JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1870–1872 Vol. 35, No. 7
0095-1137/97/$04.00+0

Determination of Genotypes of Hepatitis C Virus in Venezuela by Restriction Fragment Length Polymorphism

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Received 10 January 1997/Returned for modification 12 March 1997/Accepted 14 April 1997

Hepatitis C virus genotypes in Venezuela were analyzed by restriction fragment length polymorphism in the 5′ noncoding region. The absence of *Bst*UI digestion was found to be a useful marker for genotype 2 specimens. From 122 serum samples, 66, 20, and 2.5% were classified as genotypes 1, 2, and 3, respectively; 0.8% were classified as genotype 4; and 10% appeared to be mixed infections.

The etiologic agent responsible for the majority of non-A-, non-B posttransfusion hepatitis, the hepatitis C virus (HCV), displays a high degree of genomic variability. Different isolates have been classified into genotypes according to sequence homology. Several methodologies have been developed for HCV genotyping. Among them, restriction fragment length polymorphism (RFLP) analysis of PCR-amplified fragments of the 5′ noncoding (5′NC) region (2) allows the determination of genotypes in a large number of samples with a fairly high accuracy (16). Information about the genetic variability of HCV in South America is still scarce. The aim of this study was to analyze the genotype distribution of HCV in Venezuela by RFLP in the 5′NC region.

HCV-positive sera or plasma were collected from blood donors ($n = 19$), hemophiliac patients ($n = 13$), chronically infected patients (presenting elevated alanine aminotransferase levels for more than 6 months [$n = 43$]), and hemodialysis patients ($n = 47$) from Caracas and Maracaibo, the two largest cities in Venezuela. HCV RNA was detected by reverse transcription and single-tube nested PCR as previously described (14). Five microliters of the PCR-amplified products was incubated for 18 h in four separate reactions with 5 U (each) of the restriction enzymes *Rsa*I and *Hae*III, *Mva*I and *Hin*fI, *Scr*I at 37°C, and *Bst*UI at 60°C (2). The digested products were electrophoresed in 12.5% polyacrylamide minigels for 60 min at 160 V. Gels were fixed in methanol-acetic acid and stained with silver nitrate (10). Genotypes and subtypes were deduced from the RFLP patterns as described previously (2, 16). Sequencing was performed with the Sequenase PCR product sequencing kit (Amersham, Cleveland, Ohio). The sequences were aligned, and the distances between them were determined with CLUSTAL V.

In order to evaluate the usefulness of the *Bst*UI restriction pattern as a supplemental marker for genotype determination, a total of 437 published sequences of the 5′NC regions (classified as genotypes 1 to 11) were analyzed for *Bst*UI restriction sites. Of 77 genotype 2 sequences analyzed, all but 3 presented an A at position −99 and a T at position −69, eliminating the two *Bst*UI restriction sites present in other genotypes (2). The three exceptions were all type 2e from Indonesia. Additionally,

TABLE 1. Genotype distribution of HCV isolates among different Venezuelan populations

Population (*n*)	No. of isolates of genotype[a]:							
	1a	1b	2a	2b	3a	3b	4	Mixed
Blood donors (19)	10	**3**	3	3	0	0	0	0
Chronic patients (43)	8	**19**	7	3	2	0	1	3 (1a, 1b; 1b, 2a)
Hemodialyzed patients (47)								
Caracas (35)	12	12	5	0[b]	0	0	0	6 (1a, 1b; 1–2; 1–3)
Maracaibo (12)	0	9	2	0[b]	0	0	0	1 (1b, 3a)
Hemophiliac patients (13)								
Caracas (7)	3	0	1	1	0	0	0	2 (1b, 3a; 1a, 1b)
Maracaibo (6)	3	2	0	0	1	0	0	0

[a] The prevalence of genotype 1b among chronic patients and blood donors is shown in boldface and was significantly different ($P < 0.01$) from that of the other groups, as evaluated by the chi-square test with Yate's correction, according to a computerized Epi Info program, version 5.01b (Centers for Disease Control and Prevention, Atlanta, Ga.). There were a total of 122 isolates. Sixty-six percent of the isolates were genotype 1, 20% were genotype 2, 2.5% were genotype 3, 0.8% were genotype 4, and 10% were mixed.

[b] The absence of genotype 2b among hemodialysis patients was significant ($P < 0.05$) compared with that of the other groups.

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TABLE 2. Sequence identity of Venezuelan HCV isolates in the 5′NC region with published genotypes

Sequence (no. of nucleotides)	Genotype RFLP	Avg % similarity to genotype[a]							
		1	2a	2b	2c	3	4	5	6
YV078 (230)	2a	91.7	**98.1**	95.7	**98.2**	88.4	92.1	92.2	94.5
YV120 (220)	2a	92.9	**99.8**	97.3	**99.7**	88.2	93.2	93.4	95.9
YV153 (232)	2a	92.6	**98.9**	96.6	**99.2**	88.5	93.0	93.3	95.4
YV156 (138)	2a	89.7	**99.3**	95.5	**98.9**	83.8	90.0	91.1	92.6
YV243 (163)	2a	92.2	**99.1**	96.0	**99.1**	87.1	92.0	92.6	94.1
YV174 (231)	3a	93.3	88.8	87.0	88.3	**99.1**	94.1	93.2	90.5
YV780 (213)	4	95.5	92.0	90.1	90.5	94.3	**97.7**[b]	95.8	93.4

