direct-acting antiviral drugs and drug resistance of hepatitis C Virus. Hepatitis C Virus Protocols, Methods in Molecular Biology. Springer, New York, NY, pp. 3–32. https://doi.org/10.1007/978-1-4939-8976-8_1.

Li, D.K., Chung, R.T., 2019b. Overview of direct-acting antiviral drugs and drug resistance of hepatitis C virus. Methods Mol. Biol. Clifton NJ 1911, 3–32. https://doi.org/10.1007/978-1-4939-8976-8_1.

Manns, M.P., Buti, M., Gane, E., Pawlotsky, J.-M., Razavi, H., Terrault, N., Younossi, Z., 2017. Hepatitis C virus infection. Nat. Rev. Dis. Prim. 3, 17006. https://doi.org/10.1038/nrdp.2017.6.

Ministerio de Salud y Protección Social, 1993. Decreto 1571 de 1993, Diario oficial No 40989.

Ministerio de Salud y Protección Social, 2019. Situación de la Hepatitis C Crónica en Colombia en el Regímen Contributivo de Colombia. Cuenta de alto costo. Fondo Colombiano de Enfermedades de Alto Costo.

Ministerio de Salud y Protección Social, 2020. Situación de la Hepatitis C Crónica en Colombia en los Regímenes Subsididdado y Contributivo de Colombia. Cuenta de alto costo. Fondo Colombiano de Enfermedades de Alto Costo.

Mora, M.V.A., Romano, C.M., Gomes-Gouvêa, M.S., Gutiérrez, M.F., Carrilho, F.J., Pinho, J.R.R., 2010. Molecular characterization, distribution, and dynamics of hepatitis C virus genotypes in blood donors in Colombia. J. Med. Virol. 82, 1889–1898. https://doi.org/10.1002/jmv.21908.

Moradpour, D., Penin, F., 2013. Hepatitis C virus proteins: from structure to function. Curr. Top. Microbiol. Immunol. 369, 113–142. https://doi.org/10.1007/978-3-642-27340-7_5.

Nishiya, A.S., de Almeida-Neto, C., Ferreira, S.C., Alencar, C.S., Di-Lorenzo-Oliveira, C., Levi, J.E., Salles, N.A., Mendrone, A., Sabino, E.C., 2014. HCV genotypes, characterization of mutations conferring drug resistance to protease inhibitors, and risk factors among blood donors in São Paulo, Brazil. PloS One 9, e86413. https://doi.org/10.1371/journal.pone.0086413.

Petruzziello, A., Marigliano, S., Loquercio, G., Cozzolino, A., Cacciapuoti, C., 2016. Global epidemiology of hepatitis C virus infection: an up-date of the distribution and circulation of hepatitis C virus genotypes. World J. Gastroenterol. 22, 7824–7840. https://doi.org/10.3748/wjg.v22.i34.7824.

Piecha, F., Gänßler, J.-M., Ozga, A.-K., Wehmeyer, M.H., Dietz, J., Kluwe, J., Laschtowitz, A., von Felden, J., Sterneck, M., Jordan, S., Pischke, S., Lohse, A.W., Schulze Zur Wiesch, J., 2020. Treatment and re-treatment results of HCV patients in the DAA era. PLoS One 15, e0232773. https://doi.org/10.1371/journal.pone.0232773.

Santos, Ó., Gómez, A., Vizcaíno, V., Casas, M.C., Ramírez, M.P., Olaya, P., 2017. Genotipos circulantes del virus de la hepatitis C en Colombia. Biomédica 37, 22–27.

Sarrazin, C., Dvory-Sobol, H., Svarovskaia, E.S., Doehle, B.P., Pang, P.S., Chuang, S.-M., Ma, J., Ding, X., Afdhal, N.H., Kowdley, K.V., Gane, E.J., Lawitz, E., Brainard, D.M., McHutchison, J.G., Miller, M.D., Mo, H., 2016. Prevalence of resistance-associated substitutions in HCV NS5A, NS5B, or NS3 and outcomes of treatment with ledipasvir and sofosbuvir. Gastroenterology 151, 501–512. https://doi.org/10.1053/j.gastro.2016.06.002 e1.

