Journal of Viral Hepatitis, 2005, **12,** 405–413 doi:10.1111/j.1365-2893.2005.00605.x

Changing of hepatitis C virus genotype patterns in France at the beginning of the third millenium: The GEMHEP GenoCII Study

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Received February 2004; accepted for publication July 2004

SUMMARY. This cross-sectional study aimed to investigate, during a short period between 2000 and 2001, in a large population of patients with chronic hepatitis C, the epidemiological characteristics of hepatitis C virus (HCV) genotypes in France. Data from 26 referral centres, corresponding to 1769 patients with chronic hepatitis C were collected consecutively during a 6-month period. HCV genotyping in the 5′-non-coding region (NCR) was performed in each center using the line probe assay (LiPA, in 63% of cases), sequencing (25%) or primer-specific polymerase chain reaction (PCR) (12%). HCV genotypes 1a, 1b, 2, 3, 4, 5, non-subtyped 1 and mixed infection were found in 18, 27, 9, 21, 9, 3, 11 and 1% of our population, respectively. HCV genotype distribution was associated with gender, age, source and duration of infection, alanine aminotransferase (ALT) levels, cirrhosis, alcohol consumption, hepatitis B virus (HBV) and human immunodeficiency virus (HIV) coinfection. In multivariate analysis, only the source of infection was the independent factor significantly associated with genotype ($P = 0.0001$). In conclusion, this

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; PCR, polymerase chain reaction; LiPA, line probe assay. NCR, non-coding region.

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study shows a changing pattern of HCV genotypes in France, with i.v. drug abuse as the major risk factor, an increase of genotype 4, and to a lesser extent 1a and 5, and a decrease of genotypes 1b and 2. The modification of the HCV genotype pattern in France in the next 10 years may require new therapeutic strategies, and further survey studies.

Keywords: epidemiology, evolution, HCV genotypes, pathogenicity, treatment.

INTRODUCTION

Hepatitis C virus (HCV) is worldwide the major aetiologic agent of post-transfusion and community-acquired non-A, non-B chronic hepatitis, leading to liver cirrhosis and hepatocarcinoma [1]. In France, it is estimated that about 500 000 persons are infected but 30–40% are not aware of their infection [2]. Among them, around 20% may lead to a progressive liver disease and to cirrhosis after 20–30 years following contamination [3].

On the basis of differences in the nucleotide sequence of several HCV strains, showing between 5 and 30% variability, a classification into at least six major genotypes (1–6) and several subtypes (a–h) has been proposed in a consensus nomenclature system [4]. The emergence of the major genotypes and subtypes based on a high spontaneous mutation rate of $1.5–2 \times 10^{-3}$ per nucleotide per year was estimated at 500–2000 and 100–300 years ago, respectively [5]. This long genetic evolution of genotypes might involve variable pathogenic patterns. Indeed, several European studies suggested that the HCV genotype 1b may be linked to the severity of liver disease [6–9] but some others did not [10–12]. Thus, in a previous study, concerning a large population of 1872 HCV-infected patients in France, over a large period (from 1989 to 1997), we found no relation between genotype and cirrhosis in multivariate analysis [13]. Cirrhosis, in this study, was independently related to the age of patients at the time of exposure, the mode of contamination, the duration of contamination and high serum ALT levels. These factors were all associated to specific genotypes as sources of confusion in previous studies. On the contrary, HCV genotype has been well recognized as a major independent factor for antiviral response under treatment with interferon (IFN), pegylated-IFN and their combinations with ribavirin [12,14–17]. Indeed, response rates varied from 0 to 80% in these controlled studies regarding to the type of treatment and for each group of treatment, to the genotype; better results were obtained with genotype 2 and 3, the least with genotype 1 (and probably 4). However, we also found in our previous study that HCV genotype was varying with the mode and age of contamination, and we observed during the 1990s an increase of genotype 4 in France.

