MAJOR ARTICLE

Hepatitis C Virus Genotypes and Risk of Cirrhosis in Southern Italy

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Because hepatitis C virus (HCV) genotypes have raised considerable interest as variables that influence chronic hepatitis C progression, a case-control study was conducted to estimate their effects on patients with cirrhosis. Case patients ($n = 46$) had tested positive for anti-HCV antibody and HCV RNA and were residents of the study area who had cirrhosis recently diagnosed. Controls ($n = 138$) were drawn randomly from a residents' cohort from the same area. Demographic and other information were recorded. Presence of HCV infection, presence of HCV RNA, and HCV genotypes were assessed. Crude, stratified, and logistic regression analyses were performed. HCV genotype 2a/c occurred in 84 controls (60.9%) and 9 case patients (19.6%); HCV genotype 1b was found in 45 controls (32.6%) and 34 case patients (73.9%). HCV 1b genotype showed an independent effect on the risk of cirrhosis (odds ratio, 7.49; 95% confidence interval, 3.15–17.81). No significant effects related to other variables were observed. These results indicate that the genetic diversity of HCV phylogenetic variants may explain differences in biological behaviors.

It is estimated that <20% of patients who are chronically infected with hepatitis C virus will develop cirrhosis during a 20-year period [1]. The variables that influence the progression of chronic hepatitis C are only barely known and probably consist of a complex interplay between different factors. Known risk factors can explain only part of the observed variability. During recent years, considerable attention has been given to hepatitis C (HCV) genotypes [2] as predictors of outcome. HCV genotype 1b has been associated with advanced stages of disease and risk of hepatocellular carcinoma in patients with cirrhosis [3–5]. These associations are consistent with evidence that has implicated HCV genotype 1b with resistance to interferon [6, 7], recurrence of HCV infection in patients who have undergone transplantation [8], and reduced turnover of virus and antiviral immune responses [9, 10]. Several limitations have hampered the establishment of definite evidence for a differential pathogenicity of HCV genotypes, including the long duration of HCV-related liver disease, cohort effects in the circulation of viral types, and the lack of convincing biological bases for differences in clinical behavior among viral types.

Our study was based in an area of southern Italy that is inhabited by an extremely stable rural population, that is devoid of relevant environmental or dietary risk factors for liver disease, and that was persistently exposed to HCV until, at least, the early 1950s without appreciable cohort effects in the distribution of HCV genotypes [11, 12]. The aim of this case-control study was to estimate the effect of HCV genotype on the risk of development of clinically evident cirrhosis.

Received 15 September 2000; revised 14 November 2000; electronically published 5 June 2001.

Informed consent was obtained from each patient and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the appropriate institutional review committee.

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Clinical Infectious Diseases 2001; 33:70–5

1058-4838/2001/3301-0011$03.00

METHODS

The study base was the person-time generated by all persons who were aged ⩾30 years who lived in a local health authority (Azienda Sociosanitaria Locale Bari/5) of ~250,000 inhabitants located in the southeast of the Bari province (Apulia) of Italy from January 1991 through December 1992. The study base was ~500,000 person-years at risk. In the study area, there are several general hospitals and one specialized referral center for patients with liver and digestive diseases (Istituto di Ricovero e Cura a Carattere Scientifico "Saverio De Bellis," Pavia, Italy).

Residents in the study area who had cirrhosis recently clinically diagnosed during the accrual period and who were referred to our hospital (IRCCS "Saverio De Bellis"), as either inpatients or outpatients, were considered to be case patients. The case definition included clinical and/or histological criteria for cirrhosis. Clinical criteria were the presence of esophageal and/or gastric varices at endoscopy and thrombocytopenia (i.e., platelet count of ⩽100,000 platelets/mm^3) [13]. A liver biopsy specimen was obtained when clinically requested (17 subjects). All case patients tested positive for anti-HCV antibody and HCV RNA; case patients who tested positive for hepatitis B surface antigen were excluded from the study.