Simmonds, P., Bukh, J., Combet, C., Deléage, G., Enomoto, N., Feinstone, S., Halfon, P., Inchauspé, G., Kuiken, C., Maertens, G., Mizokami, M., Murphy, D.G., Okamoto, H., Pawlotsky, J.-M., Penin, F., Sablon, E., Shin-I, T., Stuyver, L.J., Thiel, H.J., Viazov, S., Weiner, A.J., Widell, A., 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatol. Baltim. 42, 962–973. https://doi.org/10.1002/hep.20819.

Sirin, M.C., Aridogan, B.C., Cetin, E.S., Sirin, FB., 2019. Evaluation of biochemical, hematological, RIBA and PCR assays in predicting viremia in anti-HCV positive patients. J. Infect. Dev. Ctries 13 (8), 736–743. https://doi.org/10.3855/jidc.11426. Aug 31.

Smith, D.B., Bukh, J., Kuiken, C., Muerhoff, A.S., Rice, C.M., Stapleton, J.T., Simmonds, P., 2014. Expanded classification of hepatitis C virus into 7 genotypes and 67 subgenotypes: updated criteria and genotype assignment web resource. Hepatol. Baltim. 59, 318–327. https://doi.org/10.1002/hep.26744.

Sookoian, S., Castaño, G., 2002. Evaluation of a third generation anti-HCV assay in predicting viremia in patients with positive HCV antibodies. Ann. Hepatol. 1 (4), 179–182. Oct-Dec.

Sulbarán, M.Z., Di Lello, F.A., Sulbarán, Y., Cosson, C., Loureiro, C.L., Rangel, H.R., Cantaloube, J.F., Campos, R.H., Moratorio, G., Cristina, J., Pujol, F.H., 2010. Genetic history of hepatitis C virus in venezuela: high diversity and long time of evolution of HCV genotype 2. PLoS One 5. https://doi.org/10.1371/journal.pone.0014315.

Walker, P.J., Siddell, S.G., Lefkowitz, E.J., Mushegian, A.R., Adriaenssens, E.M., Dempsey, D.M., Dutilh, B.E., Harrach, B., Harrison, R.L., Hendrickson, R.C., Junglen, S., Knowles, N.J., Kropinski, A.M., Krupovic, M., Kuhn, J.H., Nibert, M., Orton, R.J., Rubino, L., Sabanadzovic, S., Simmonds, P., Smith, D.B., Varsani, A., Zerbini, F.M., Davison, AJ., 2020. Changes to virus taxonomy and the statutes ratified by the international committee on taxonomy of viruses. Arch Virol. 165 (11), 2737–2748. NovPMID: 32816125.

Wiese, M., Berr, F., Lafrenz, M., Porst, H., Oesen, U., 2000. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in germany: a 20-year multicenter study. Hepatol. Baltim. 32, 91–96. https://doi.org/10.1053/jhep.2000.8169.

World Health Organization, 2021b. Interim Guidance for Country Validation of Viral Hepatitis Elimination. World Health Organization, Geneva.

Wyles, D.L., 2017. Resistance to DAAs: when to look and when it matters. Curr. HIV/AIDS Rep. 14, 229–237. https://doi.org/10.1007/s11904-017-0369-5.

Yamashita, T., Takeda, H., Takai, A., Arasawa, S., Nakamura, F., Mashimo, Y., Hozan, M., Ohtsuru, S., Seno, H., Ueda, Y., Sekine, A., 2020. Single-molecular real-time deep sequencing reveals the dynamics of multi-drug resistant haplotypes and structural variations in the hepatitis C virus genome. Sci. Rep. 10, 2651. https://doi.org/10.1038/s41598-020-59397-2.

Zein, N.N., 2000. Clinical significance of hepatitis C virus genotypes. Clin. Microbiol. Rev. 13, 223–235.

Zein, N.N., Rakela, J., Krawitt, E.L., Reddy, K.R., Tominaga, T., Persing, D.H., 1996. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative study group. Ann. Intern. Med. 125, 634–639. https://doi.org/10.7326/0003-4819-125-8-199610150-00002.