The aim of our study, at the beginning of the new millenium (2000–2001), was to evaluate the evolution of HCV genotypes in France in a short period (6 months), in a large cohort of patients (1769), in several regions (seven) and centres (26), with regard to their epidemiological characteristics and the new treatment using pegylated-IFN and ribavirin combination.

MATERIAL AND METHODS

Data collection

Data were collected uniformly from 26 main hospitals in France between September 2000 and April 2001. The first 100 consecutive HCV-genotyped patients or all genotyped patients during that period if below 100 were included anonymously in each centre. At the end of this period, 1769 patients with chronic HCV infection (i.e. anti-HCV positive and with detectable serum HCV-RNA) were included in this cross sectional study (GenoCII study). Most of the patients had been referred by a hospital hepatologist, but also by their physician or by infectious disease units, for pretreatment check-up. Six geographical regions in France were reported as: north (two centres, 154 patients), north-east (two centres, 106 patients), west (three centres, 135 patients), south-east (five centres 354 patients), south-west (two centres, 158 patients), central (three centres, 105 patients) and Paris and area (10 centres, 757 patients). Among the 1769 patients, geographical origin was identified in 1340 (1052 originating from France and 11 from other western European countries, 49 from southern Europe, 112 from North Africa, 67 from Central Africa, 25 from Asia and 14 from South America). Patients' characteristics are shown in Table 1. Data from 26 variables such as age, gender, risk factors for HCV acquisition, duration of infection, serum ALT levels, hepatitis cofactors such as alcohol consumption, hepatitis B or impaired immunity associated to HIV coinfection or renal transplantation, liver histology findings at the time of presentation, and genotyping indication were recorded for each patient when available (representing 37 756 recorded data and 82% exhaustivity in this study). Liver histology, reported for 73% of the patients, was classified into five groups regarding the *F*-score of METAVIR performed independently in each of the 26 centres: normal liver designed as F0; mild hepatitis, F1; moderate hepatitis, F2; severe hepatitis, F3; and cirrhosis, F4 [18]. If done, previous treatment for chronic hepatitis C for each patient was also recorded with Interferon doses, ribavirin association, treatment duration and virological response (defined as a negative polymerase chain reaction (PCR) 6 months after the end of treatment).

Table 1 Characteristics of the 1769 patients with chronic hepatitis C in the GenoCII study

Characteristics	Data
Men/Women	1065/699
Mean age (years*)	45 ± 12
Source of HCV infection (%)	
Blood transfusion	27
Intravenous drug addiction	38
Other parenteral exposure to blood	10
Unknown	25
Duration of HCV infection (years*)	11 ± 11
Serum HCV RNA (log_{10} UI/mL*)	5.86 ± 0.83
Serum ALT (×normal*)	2.27 ± 2.05
Liver histology (done %)	73
Normal	6
Mild active hepatitis	39
Moderate active hepatitis	28
Severe active hepatitis	14
Cirrhosis	13
HIV coinfected (%)	8.4
HBV coinfected (%)	1.2
Alcohol abuse (>15 g/day, %)	22
Previous hepatitis C treatment (%)	13

*Mean ± SD (the upper limit of normal value).
ALT, alanine aminotransferase; HCV, hepatitis C virus.

Detection and quantification of serum HCV-RNA

Serum HCV-RNA was detected in each centre using the one-step reverse transcription-polymerase chain reaction (RT-PCR) with biotinylated primers located in the 5′-non-coding region (5′-NCR) of the HCV genome as recommended by the manufacturer (Amplicor HCV 2.0, with a positive cut-off at 50 UI/mL kit; Roche Diagnostics, Neuilly, France) [19]. Serum HCV-RNA quantification was performed in the 26 centres for 65% of the patients, using the improved quantitative branched DNA (bDNA) signal amplification assays (Quantiplex HCV RNA 2.0 and 3.0; Bayer-Chiron Diagnostics, Eragny sur Oise, France) [18] or the RT-PCR-based method (Monitor HCV RNA 2.0; Roche Diagnostics). The quantification cut-off of the bDNA 2.0 assay is 0.2×10^6 HCV Meq/mL or 30 000 UI/mL; cut-off of the bDNA 3.0 assay and the Monitor 2.0 is similar at 600 IU/mL. Whatever was the reported assay in the centres, HCV-RNA viral load was expressed in log_{10} UI/mL.