Controls were randomly drawn from a cohort (age, ⩾30 years; residents of the area) enrolled from 1985 through 1986 and followed up from 1989 through 1992 [11]. Control sampling was done without replacement, taking into account the time of diagnosis or follow-up. All controls underwent the same procedures, but only follow-up information was used and incident HCV infections were excluded. All controls tested positive for anti-HCV antibody and HCV RNA and all were free of cirrhosis, as assessed by physical examination, ultrasound scan of the liver, routine laboratory tests, and review of medical records. Three unmatched controls were selected for each case patient.

A validated semiquantitative food frequency questionnaire was administered; questions about alcohol intake sought information regarding type of alcoholic beverages consumed, frequency of alcohol use, and glass size. [14]. Blood samples were obtained at enrollment and frozen until the time of testing.

Serum samples were screened by use of HCV ELISA and confirmed by use of RIA (Ortho Diagnostic Systems). Serum HCV RNA was tested by use of nested reverse transcriptase–PCR with conserved primers in the 5′-noncoding region [4]. HCV genotyping was done with type-specific primers in the core region according to the method by Okamoto and subsequent modifications [4, 15]. Hepatitis B surface antigen was assessed by use of ELISA.

We established the sample size after taking into account the distribution of HCV genotypes in this area [12]. The following variables were used for analysis: sex, age at diagnosis (<45 years, 45–59 years, and ⩾60 years), birth cohort (before 1930, 1930–1939, 1940–1949, 1950 or after), alcohol intake (as continuous [grams per day] and categorical [none, moderate, excessive]), and job (farmer, salaried position, pensioner, other). Cutoff points for alcohol intake were 60 g/day and 30 g/day for men and women, respectively.

For comparison between continuous and categorical variables, we used the t test, assuming unequal variances, and the χ^2 test, respectively. To estimate the risk of cirrhosis, crude ORs and 95% CIs were calculated. A stratified analysis, by use of Mantel-Haenszel OR pooled estimates, was done to account for confounding [16]. Finally, multivariable unconditional logistic regression analysis was used to estimate the association between variables and the risk of cirrhosis.

Because nearly all of the case patients and the controls were infected with HCV genotypes 1b or 2a/c, analysis was limited to these genotypes. HCV genotype was considered to be the exposure variable, with HCV 2a/c being the baseline. Statistical significance was established at $P < .05$.

Table 1. Characteristics of Italian patients in study of hepatitis C virus genotype and risk of cirrhosis.

Characteristic	Controls (n = 138)	Case patients (n = 46)
Sex		
Male	74 (53.6)	23 (50.0)
Female	64 (46.4)	23 (50.0)
Age, years		
<45	10 (7.2)	5 (10.9)
45–59	56 (40.6)	23 (50.0)
⩾60	72 (52.2)	18 (39.1)
Age, mean years ± SD	58.8 ± 9.4	56.9 ± 8.9
Year of birth		
Before 1930	57 (41.3)	23 (50.0)
1930–1939	46 (33.3)	16 (34.8)
1940–1949	29 (21.0)	7 (15.2)
1950 or after	6 (4.3)	0 (0)
Alcohol intake[a]		
None	28 (20.3)	26 (56.5)
Moderate	85 (61.6)	9 (19.6)
Excessive	25 (18.1)	11 (23.9)
Alcohol intake, mean g/d, ± SD	37.5 ± 32.0	29.6 ± 41.8
Job[a]		
Farmer	25 (18.1)	8 (17.4)
Salaried position	36 (26.1)	22 (47.8)
Pensioner	70 (50.7)	14 (30.4)
Other	7 (5.1)	2 (4.3)

NOTE. Data are no. (%) of patients, unless otherwise noted.

[a] $P < .05$; all other comparisons were not significant.

RESULTS

During the accrual period, 104 new patients had cirrhosis diagnosed and were referred to our hospital; 58 of these patients tested positive for hepatitis B surface antigen and were excluded (table 1). Eight case patients came from the cohort enrolled in 1985–1986, 4 of whom were excluded. There was no difference between case patients and controls with respect to sex, age, and birth cohort. Mean levels of alcohol consumption were higher among controls; most of the controls (85 [61.6%]) had a moderate level of alcohol intake, whereas 26 case patients (56.5%) did not drink at all. HCV genotype distribution was markedly different between groups (table 2). HCV genotype 2a/c infection occurred in 84 controls (60.9%) and 9 case patients (19.6%), whereas HCV genotype 1b was found in 34 case patients (73.9%) and 45 controls (32.6%). Other variables considered were not differentially distributed according to the infecting HCV genotype. Forty-three case patients and 129 controls were considered for risk analysis.

