Research Article

Intervirology

Intervirology 2019;62:191–197
DOI: 10.1159/000505065

Received: July 17, 2019
Accepted: November 24, 2019
Published online: December 20, 2019

Variation in Hepatitis C Virus Subtype Distribution during 20 Years in Venezuela

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Keywords
Hepatitis C virus · Genotype · Subtype · NS5B · 5′-noncoding region

Abstract
Objective: Hepatitis C virus (HCV) infection is a public health problem and a major cause of chronic hepatitis. This virus exhibits a great genetic variability, with 8 genotypes and numerous subtypes. The aim of this study was to evaluate the fluctuations of HCV subtypes during 2 decades in Venezuela. ***Methods:*** HCV genotypes were determined by direct sequencing of the 5'-noncoding region in 392 isolates circulating in patients attended during the years 2014–2015. HCV subtype assignment was confirmed in a subset of samples ($n = 24$) by partial sequencing of the NS5B region. The genotype distribution was compared with the one observed in a previous study of patients followed up during the years 1994–1996 and 2005–2006. ***Results:*** Some variation was observed in the HCV genotype distribution over these 20 years. HCV genotype 1b prevalence was reduced significantly from 1994–1995 to 2004–2005, as previously described, and then remained constant. During the last 10 years, a significant decrease of HCV subtype 2b (36/237 in 2005–2006 vs. 24/392 in 2014–2015, $p < 0.001$) was observed. Patients infected with HCV G2acj were significantly older than the ones infected with G1 (53 vs. 47 years, $p = 0.004$), and male sex was significantly more prevalent among G3a-infected patients compared to the other ones (71 vs. 47%, $p = 0.047$). ***Conclusions:*** Fluctuations in HCV subtype distribution have been observed over 2 decades in Venezuela. Different major mode of transmission and susceptibility to the available HCV treatment during each period might be playing a role in the observed fluctuations in HCV subtype distribution.

Introduction

Hepatitis C virus (HCV) infection is a public health problem and a major cause of chronic hepatitis. Around 177.5 million persons are infected by HCV worldwide [1]. Hepatocellular carcinoma (HCC) is the most common cause of death in patients with chronic HCV infection [2]. In Latin America, HCV seroprevalence is moderate to low depending on the region (1.0–2.7%) [3], and it is around 1.5% in Venezuela [4].

Eight HCV genotypes, with the most recently discovered genotype 8 (G8) circulating in India, and a large number of subtypes have been recognized, based on the sequence of the viral genome that differs from the other genotypes by 29–33% [5]. Globally, the most common

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Table 1. HCV subtype distribution in Venezuelan patients during 2014–2015

	HCV genotypes								
	1ac	1b	2acj	2b	3a	3b	4	6	total
Subjects, *n* (%)	133 (33.9)	91 (23.2)	115 (29.3)	24 (6.1)	21 (5.4)	2 (0.5)	4 (1)	2 (0.5)	392
	HCV genotypes								
	1		2		3				
Subjects, *n* (%)	224 (57.1)		138 (35.5)		23 (5.9)				

1ac stands for subgenotype 1a or 1c. 2acj stands for subgenotype 2a, 2c, or 2j.

Table 2. Age and sex according to HCV subtypes in Venezuelan patients

Subtype	1ac	1b	2acj	2b	3a	*p*
Mean age ± SD, years[a]	46.6±15.5	47.2±15.9	52.7±16.2	47.5±13.3	51.9±10.1	1 vs. 2acj $p = 0.04$
Male sex, *n*/*N* (%)	65/131 (50)	42/91 (46)	50/114 (43)	11/24 (46)	15/21 (71)	3a vs. others $p = 0.047$

[a] Information available for 327/392 patients.

genotype is G1 (49%), followed by G3 (18%), G4 (17%), and G2 (11%) [1]. Subtype 1b accounted for 22% of all infections worldwide [6]. In Latin America, the dominant genotype is G1 (74.3%), followed by G3 (14.2%) or G2 (10.4%), depending on the country [1, 7]. In Venezuela, G1 is the most common genotype, followed by G2 and G3. In addition, the most frequent subtype of G2 is G2j, a subtype quite rare in other countries [8, 9]. Some HCV genotypes have been associated with a more frequent development of liver damage (HCC) in patients with chronic infection, i.e., G1 and G3. In contrast, people infected with HCV G2 appear to have better survival [2].