HCV genotyping assays

Hepatitis C virus genotyping was performed on serum collected during the period study, using mostly (63% of the genotypes in this study) reverse hybridization in the 5′-NCR of the HCV genome with the line probe assay (LiPA) combined to the qualitative Amplicor assay (InGeN, Rungis, France) [20]. The HCV LiPA contains 15 probe lines, allowing the identification of HCV types 1–10 and subtypes la, lb, 2a/2c, 2b, 3a, 3b, 4a, 4c/4d, 5a and 6a, according to the classification of Simmonds *et al.* [4]. Subtyped 1a/1b or non-subtyped 1 were referred to as genotype 1 in this study. Other genotyping methods such as sequencing (25%), primer-specific PCR or enzyme-restriction PCR (12%) in the 5′-NCR of HCV were used in this study. All these methods have been validated in the French ANRS panel quality control study in 2000 with about 98% concordance [19].

Statistical analysis

For the descriptive analysis, all the 1769 genotypes were used. Statistical analysis was performed using the main genotypes found (la, lb, 1, 2, 3, 4 and 5), corresponding to 99% of the patients in this study. The chi-square test and Student's *t*-test were used to compare categorical and quantitative data, respectively. To test two different effects on a quantitative parameter, a two-way analysis of variance (ANOVA) was performed. A Kruskal–Wallis non-parametric test was used for variables with a non-Gaussian distribution. Logistic regression analysis was used to evaluate the independent predictors of cirrhosis. All analyses were performed using BMDP (BMDP Statistical Software Inc., Berkeley, University of California, CA, USA).

RESULTS

Geographical distribution of HCV genotypes in France

Hepatitis C virus genotype lb was the most common in our study (485 patients, 27.4%); genotype 3 was found in 368 patients (20.8%), genotype 1a in 325 patients (18.4%), genotype 1 in 202 patients (11.4%), genotype 2 in 164 patients (9.3%), genotype 4 in 158 patients (8.9%), genotype 5 in 48 patients (2.7%), genotype 6 in three patients (0.2%) and mixed genotypes in 16 patients (0.9%). Although we observed a relatively homogenous genotype distribution in the different geographical regions (Fig. 1), some significantly different distribution was found ($P < 0.0001$). Genotype 2 was found more frequently in the south-west than in the other regions (15.8 *vs* 5.4 to 11.2%). Genotype lb was found less frequently in southern regions and in Paris (22.9–26.6 *vs* 34.4–39.6% for other regions) and genotype 4 was predominant in Paris (10.9 *vs* 5.2–8.8%). A sporadic genotype 5 infection was noted in central France during the period study, with 17% of cases in one centre (0.7–2.6% for the others), increasing the global rate of genotype 5 in France from 2.1 to 2.7%. HCV genotype distribution varied significantly according to the origin of the patients (Fig. 2) ($P < 0.0001$). Genotypes 2 and 4 were mostly found in patients from Central Africa (28.6 and 30.6%, respectively) and the further highest values were found in those from southern Europe (14.3 and 17.9%, respectively). Genotype 1b was predominant in

