

## Prevalence of Hepatitis C Virus Genotypes in Italy

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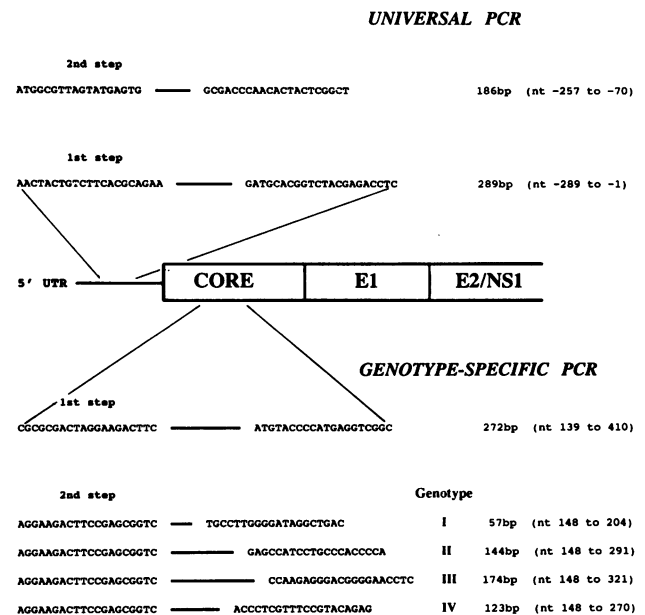
**Hepatitis C viruses (HCV) present in 110 Italian patients were characterized by genotype-specific PCRs. Among the 65 cases of community-acquired hepatitis, HCV genotype II was dominant (60%), followed by genotypes IV (15%), III (11%), and I (3%). Among the 45 hemophilia-associated cases, the distribution of the four HCV genotypes was markedly different: genotype I was the most prevalent (61%), followed by genotypes II (25%), III (4%), and IV (2%). Double infections were observed in eight patients. Two HCV remained unclassified. For the 45 community-acquired cases from which a liver biopsy was available, genotype II was associated with more severe liver damage than the other types.**

Hepatitis C viruses (HCV) are the major etiologic agents of posttransfusion and community-acquired or sporadic non-A, non-B hepatitis. Since the viral genome was cloned a few years ago (4), extensive use of molecular techniques has led to a detailed characterization of its structure, even though many aspects of the biology and epidemiology of the virus remain elusive (5).

On the basis of differences in the nucleotide sequence of the core gene, Okamoto et al. have grouped the HCV detected in different patients into at least four genotypes, which can be readily recognized by the use of genotype-specific PCR (8). The limited epidemiological data available indicate that the relative prevalences of such genotypes may vary considerably in different regions of the world (2). Genotype I appears to be predominant in the United States (70%, also called American prototype), genotype II appears to be predominant in Japan (77%, Japanese K1), while genotypes III and IV (Japanese K2a and K2b, respectively) show lower prevalences in Japan (16 and 5%, respectively) and are rarely found in the United States and western countries (10% for genotypes III and IV) (12). Other HCV classifications have been proposed. For example, Simmonds et al. identified six genotypes on the basis of the sequence configuration in the 5'-untranslated region (3, 11); according to this classification, genotypes I and II of Okamoto et al. are grouped into type 1 (1a and 1b subtypes, respectively), genotypes III and IV of Okamoto et al. are grouped into type 2 (2a and 2b subtypes, respectively). However, the classification proposed by Okamoto et al. is still widely used.

Studies are now in progress to investigate whether HCV genotypes differ in routes of transmission or result in different clinical outcomes (1, 13). In this study, we have examined the distribution in 110 Italian patients of the four HCV genotypes identified by Okamoto et al. These subjects had previously been found to be viremic by a nested reverse transcriptase PCR which recognizes most if not all HCV (6), as it uses primers covering the conserved 5'-untranslated region of the viral genome (Fig. 1, universal PCR). Sixty five patients had community-acquired infections, while 45 were hemophiliacs who had been extensively treated with com-

mercial coagulation factor concentrates in previous years. The patients lived mainly in northern and central Italy and were aged 15 to 84 years. After collection, the sera were stored at -80°C until analyzed. According to a published procedure (10), to minimize the risk of contamination, genotyping was performed directly on serum, since prior studies had shown that RNA extraction could be omitted with no apparent loss of sensitivity. Viral RNA was first reverse transcribed and then amplified for 35 cycles (melting at 94°C for 3 min in the first cycle and for 30 s in the following ones, annealing at 45°C for 1 min, and extension at 72°C for 3 min) with the first-step primers shown in Fig. 1 (genotype-specific PCR). The product of such a reaction was then reamplified for 24 cycles (the temperature and duration of each step were as described above) with a mixture of genotype-specific, second-step primers (Fig. 1). The amplified products were finally analyzed by gel electrophoresis in 4% agarose and



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FIG. 1. Primers used for universal and genotype-specific PCR analyses. nt, nucleotides; 5'-UTR, 5'-untranslated region.