Variations in genotype and subtype distribution over time have been observed in several countries [10]. In Venezuela, a significant reduction in HCV G1b circulation was observed in the middle of the 2000s, compared to 1994–1995, accompanied by an increase in G2, particularly G2j [9, 11]. Since then, the implementation of direct-acting antivirals (DAA) against HCV infection [12] might have influenced the relative distribution of viral subtypes in the country. The aim of this study was to evaluate the fluctuations in HCV subtype distribution during 2 decades in Venezuela.

Materials and Methods

Blood Samples

This study was approved by the Bioethical Committee of Instituto Venezolano de Investigaciones Científicas (IVIC). Plasma from 392 patients (188 males and 204 females, average age 48.7 years, range 9–85 [age information available for 327/392 patients]) was collected between 2014 and 2015 after written informed consent from the donor. The studied population (2004–2005 [11] and 2014–2015) was from 14 out of the 24 Venezuelan states, including those with the highest population density. Patients analyzed in the years 1994–1996 [8] were from the most populated cities in Venezuela, Caracas and Maracaibo. Patients were anti-HCV and HCV-RNA positive and required genotype assignment to initiate antiviral treatment. Samples were stored at –30 °C until use.

PCR and Sequencing

In the 392 samples, the HCV genotype was determined by direct sequencing (performed by Macrogen Service Center, Seoul, Korea) and phylogenetic analysis of a PCR-amplified product from the 5′-noncoding region (5′NC) [11]. Genotype distribution was compared to the one observed in 110 HCV-infected individuals followed up during the years 1994–1996 and in 237 patients followed up during the years 2005–2006 [8, 11] for a total of 739 samples. In the samples collected during 1994–1996, the genotype was determined by restriction fragment length polymorphism (RFLP) analysis and confirmed in some isolates by sequencing of the 5′NC region with a 100% correlation [4, 9, 11]. The confirma-

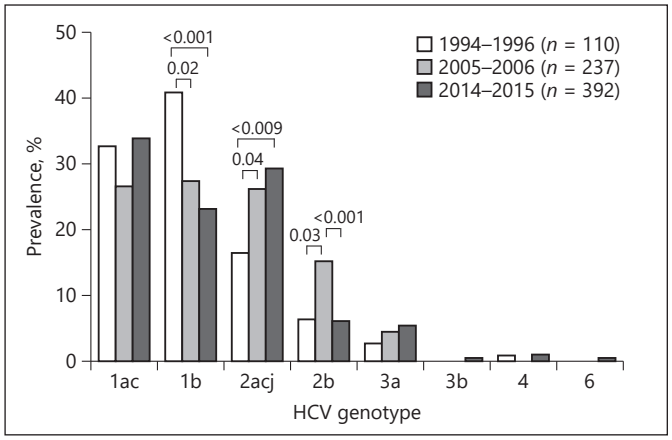

Fig. 1. Prevalence of HCV genotypes and subtypes in Venezuelan HCV-infected patients. The HCV genotypes obtained from HCV-infected patients followed up during the years 2014–2015 ($n = 392$) were compared to the ones reported during the years 1994–1996 ($n = 110$ [8]) and 2005–2006 ($n = 237$ [11]).

tion of HCV genotype and appropriate identification of subtypes was determined by direct sequencing of a PCR-amplified product from the NS5B region [9] in 181/739 total samples.

Sequence alignment and phylogenetic analysis by the neighbor joining method (500 bootstrap replicas, with genetic distances estimated with Kimura 2 parameters correction) were performed using DNAMAN 5.2.2 (Lynnon Biosoft, Canada). The GenBank accession numbers are MK984693–MK984716 and those previously reported [9].

Statistical Analysis

Statistical differences were evaluated by Student *t* test or by χ^2 test.