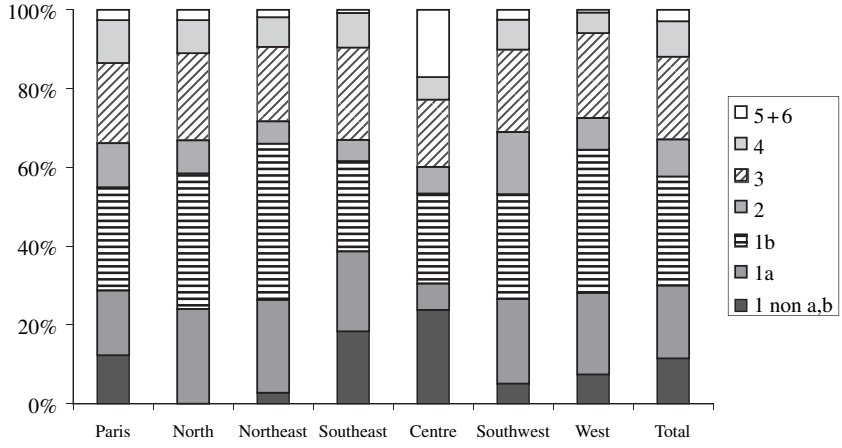

Fig. 1 Regional French distribution of hepatitis C virus (HCV) genotypes ($P < 0.0001$). Genotype 1b was observed more frequently in the north, north-east and west of France, genotype 2 in the south-west and 4 in Paris and south-east. Note the incidence of epidemic genotype 5a HCV infection observed in central France during spring 2001 showing an unusual prevalence of 17% in this region.

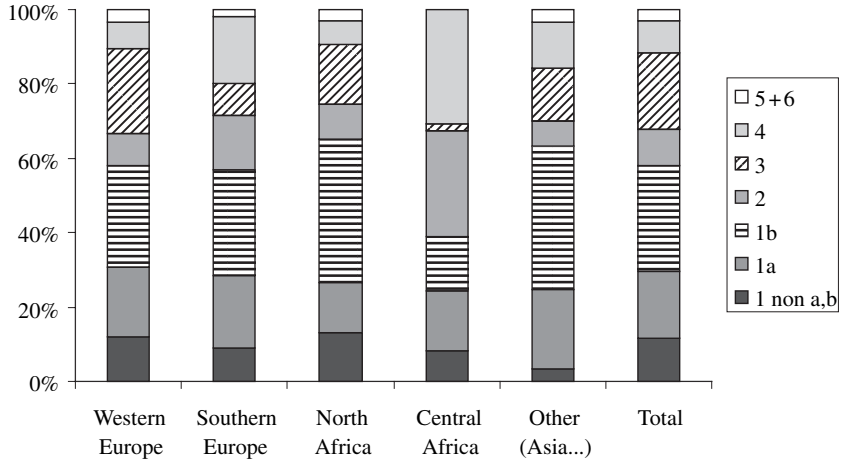

Fig. 2 Geographic origin of hepatitis C virus (HCV) genotypes ($P < 0.0001$). Predominant genotype 1b was observed in North Africa, genotype 3 with 1b in western Europe and genotypes 2 and 4 in Central Africa. Note the increase of genotypes 2 and 4 in southern Europe as well as in Paris in the Black communities.

patients from northern Africa and from Asia (38.8 and 38.6%, respectively) and genotype 3, in those from western Europe including France (22.7%).