Only HCV genotype was associated with a statistically significant risk for cirrhosis (OR, 7.1; 95% CI, 3.1–15.6; table 3). Stratified analysis (table 4) showed substantially unchanged measures of HCV genotype effect, except for the cohort of birth, which was larger than other estimates (OR, 8.4; 95% CI, 3.5–20.5), but the uniform effect hypothesis held ($P = .90$). Results of multivariable analysis are shown in table 5. HCV genotype showed an independent effect on the risk of cirrhosis (OR, 7.49; 95% CI, 3.15–17.81). No significant effect was observed with regard to sex, age at the time of diagnosis, or daily alcohol intake. The cohorts of birth and job were dropped from the model because their contribution to the reduction of log likelihood was minimal.

DISCUSSION

This study shows that in this area of southern Italy, chronic infection with HCV genotype 1b is associated with a high risk

Table 2. Hepatitis C virus (HCV) genotype distribution among 138 controls and 46 case patients from southern Italy, according to selected variables.

	Controls with HCV genotype			Case patients with HCV genotype		
Characteristic	2a/c	1b	Other	2a/c	1b	Other
Sex						
Male	45 (53.6)	23 (51.1)	6 (66.7)	5 (55.6)	18 (52.9)	0 (0)
Female	39 (46.4)	22 (48.9)	3 (33.3)	4 (44.4)	16 (47.1)	3 (100)
Age, years						
<45	9 (10.7)	1 (2.2)	0 (0)	1 (11.2)	3 (8.8)	1 (33.3)
45–59	25 (29.8)	29 (64.4)	2 (22.2)	4 (44.4)	17 (50.0)	2 (66.7)
⩾60	50 (59.5)	15 (33.4)	7 (77.8)	4 (44.4)	14 (41.2)	0 (0)
Year of birth						
Before 1930	37 (44.1)	13 (28.9)	7 (77.8)	7 (77.8)	16 (47.1)	0 (0)
1930–1939	24 (28.6)	20 (44.5)	2 (22.2)	1 (11.1)	13 (38.2)	2 (66.7)
1940–1949	18 (21.4)	11 (24.4)	0 (0)	1 (11.1)	5 (14.7)	1 (33.3)
1950 or after	5 (5.9)	1 (2.2)	0 (0)	0 (0)	0 (0)	0 (0)
Alcohol intake						
None	17 (20.2)	8 (17.8)	3 (33.3)	4 (44.5)	20 (58.8)	2 (66.7)
Moderate	52 (61.9)	29 (64.4)	4 (44.4)	2 (22.2)	7 (20.6)	0 (0)
Excessive	15 (17.9)	8 (17.8)	2 (22.3)	3 (33.3)	7 (20.6)	1 (33.3)
Job						
Farmer	14 (16.7)	10 (22.2)	1 (11.1)	2 (22.2)	6 (17.6)	0 (0)
Salaried position	23 (27.4)	13 (28.9)	0 (0)	3 (33.4)	16 (47.1)	3 (100)
Pensioner	43 (51.2)	19 (42.2)	8 (88.9)	4 (44.4)	10 (29.4)	0 (0)
Other	4 (4.7)	3 (6.7)	0 (0)	0 (0)	2 (5.9)	0 (0)
Cirrhosis diagnosis						
Histological				5 (55.6)	12 (35.3)	0 (0)
Clinical				4 (44.4)	22 (64.7)	3 (100)
Total	84 (60.9)	45 (32.6)	9 (6.5)	9 (19.6)	34 (73.9)	3 (6.5)

NOTE. Data are no. (%) of patients with respective genotype.

Table 3. Crude ORs for cirrhosis among Italian patients who were infected with hepatitis C virus (HCV), by selected variables.