Results

The 5'NC sequences were analyzed in 392 HCV isolates collected between 2014 and 2015. Phylogenetic analysis showed that HCV G1 was the most prevalent genotype (225/392, 57.4%), followed by G2 (138/392, 35.2%) and G3 (23/392, 5.9%). Subtype 1a was more prevalent than subtype 1b (135/225, 60%). Four isolates of G4 (1%) were also identified, and to the best of our knowledge, for the first time in Venezuela, 2 isolates of G6 were identified (Table 1). Patients infected with HCV G2acj were significantly older than those infected with G1. The prevalence of male patients was significantly higher in those infected with HCV G3a compared to other genotypes (Table 2).

The subtype distribution found in 2014–2015 was compared to the one obtained in 2004–2005 and in 1994–1996 [8]. HCV genotype distribution was similar during 2014–2015 to the one obtained in 2004–2005 (Fig. 1), G1 being the most prevalent genotype, followed by G2. However, a significant G2b decrease (36/237 in 2005–2006 vs. 24/392 in 2014–2015, $p < 0.001$) was observed during the years 2014–2015, the frequency of G2b being similar to the one observed in 1994–1996 [5]. No change was observed in the prevalence of the other HCV subtypes. Then, the reduction in G1b and increase in G2acj observed during the first decade was stabilized in the present decade (Fig. 1).

A total of 181 sequences were available in the NS5B region. The same genotypes were found in the samples analyzed by NS5B sequences as by the genotype assignment in the 5'NC region. Among the G2acj samples, the most prevalent subtype was again G2j. No particular clade was observed for any of the studied genotypes during any period of time (Fig. 2).

Discussion

It has been suggested that HCV is not autochthonous to Latin America, which is supported by the low prevalence of infection in native groups in this region [7]. In Venezuela, HCV genotype distribution has been maintained during the last 20 years (G1 > G2 > G3) and is similar to that reported in other Latin American countries, such as Argentina and Mexico, and in Europe, specifically in southern Italy, with a significantly higher frequency of G2 compared to G3 [1, 13].

Analysis of the phylogeographic patterns of HCV subtypes 1a and 1b circulating in the world reveals that the source of epidemic spatial dispersion of these subtypes was possibly initiated and sustained by a first wave of transmission due to an increase in parenteral iatrogenic procedures during and after World War II, which facilitated the dissemination of HCV G1 in developed countries and its subsequent propagation to the developing world [14]. Bayesian coalescence analysis of Venezuelan isolates suggests that the most recent common ancestors of G1a and G1b subtypes date from the years around 1922 and 1869, respectively, similar to those observed in Brazil and the United States [9]. Likewise, a substantial increase in infection by subtypes 1a and 1b was evidenced before the middle of the 20th century [9]. This increase might be due to an important migration from Europe to Venezuela and through imported blood derivatives.

Even if HCV genotype distribution in Latin America is not as diverse as in Africa [7], in a few countries of the region, quite unique subtypes circulate, particularly G2