Epidemiological characteristics of the patients and HCV genotypes

Hepatitis C virus genotype distribution was significantly different according to gender (Table 2) ($P < 0.0001$). Genotype lb was found more frequently in women than in men (32.9% *vs* 24.1%) and genotype 3 was found more frequently in men than in women (24.5% *vs* 15.8%). Genotype 4 was also more often found in men and genotype 2 in women. HCV genotype distribution was also significantly different according to age (Fig. 3) ($P < 0.0001$). Genotypes la, 3 and 4 were more common in patients <40 years than in those >50 years (27.1, 27.3 and 10.4% *vs* 8.6, 6.8 and 4.2%). Conversely, genotypes lb and 2 were less common in patients <40 years than in those >50 years of age (21.3 and 3.8% *vs* 42.1 and 17.9%, respectively). However, we observed a trend to genotype 1b increase (24.3%) and genotype 3 decrease (23.6%) in patients <30 years, although statistically significant differences were not reached. HCV genotype distribution was also significantly different according to the source of infection (Fig. 4) ($P < 0.0001$). Genotypes lb, 2 and 5 were found more frequently among patients with a history of blood transfusion than among patients with a history of intravenous drug use (IVDU) (39.5, 13.4 and 5.9% *vs* 14.2, 2.4 and 0.1%, respectively). Conversely, genotypes la, 3 and 4 were found more frequently among patients with a history of IVDU than of blood transfusion (26.9, 36 and 10.5% *vs* 12.3, 9.8 and 6.4%, respectively). Finally, the HCV genotype distribution in the patients with nosocomial or unknown source of infection was similar to that in patients with a history of blood transfusion. Genotype distribution was different according to the immune status (Table 2) ($P < 0.0001$). The genotype distribution in transplanted and immunocompetent patients was comparable with that in patients with a history of blood transfusion whereas genotype distribution in HIV-coinfected patients was similar to that in patients with a history of IVDU (Fig. 4). The duration of infection was also significantly associated with HCV genotype ($P = 0.01$). In patients infected with genotypes la or 5,

Table 2 Hepatitis C virus (HCV) genotype distribution in France in 2001

	HCV genotype								
	1a	1b	1	2	3	4	5	*n*	*P*-value
Number (%)	325 (18.6)	482 (27.6)	202 (11.6)	163 (9.3)	368 (21.1)	158 (9.0)	48 (2.7)	1750	<0.0001
Men/women (%)	19.2/17.7	24.1/32.9	12.4/10.3	7.8/11.7	24.5/15.8	10.0/7.5	2.1/4.1	1058/692	<0.0001
Infection duration (year*)	12.1 ± 10.8	11.6 ± 12.2	9.3 ± 11.6	11.1 ± 13.5	11.5 ± 10.0	9.6 ± 10.1	13.1 ± 12.1	1352	0.01
HCV-RNA (log_{10} UI/mL*)	5.88 ± 0.89	5.89 ± 0.83	6.07 ± 0.75	5.67 ± 0.88	5.78 ± 0.80	5.76 ± 0.82	5.75 ± 0.79	1296	0.001
ALT (×normal*)	2.03 ± 1.40	2.22 ± 2.15	2.19 ± 1.64	2.38 ± 2.58	2.50 ± 2.08	2.32 ± 2.64	2.42 ± 2.14	1736	0.003
HIV coinfected (%)	35.1	14.2	3.4	4.1	28.4	14.9	0	147	<0.0001
Liver histology (%)								1263	
Normal	28	23	11	15	19	4	1	72	0.006
Mild hepatitis	20	30	10	8	20	10	2	492	
Moderate hepatitis	17	26	11	7	21	12	5	354	
Severe hepatitis	15	34	13	10	21	4	3	176	
Cirrhosis	15	27	11	10	29	5	2	169	

*Mean ± SD; Percentage is reported to the number (*n*) of patients per class; Significant *P*-value is below 0.05.