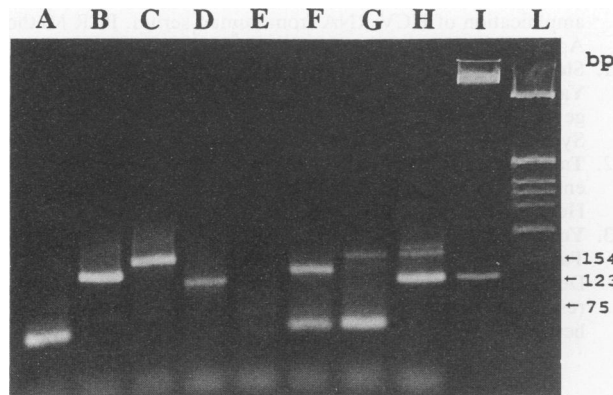


FIG. 2. Representative results of the genotype-specific PCR. Lanes: A to D, single infections with genotype I (57 bp), II (144 bp), III (174 bp), or IV (123 bp), respectively; E, infection with an unclassified genotype; F to H, double infections with genotypes I and II, I and III, or III and IV, respectively; I and L, DNA molecular size markers (123-bp ladder and *Hinf*I-digested pBR322, respectively).

stained with ethidium bromide. The sizes of amplicons were determined by comparison with appropriate molecular size markers (100-bp ladder [Pharmacia Biotech Europe], 123-bp ladder [GIBCO BRL], and *Hinf*I-digested pBR322) (Fig. 2). Each serum specimen was examined at least twice, and no conflicting results were observed.

The results are summarized in Table 1. The great majority of the HCV examined (98%) were typed by the procedure used. Among the community-acquired infections, HCV genotype II was largely dominant (60%), followed by genotypes IV (15%), III (11%), and I (3%). Thus, it would appear that the prevalence of HCV genotypes in Italy is similar to that reported for Japan (8) and differs substantially from the distribution observed in the United States (3, 12).

For the hemophilia-associated cases, the distribution of the four HCV genotypes was markedly different: genotype I was the most prevalent (61%), followed by genotypes II (25%), III (4%), and IV (2%). This result most likely reflects the fact that the concentrates used by these patients were largely prepared from blood purchased abroad (9). Alternative explanations should, however, be considered. These include the possibility that different HCV genotypes preferentially use different means of transmission or react differently to the treatments used for the preparation of concentrates. Fifteen hemophiliacs were also infected with human immunodeficiency virus type 1. No differences were noted in the distribution of HCV genotypes in these patients com-

TABLE 1. HCV genotypes in Italian patients with community-acquired or hemophilia-associated hepatitis C

Type of hepatitis	No. of patients examined	No. of patients with the following HCV genotype:					
		I	II	III	IV	Mixed	Unclassified
Community acquired	65	2 <sup>a</sup>	39 <sup>a</sup>	7	10	5 <sup>b</sup>	2
Hemophilia associated	45	28	11	2	1	3 <sup>c</sup>	

<sup>a</sup> Significantly different from the hemophilia-associated group (chi-square test;  $P < 0.001$ ).

<sup>b</sup> Genotypes I and II, I and III, and III and IV in one patient each and II and III in two patients.

<sup>c</sup> Genotypes I and II, I and IV, and III and IV in one patient each.

TABLE 2. HCV genotypes in Italian patients with community-acquired hepatitis C, grouped according to results of liver biopsies

Result of liver biopsy	No. of patients examined	No. of patients with the following HCV genotype:				
		I	II	III	IV	Mixed
Persistent chronic hepatitis	12	2	3 <sup>a</sup>	2	4	1 <sup>b</sup>
Active chronic hepatitis	25		17	2	3	3 <sup>c</sup>
Cirrhosis	8		7	1		

<sup>a</sup> Significantly different from the groups of patients with severe infections (active chronic hepatitis and cirrhosis) (chi-square test;  $P < 0.01$ ).

<sup>b</sup> Genotypes I and III.

<sup>c</sup> Genotypes II and III in two patients and genotypes III and IV in one patient.

pared with the human immunodeficiency virus-seronegative ones (data not shown). The concomitant presence of two viral genotypes was observed in three hemophiliacs as well as in five community-acquired cases.

A liver biopsy was available for 45 patients with community-acquired hepatitis. As shown in Table 2, patients with more severe degrees of liver damage (active chronic hepatitis or cirrhosis) showed a significantly higher prevalence of genotype II than patients with persistent chronic hepatitis. Although the numbers of patients examined are still too low to draw firm conclusions on this important matter, these data and other reports encourage further studies to better evaluate the pathogenic potential of the various HCV genotypes (1, 7, 13).

The two HCV which remained unclassified by the procedure described above were not amplified, even when examined by use of the four genotype-specific primers separately. The fact that under identical conditions of reaction such viruses were consistently amplified by the universal primers seems to exclude the possibility that the failure of typing was due to the presence of inhibitors in the sera or to degradation of the viral genome and suggests that they belong to genotypes not covered by the primers used. Sequencing the core region of these HCV might lead to the recognition of additional, hitherto-unrecognized HCV genotypes.

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