[a] A total of 20 published 5′NC regions were used for the sequence comparison, broken down as follows: 1 each of genotypes 1a (M86765), 1b (D10074), and 1c (D16191); 2 each of genotypes 2a (D10075 and D00944), 2b (D10077 and D10988), and 2c (L38320 and L38329); 2 genotypes 3a (D17763, D12355), 5 genotypes 4 (4a [M84848], 4b [M84845], 4c [M84862], 4f [M84829], and a Canadian genotype 4 [U33432]), 1 genotype 5 (L29585), and 1 genotype 6 (U33431). Numbers in boldface refer to the highest percentage of sequence identity.

[b] The highest percentage of identity of YV780 (98.1%) was found with genotypes 4c and 4f and the Canadian isolate.

none of the 25 Venezuelan specimens which presented genotype 2 RFLP patterns were digested with *Bst*UI. Moreover, all but one genotype 1 specimen ($n = 131$) presented at least one restriction site for *Bst*UI, and the one which did not is presumed to be a recombinant with a genotype 2 5′NC (16). Previous sequence analysis predictions showed that the standard panel used for RFLP (*Mva*I-*Hin*fI pattern) is fairly reliable for genotype 2 assignment, although it failed in at least one sequence (16). *Bst*UI digestion might be particularly useful for the discrimination of genotypes 1 and 2 in countries in which these are the predominant types. Other genotype groups were more polymorphic in terms of the number of *Bst*UI restriction sites; for example, most genotype 4 isolates presented one (45 of 81) or two (31 of 81) restriction sites, while two restriction sites were predominant in genotype 3 (71 of 81) or 5 (23 of 24) isolates. The absence of a restriction site for this enzyme was frequently found in genotype 6 isolates (8 of 12).

A total of 122 Venezuelan serum samples were analyzed: 66% were classified as genotype 1 (29% subtype 1a, 37% subtype 1b), 20% as genotype 2 (15% 2a and 5% 2b), and 2.5% as genotype 3a, and 10% appeared to be mixed infections. Nine of the 12 (75%) specimens with putative mixed infections were found in high-risk groups (Table 1). One genotype 4 isolate, confirmed by sequence analysis (Table 2), was found in an HCV-infected women with an autoimmune disorder (1). Additionally, sequence identity analysis corroborated five genotype 2a RFLP patterns and one genotype 3a RFLP pattern (Table 2). Moreover, specific sequence motifs have been reported that are characteristic for isolates belonging to genotypes 2, 3, and 4 (6, 17), and these were consistently present with the RFLP genotype designation in our sequenced isolates (data not shown).

A significantly higher prevalence of genotype 1b was found among chronic patients than the prevalence observed in blood donors ($P = 0.01$). No significant difference was observed in the average age of genotype 1b chronic patients compared to that of patients infected with other genotypes (data not shown). This agrees with the higher frequency of genotype 1b among patients with elevated alanine aminotransferase levels and evidence of chronic liver disease previously reported (15). It could be speculated that patients infected with genotype 1b might be more prone to seek the assistance of a gastroenterologist. The differential distribution of HCV genotypes stresses the importance of studying different populations when analyzing the genetic variability of HCV.

Genotype 2b was not found among hemodialysis patients, although it was present in blood donors, chronic patients, and hemophiliacs (0 of 46 versus 7 of 74; $P = 0.04$ [Table 1]), which is in agreement with the absence of this subtype in hemodialysis patients reported previously (8, 13, 18, 19). A systematic reduction of virus concentration after hemodialysis has been described (11). It would be interesting to test whether genotype 2b isolates are more susceptible to damage by hemodialysis treatment. Alternatively, strains of this subtype might be more frequently cleared because they generally circulate at lower concentrations (9).

From these preliminary data, the genotype distribution in Venezuela seems to be very similar to those found in North America (7), Western Europe (3), and other countries of South America (5, 12). An interesting finding was the presence of a less common genotype, 4, in a woman who presented an autoimmune disorder but without a history of origin in or travel to Africa. Sequence analysis of this isolate corroborated the genotype assignation by RFLP. In a recent study, all patients infected with HCV genotype 4 had cryoglobulins (4). Even if cryoglobulins were not present in this particular Venezuelan patient (1), the association of genotype 4 with autoimmune disorders deserves further studies.

Nucleotide sequence accession number. Nucleotide sequence data have been assigned to the GenBank database under accession no. U81278 to U81284.

This work was supported by grant S1-96000064 from CONICIT and grant 1722-95 from Proyecto LUZ-CONDES, Venezuela.

We thank Graciela León, Arlette de Saez, and Jose Luis López, Banco Municipal de Sangre; and Freya Capriles, Unidad de Hemodiálisis Crónica de Caracas, for providing serum samples. We also thank Donald Smith, University of Edinburgh; Claudio Argentini, Istituto Superiore di Sanita; and Donald Murphy, Laboratoire Sante Publique du Quebec, for providing some of the sequences analyzed in this study. We thank Gustavo López for technical assistance in sequencing and Howard Takiff for careful reading of the manuscript.

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