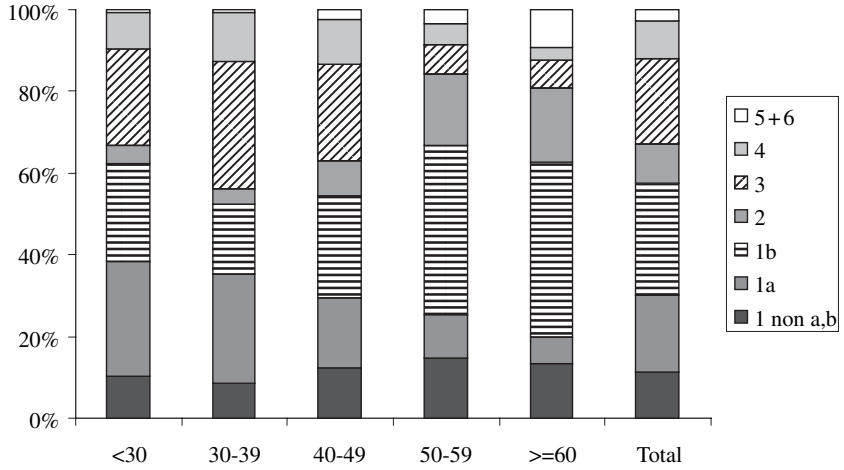

Fig. 3 Hepatitis C virus (HCV) genotype distribution according to the age (in years) of the patients ($P < 0.0001$). Genotypes 1b and 2 were observed more frequently in the older patients. Conversely, genotypes la, 3 and 4 were also observed more frequently in younger patients. Note the reincrease of genotype 1b in the youngest group.

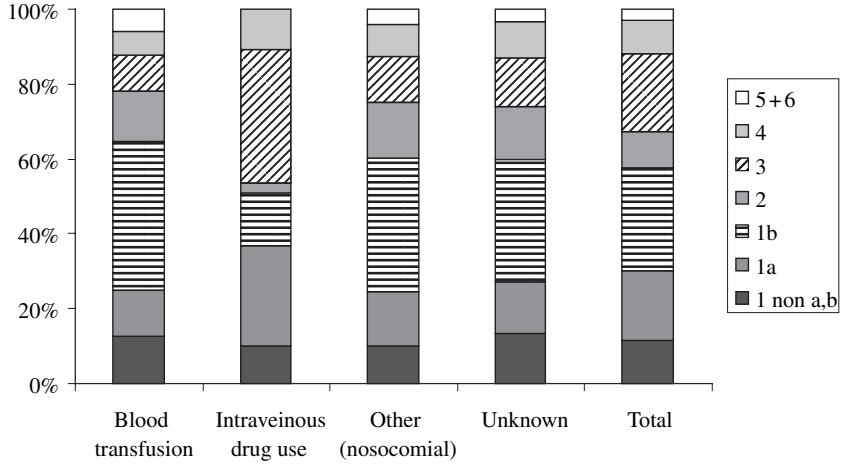

Fig. 4 Hepatitis C virus (HCV) genotype distribution according to the source of infection ($P < 0.0001$). Large modification in genotype distribution was observed in blood-transfused patients with mostly genotypes 1b, 2 and 5 till the late 1980s compared with intravenous drug users with increased genotypes 1a, 3 and 4. Note the similar distribution in nosocomial or unknown source and blood transfusion.

the average duration of infection was longer when comparison with the other genotypes. However, we found a variable genotype distribution according to the period of contamination ($P < 0.0001$), with genotypes 1b and 2 mostly found in patients with a longer period (36.5 and 14.8%, respectively, if time exposure over 20 years *vs* 23.5 and 5.3% if below) and 1a and 3 within a shorter period (24.3 and 26.0% if below 20 years *vs* 13.9 and 15.0 if more). HCV genotype distribution was different according to serum HCV-RNA levels but not if the genotype 1 group was withdrawn (Table 2). However, using multivariate analysis, the association between genotype and gender, age, duration of infection, immune status was related to the source of infection, which was the only factor independently and significantly associated with HCV genotype ($P = 0.0001$).

Liver disease severity and HCV genotypes

Serum ALT levels were significantly higher in the group of patients infected with genotype 3 when compared with the others (Table 2) ($P = 0.003$). Genotypes 1a and 3 were associated with alcohol consumption, whereas genotypes 1b and 4 were mostly associated with hepatitis B coinfection ($P < 0.0001$). Data allowing histological classification were available for 1292 patients (73%) using the METAVIR score. Normal histology was found in 78 patients (6%), mild hepatitis in 503 (39%), moderate hepatitis in 361 (28%), severe hepatitis in 181 (14%) and cirrhosis in 168 (13%). HCV genotype distribution was different according to liver fibrosis score ($P = 0.006$) but not to the inflammatory activity score (Table 2). Genotypes 1a and 2 were mostly observed in patients with normal or mild hepatitis, genotype 1b in those with more severe hepatitis and genotype 3 in those with cirrhosis. Cirrhosis was found more frequently in patients infected with genotypes 1b and 3 (27 and 29%, respectively) compared with patients infected with genotypes la, 2 or 4 (15, 10 and 5%, respectively). However, in multivariate analysis, HCV genotype distribution was not related to cirrhosis. Cirrhosis was associated with age >40 years at exposure ($P = 0.01$), high serum ALT level ($P = 0.001$), immune-compromised status (i.e. HIV coinfection) ($P = 0.001$) or hepatitis cofactors (alcohol or hepatitis B) ($P = 0.0001$).