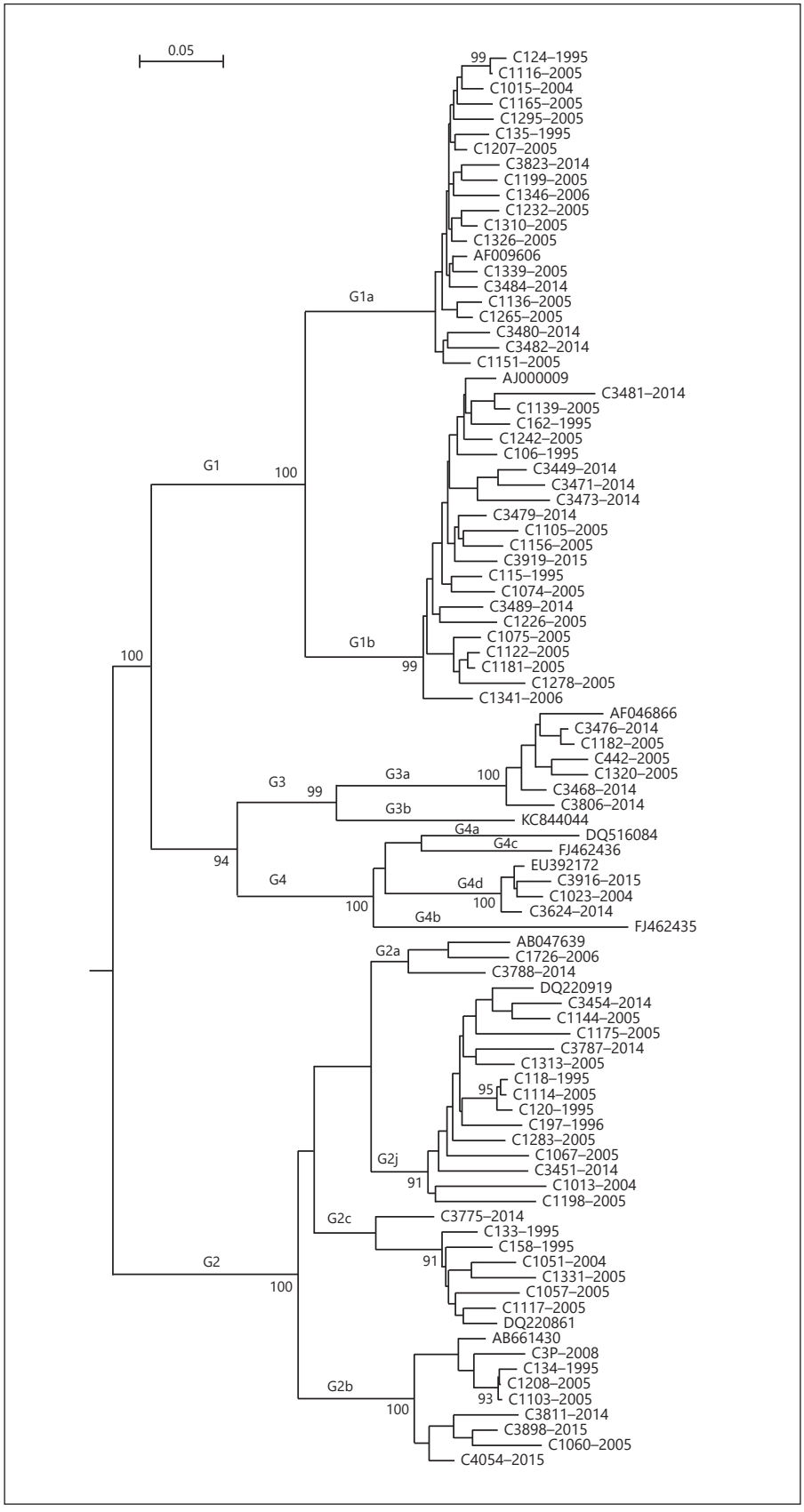

Fig. 2. Phylogenetic analysis of HCV NS5B sequences (255 bp). Genetic distance was estimated by Kimura 2 parameters, and the phylogenetic tree was constructed with the neighbor joining method. Numbers at each node correspond to bootstrap values (greater than 70%) obtained with 500 replicates. Venezuelan isolates are designated with their name and year of collection. Isolates are designated by their GenBank accession number if they are not from Venezuela.

subtypes. For example, in Martinique, there is a diverse circulation of G2 [15], different from the one circulating in Venezuela. HCV G2j has been reported in other countries of America, such as Canada [16], Argentina [17], and Mexico [18]. However, the high prevalence of HCV G2j in Venezuela is a unique situation, as a possible consequence of the slave trade from West Africa around 1785 [9]. In Brazil or Colombia, countries also subject to African immigration through the slave trade, the presence of this or other exotic subtypes has not been reported [19]. In other Latin American countries, such as Brazil, Chile, and Peru, G3a can even be more frequent than G2 (and G2 is mostly G2a, G2b, or G2c) [1]. This might be associated with a higher prevalence of intravenous drug users in these countries, since it is known that G3a is frequent among intravenous drug users [20]. In countries with the largest drug-injecting populations, like the United States and Brazil [20], the main HCV genotypes in intravenous drug users are G1 and G3, without G2 [21–26]. In Mexico, a country with a high HCV prevalence, an increase in G3 and intravenous drug use (IDU) have been observed [27]. In Venezuela, G3a is still maintaining a low prevalence, as shown in this study. Indeed, IDU does not seem an important risk factor. The low frequency of IDU might also contribute to the lower HCV coinfection prevalence among HIV-infected patients compared to HBV coinfection [28], while the opposite scenario is observed in many countries of the region where IDU is more frequent. However, even if the prevalence of G3a remains relatively low in Venezuela, this subtype was significantly more frequent among males than the other subtypes. This difference might be associated with IDU as a risk factor among these patients (although not revealed in the questionnaire), since there is also a strong association between IDU and male sex [29].

