

Genotyping of hepatitis C virus isolates from Basque Country, Spain

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SUMMARY

The genotype of HCV was determined in 161 chronic HCV-infected patients. The patients were classified into three groups on the basis of the origin of the HCV infection: 50 patients had a history of intravenous drug use (IVDU) but no HIV infection; 41 patients had received blood transfusions, and 70 patients had no known exposure. The distribution of HCV genotypes was associated with the origin of infection and age of patients: genotype 1b was predominant among patients who had received blood transfusions and those without evidence of parenteral exposure (84.6% and 67.7%, respectively), whereas genotype 3a was present in 65.3% of IVDUs. Patients with genotype 1b were older than those with genotypes 1a or 3a: 50.3 ± 12 vs. 34.1 ± 9.9 and 31 ± 5.4 years, respectively. These findings suggest that the pattern of HCV genotypes in our region is changing and that genotype 1b may be substituted by 3a as the dominant genotype in the future.

INTRODUCTION

HCV is a single-stranded, positive sense RNA virus with a genetic organization similar to that of the flaviviruses and pestiviruses [1]. It is the main cause of post-transfusional and sporadic non-A non-B hepatitis [2, 3]. There is a high degree of nucleotide sequence diversity among different isolates of HCV. The major groupings of sequence variants are designated HCV types and the more closely related groups observed within some types are termed subtypes following the classification system proposed by Simmonds and other researchers [4]. The genotyping of HCV isolates has become important for epidemiological purposes [5–7]. Several studies have suggested the existence of biological differences between HCV genotypes which affect their capacity for producing liver disease and sensitivity to interferon treatment [8–10].

Little information is available about the genotypes causing HCV infection in Spain. This study was

undertaken for the purpose of analyzing the distribution of HCV genotypes and source of infection related in persons infected with this virus in Gipuzkoa, Basque Country, Spain.

PATIENTS AND METHODS

Gipuzkoa is in the northeastern area of the Basque Country, bordered by the Cantábrico Sea (Atlantic Ocean) and France to the north (1997 square kilometres, population 671 774). A total of 161 chronic HCV-infected patients with 1 year or more of follow-up (49 females, 112 males; mean age: 42.6 ± 13.5 , range 22–69 years) were recruited from January 1990 to December 1994. The patients were classified into three groups on the basis of the source of their HCV infection: patients with a history of intravenous drug use (IVDU) ($n = 50$, mean age 30.7 ± 4.2 years); patients who had acquired the infection through blood transfusion ($n = 41$, mean age = 50.7 ± 11.4

years); and patients with no evidence of parenteral exposure or other known origin ($n = 70$, mean age 46.1 ± 13.3 years). Anti-HIV positive patients were excluded from these study groups.

All patients were anti-HCV positive by ELISA (Ortho HCV 3.0 ELISA System, Ortho Diagnostics Systems, Raritan, NJ, USA) and serum HCV RNA-positive by reverse transcription-polymerase chain reaction (Amplicor HCV test, Roche Diagnostic System, Inc., Branchburg, NJ, USA). Genotyping was performed on biotinylated PCR fragments using a method of reverse hybridization with a line probe assay using oligonucleotide probes specific for the major HCV types and subtypes in the 5' untranslated region (INNO-LIPA HCV, Innogenetics NV, Zwijnaarde, Belgium).

Standard statistical methods were used for data analysis. The χ^2 distribution was used to compare prevalences. Variance analysis (ANOVA) was used to compare the ages of patients. The level of significance chosen was ($\alpha = 0.05$).

RESULTS

The genotype distribution obtained is shown in Table 1. Genotype 1 was the most frequent, occurring in 108 of the 161 patients included in the study (67.1%): 83 had genotype 1b (51.6%) and 17 had 1a (10.6%). Subtyping was not possible in 5 patients with genotype 1 (3.1%). Three subjects had co-infection by subtypes 1a and 1b (1.9%) and were the only cases of mixed infection detected. Forty-three patients had infection by genotype 3a (26.7%). Infection by other genotypes was unusual: genotype 4/5 was found in 8 cases (5.0%) (the test used did not discriminate adequately between genotypes 4 and 5) and genotype 2a in 2 cases (1.2%). Patients with co-infection and patients not subtyped were excluded from statistical analysis.

The distribution of HCV genotypes was associated significantly with source of infection: in the group of patients with a history of blood transfusion and in the group of patients without evidence of parenteral exposure, the prevalence of genotype 1b was 84.6% (33/39) and 67.7% (44/65) respectively, but only 12.2% (6/49) of IVDUs had genotype 1b ($P < 0.001$). In contrast, genotype 3a was present in 65.3% (32/49) of IVDUs, versus 5.1% (2/39) of blood transfusion patients and 13.8% (9/65) of patients without evidence of parenteral exposure ($P < 0.001$). The number of patients infected with other genotypes was insufficient to evaluate their association with origin of infection.