DISCUSSION

This cross-sectional study showed, on a large French population with chronic hepatitis C attending medical care in 26 main hospitals in France, taken in a short period between 2000 to 2001, comprising mostly recently diagnosed and thus untreated patients (<4 years), that HCV genotype distribution is changing in France. Although genotype 1b is still the most common one (27.4%) and found mainly in patients infected by blood transfusion (39.5%) and genotype 3 is still the second most common (20.8%) and found mainly among patients infected by IV drug use (36%), we observed a large decrease of genotype 1b since our previous study (−13%) [13], whereas genotype 3 remained stable (−1%). On the contrary, genotypes 1a, 4 and 5 increased (+2, +5 and +2%, respectively) whereas genotype 2 decreased (−2%).

With regard to these genotype variations, we observed in this study for the first time that the most predominant reported mode of contamination in France was the i.v. drug use (38%). It is well known that nowadays the major risk factor of contamination is IVDU but this study reported cases of patients infected in the 1980s and in the early 1990s when HCV screening was mandatory in blood donors. Thus, genotypes 1a, 3 and to a lower extent, genotype 4, were mostly associated to IVDU as reported in an HCV genotype observatory study in France [21]. Furthermore, these genotypes were mostly found in HIV coinfected patients who shared the same mode of contamination. However, genotype 3 was noticed in older patients (between 30 and 40 years) whereas genotype 1a was observed in younger patients. Indeed, genotype 3 is associated with contamination after the late 1970s with IVDU coming from India, where this genotype is predominant [22] and genotype 1a is known to be the major one in North America [23], whereas genotypes 1b and 2 were associated to contamination by transfusion from the early 1960s to the late 1980s [13]. It is of note in this study that similar genotypes were found in transfused, transplanted and nosocomial infected patients, with 1b as the most prominent genotype.

Surprisingly, we also noticed a re-increase of genotypes 1b and 3 in young adults (<30 years). In these cases, transfusion was the most common related mode of contamination. This re-increase might be related to the HCV infection screening programme in the late 1990s in France. Although genotype distribution was fairly uniform in France as in our previous study [13], some variations were observed with a higher proportion of genotypes 2 and 4 in the southern regions and in Paris where larger immigration is found. Indeed, these genotypes were mostly found in patients of Central African origin as reported previously [24–26].

We were surprised to observe that mixed genotypes were stable and rare with <1% of cases. Indeed, as IVDU is now the major risk factor of contamination, HCV reexposure is expected to increase leading to mixed infections. This is not the case here. However, emergence of these mixed genotypes might take more time and should be therefore followed on a longer period. Although HCV genetic evolution is known to involve punctual mutations at a rate of about 10^3 per nucleotide per year, recent recombinant strains have been reported in Russia from 1a–3 mixed genotype-infected patients [27]. Numerous questions concerning these recombinant viruses will remain: how often do their occur? what is the consequence in genotyping study and assays? is there a specific resistance to antiviral treatments? Moreover, genotype follow-up of mixed infection has to be maintained. We were also surprised to note in this study a high proportion of non subtyped genotype 1 (about 10%) involving an increase of diversity in genotype 1 or a technical typing problem. Indeed, genotyping in the HCV 5′-NCR showed little gene modification from 1a to 1b or other genotypes 1 (only one mutation and even no mutation in some strains) compared to HCV NS5b region. However, these non-subtyped genotype 1 patients showed a similar epidemiological pattern to that of HCV 1b-infected patients.