An interesting observation was that G2j-infected patients were older than those infected with other genotypes/subtypes, including those infected with G2b. Melo et al. [30] also found in São Paulo, Brazil, that patients infected with G2 were older than those infected with G3. However, in this case, there were other G2 subtypes, since G2j has not been described in Brazil. The older age found in G2j-infected patients from Venezuela, compared to those infected with other genotypes and subtypes, might be associated with infection by parenteral transmission in the last century, through the shared use of glass syringes for medicinal purposes, as described previously for G2c in Italy [31].

Analyzing the dynamics of HCV subtypes during 2 decades in Venezuela, it can be said that the initial decrease in G1b and increase in G2j prevalence was stabilized. For HCV G2b, which increased during the first decade, like G2j, the prevalence significantly decreased during the last decade. In contrast to Venezuela, HCV G2b prevalence is increasing in Japan, although the reason for this increase is not known [32]. At first glance, it could be suggested that the reduction in HCV G2b prevalence in Venezuela might be associated with the sensitivity of this subtype to antiviral treatments based on interferon, which was applied in the country during the last decade. It is known that HCV G2 displayed high sustained virologic response to antiviral treatment before the DAA era [33]. Indeed, the rate of sustained response to interferon-based regimes in HCV G2-infected patients in Venezuela (most of them infected with HCV G2j, but also G2b) is similar to that observed in other countries [León, R., pers. commun., June 16, 2017], suggesting that HCV G2j is susceptible to interferon treatments as other G2 subtypes. However, in Venezuela, around 0.3–0.5% of the HCV-infected patients have been treated with interferon-based regimes or DAA [León, R., pers. commun., June 16, 2017]. This implies that antiviral treatment might not play a significant role in reducing the prevalence of susceptible HCV types.

In addition, although heterosexual transmission of HCV is an infrequent event [34], it has been described in long-time couples. Sexual transmission has been described as a possible route of transmission of HCV G2 in Guinea Bissau [35]. We have no evidence that sexual transmission may be playing a role in HCV G2j dissemination, but this could explain both the HCV G2b and G2j prevalence emerging in the first decade of this study, while G2j was maintaining a relatively high frequency in the last decade. It is also not known if some HCV subtypes might be more prevalent in some specific socioeconomic groups. Long-term exposure to HCV patients and low family income have been correlated with HCV familial clustering, whereas blood transfusion does not seem to play a role [36, 37].

Conclusion

Fluctuations in HCV subtype distribution have been observed over 2 decades in Venezuela. Some unapparent routes of transmission might be contributing to the dissemination of particular subtypes. Even if highly efficient DAA-based treatments are now available, we are still far from identifying most of the infected patients and providing them with effective treatment, particularly in Venezuela.

Statement of Ethics

This study was approved by the Bioethical Committee of Instituto Venezolano de Investigaciones Científicas (IVIC). Samples were collected after written informed consent from the Venezuelan patients of this study.

Disclosure Statement

All authors declare that they have no conflicts of interest associated with the present work.

Funding Sources

This work was supported by grants 650 and 1108 from IVIC, Venezuela.

Author Contributions

F.H.P. and R.C.J. performed the conception and design of the study. C.L.L. carried out the molecular genetic studies. C.L.L., R.C.J., and F.H.P. performed the sequence alignment and phylogenetic analysis. C.L.L., R.C.J., and F.H.P. drafted the manuscript. All authors read and approved the final manuscript.

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