Patient age was related to the HCV genotype (Fig. 1). Patients with genotype 1b were older than those with genotypes 1a or 3a: 50.3 ± 12 vs. 34.1 ± 9.9 and 31 ± 5.4 years, respectively ($P < 0.001$). Of the patients infected with genotype 1b, 25.3% (21/83) were under 40 compared with 95.3% (41/43) of patients infected with genotype 3a ($P < 0.001$). Among non-IVDU patients, the results were similar, the mean age of the 77 patients infected with genotype 1b being 52.1 ± 10.8 years compared with 34.9 ± 11.9 and 34.5 ± 7.2 years for patients infected with genotypes 1a and 3a, respectively. Mean age did not differ between patients who received blood transfusions and patients without evidence of parenteral exposure (50.7 ± 11.4 vs. 46.1 ± 13.3 years, respectively), but IVDUs were significantly younger (30.7 ± 4.2) ($P < 0.001$).

DISCUSSION

Although it was suggested initially that different HCV genotypes were confined to continents or specific geographic regions, more recent studies have shown the coexistence of different genotypes in each zone [11–14]. HCV infection is common in Spain, the prevalence of anti-HCV in the general population being about 2% [15–17]. The genotype of Spanish isolates of HCV is little known but has sometimes been mentioned in international collaborative surveys [13, 14] or in studies of HIV-infected IVDUs [18]. The genotype most often detected in these studies was genotype 1. Overall, the distribution of HCV genotypes found in our study shows a predominance of genotypes 1b, 3a and 1a, which were responsible for 90.7% of HCV infections. The genotype most frequently detected was 1b, which is the genotype most widely extended worldwide and has a high incidence in Europe, Asia and America [6, 7, 9, 12, 19–21]. Genotype 3a was the second most frequent and was observed mainly in IVDUs, among whom it was found in 64%. In contrast, it was detected in only 9.9% of the subjects who did not have a factor of risk of drug addiction. Genotype 3a also has been associated with intravenous drug use in other western countries: 63% of the isolates of HCV obtained from France by Pawlotsky and colleagues were genotype 3a [7] compared with 44% and 21% in recent studies in IVDUs of Germany [22] and Italy [23], respectively. Genotype 3a has been detected for the first time in Thailand [14], where it is predominant [6, 12, 25], but it seems to be exceptional in other Asian countries, such as Japan or China [19, 20]. It

Table 1. Prevalence of hepatitis C virus genotypes in three groups of patients: blood transfusion receptors, intravenous drug users and patients with no history of parenteral exposure

Group of patients	Number	1a		1b		1a + 1b*		Int†		2a		3a		4/5‡	
		Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Blood transfusion	41	0	33 (80.5)	1 (2.4)	1 (2.4)	1 (2.4)	1 (2.4)	1 (2.4)	1 (2.4)	1 (2.4)	2 (4.9)	3 (7.3)			
Without parenteral exposure	70	10 (14.3)	44 (62.9)	1 (1.4)	1 (1.4)	4 (5.7)	1 (1.4)	1 (1.4)	9 (12.8)	1 (1.4)	9 (12.8)	1 (1.4)			
Intravenous drug users	50	7 (14.0)	6 (12.0)	1 (2.0)	1 (2.0)	0	0	0	32 (64.0)	4 (8.0)	4 (8.0)	4 (8.0)			
Total	161	17	83	3	3	5	2	2	43	8					

* Mixed infections.
 † Genotype 1 non subtyped.
 ‡ Genotype 4 or 5.

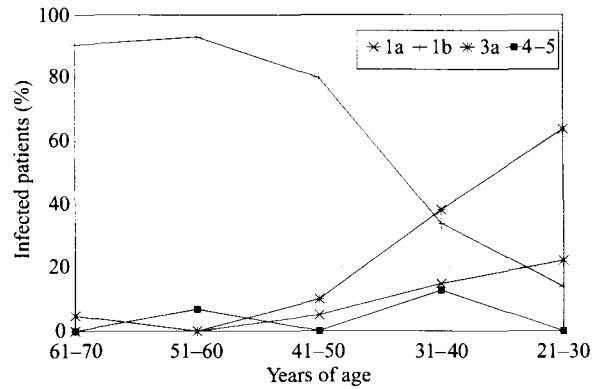


Fig. 1. Prevalence of hepatitis C virus genotypes in relation to age in 161 chronic HCV-infected patients.

was probably transmitted to Europe through intravenous drug use. Genotype 1a is predominant in the US, Canada and Northern Europe [5, 6, 26]. It has been associated with transmission by stored blood products [27] and intravenous drug use [7, 23]. In this study genotype 1a was detected in only 9% of the non-IVDU population and in 14% of IVDUs ($P > 0.05$).

It has been reported that the pattern of HCV genotypes is changing in Europe. Although this idea is based mainly on cross-sectional studies, findings obtained in various countries support this tendency. In general, genotype 1b has been more prevalent in patients who acquired the infection decades ago [9], whereas 3a is prevalent in subjects more recently infected and of younger age [7, 23]. Our study yielded similar results: 25.6% of patients infected with genotype 1b were under 40 vs. 95.2% of those infected with genotype 3a. The mode of transmission of each genotype and the effectiveness of control measures in preventing transmission are key factors contributing to the change in the pattern of HCV genotypes. Screening for anti-HCV in blood banks has reduced the incidence of post-transfusional hepatitis in Spain from about 16% in the early 1980s [28] to about 1% ten years later [29]. The transmission of hepatitis C between IVDUs is more difficult to control because of their lifestyle and the rapid transmission of HCV through needle-sharing [22]. In such circumstances, it is logical that the percentage of HCV patients related with IVDU has increased in recent years. Our findings suggest a relative increment in the prevalence of genotype 3a, which could become the dominant genotype in the future.

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