As shown previously [13], we reported no relation of genotype to liver disease. Discrepant results have been published suggesting that type 1b could be associated to cirrhosis [6–13]. In a European study on a 7-year follow-up of patients, genotype 1b and 2 were the most common in cirrhotics and genotype 1b was related to a higher risk for decompensation but was not associated to hepatocellular carcinoma development [9]. We showed in our study that cirrhosis in multivariate analysis was associated to the age of patients at time of exposure, to the duration of infection but not to the genotype. Indeed, genotypes 1b and 2 were found in patients showing the oldest HCV infection duration. Furthermore, cirrhosis was independently associated with hepatitis cofactors such as alcohol consumption and HBV or HIV coinfection. It is also well known that immune deficiency in HIV coinfection increases the risk of rapid progression to cirrhosis in HCV infection [9]. In these cases, genotypes 1a, 3 and 4, are mostly found. Indeed, we found a close relation between genotype 3, ALT increase and cirrhosis in univariate analysis, but only high ALT levels and cirrhosis were related in multivariate analysis. These findings showed that many host characteristics might induce confusion between genotype and liver disease association.

Genotype 1 has been well recognized as a strong predictor of bad response to IFN treatment, with 9–46% of response regarding to IFN monotherapy, IFN pegylated forms or combined therapy with ribavirin, compared with 40–80% of response with genotype 2 or 3 [14–17]. It seems that no difference in response has been reported between subtype 1a and 1b, and that a low response is noticed with genotype 4 [25]. Little is known concerning types 5 and 6. However, these genotypes have been associated to types 1 and 4 in standard therapeutic schedules in the last HCV consensus conference in Paris, in February 2002 [2]. We noticed an increase of genotypes 1a, 4 and 5 in our study for which no complete data are available concerning their response to

treatment. This observation of genotype evolution should be confirmed in the next 10 years in regard to the yet standard pegylated-IFN and ribavirin combination and future antiviral treatments.

In conclusion, our results show, in a large cohort of patients, prospectively recorded in a short period of time, that HCV genotype is changing in France, with an increase of genotype 4 at a level similar to genotype 2. This modification is related to the source of infection that has been changing in the past 20 years in France, with HCV antibodies blood screening and maintenance of IVDU [28]. Immigrants also participate largely to new genotype diffusion. However, HCV genotype is a strong predictor for sustained response to therapy but is not a prognostic factor for the severity of liver disease. Our study reinforces the need of a HCV genotype evolution survey in regard to modifications of epidemiological characteristics, to liver disease progression and to new therapeutic protocols.

ACKNOWLEDGEMENTS

This project was supported by the Groupe d'Etude Moléculaire des Hépatites (GEMHEP), France. We wish to thank colleagues who participated to this study: Pr P. Lebon, Pr D. Sicard (GH Cochin St Vincent de Paul, Paris), Dr J. Moussalli, Dr K. Gourlain (GH Pitié-Salpétrière, Paris), Dr X. Amiot (Hôpital Tenon, Paris), Pr Fleury, Dr J. Foucher, Dr P. Bernard (Hôpital Pellegrin, Bordeaux), Pr D. Ingrand, Pr S. Naveau, Dr P. Mathurin, Pr F. Boue, Dr V Chambrin (Hôpital Antoine Béclère, Clamart), Dr M. Bouvier-Alias (Hôpital Henri Mondor, Créteil), Pr F. Denis (Limoges), Dr A. Bourgoin (Poitiers), Dr S. Levy (Reims), Dr I. Mendel, Dr O. Goria (Rouen), Pr B. Pozzetto (St Etienne), Pr D. Vetter (Strasbourg), Dr K. Sandres (Toulouse). We also wish to thank the 'Comité de la Ligue contre le Cancer de la Vienne' for financial support and Mrs Anne Kent for reviewing this paper.

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