Characteristic	Controls (n = 138)	Case patients (n = 46)	OR	95% CI
HCV genotype				
2a/c	84	9	Referent	
1b	45	34	7.1	3.1–15.6
Sex				
Male	68	23	Referent	
Female	61	20	0.9	0.5–1.9
Age, years				
<45	10	4	Referent	
45–59	54	21	0.9	0.3–3.2
⩾60	65	18	0.7	0.2–2.3
Year of birth				
Before 1930	50	23	Referent	
1930–1939	44	14	0.7	0.3–1.5
1940–1949	29	6	0.5	0.2–1.2
1950 or after	6	0	Undefined	
Daily alcohol intake				
None	25	24	Referent	
Moderate	81	9	0.1	0.0–0.3
Excessive	23	10	0.5	0.2–1.1
Job				
Farmer	24	8	Referent	
Salaried position	36	19	1.6	0.6–4.1
Pensioner	62	14	0.7	0.2–1.8
Other	7	2	0.9	0.0–4.5

NOTE. Data are no. of patients, unless otherwise indicated.

of cirrhosis. Similar results from another Italian area have been recently published [17]. In addition, anti-HCV antibody prevalence in Italy is presumed to be >20% among people aged ⩾60 years [11, 18, 19].

Precision and validity are important issues in epidemiology, particularly with regard to research on chronic hepatitis C, which is affected by unavoidable limitations due to a long and variable clinical course and, often, to the inability to account for duration of infection. In this sense, our data were collected to reduce random and systematic errors. Sample size was calculated in a conservative way, by use of regional information about HCV genotype distribution [12]. Our findings are also reliable on statistical grounds, as reflected by the relatively small confidence intervals for the estimate of the HCV-genotype effect.

Because our institution is the only specialized center in this large area, it is expected that most patients would be referred to it. The rate of patient loss due to transfer to other areas has been minimal, and it is unlikely that this loss was selective for patients with non–HCV genotype 1b cirrhosis. When patient accrual was completed, information on HCV genotypes was not available; therefore, differential referral or hospitalization rates were unlikely, which would have made selection bias negligible. With regard to exposure classification, similar HCV genotype distributions have been observed in other cohorts from both southern and northern Italy [4, 18, 20–22].

Possible bias might derive from disease misclassification; however, the diagnosis of cirrhosis was made mainly according to clinical signs of liver decompensation, and all controls underwent an ultrasound scan of the liver. Sensitivity of clinical diagnosis of cirrhosis is high [13]; therefore, misclassification, if present, would be nondifferential.

Previous studies that have showed associations between infections with HCV genotype 1b and progressive liver disease have been criticized because the results might be explained as cohort effects in HCV genotype distribution [3]. Cohort effects in HCV genotype distribution exist, and they have been consistently demonstrated throughout Europe for HCV genotypes 1a and 3a, which were introduced during the early 1960s by means of injection drug abuse [23, 24]. However, no evidence was ever found regarding a differential cohort distribution of endemic HCV genotypes, such as 1b and 2a/c, which have been present in our country for a long time. Indeed, we have shown that HCV genotypes 1b and 2a/c have circulated together in this area since the early 20th century and that their distribution is unrelated to age [12], which is in agreement with other Italian studies [4–6, 18, 20].

The duration of infection was not known in this study. To account for time from infection, we performed stratified and multivariable analyses, but no age or cohort effect was found. In this area, >45% of 60-year-old persons tested positive for anti-HCV antibody, which indicates that this population has been heavily and widely exposed [11]. Therefore, it can be assumed that the modes of HCV transmission in this population were comparable, given the extremely uniform social structure, the almost complete absence of migration, and the low frequency of drug abuse.

Alcohol consumption, which many studies have indicated to be a relevant variable of disease progression [25], was considered as a confounder, but no significant effect was observed. Although alcohol intake was measured by means of a validated questionnaire [14], some confounding may still have been present. Case patients were more likely than controls to have changed their levels of alcohol intake when clinical signs of cirrhosis appeared. Furthermore, levels of alcohol intake may have been underestimated because one would expect a bias toward "socially desirable" answers, but the effect would not have been differential. Unlike other populations [26, 27], for persons in the area that we studied, it is likely that alcohol consumption plays a minor role in the progression of chronic hepatitis C. In this area, the drinking pattern is characterized

Table 4. Odds ratios for cirrhosis among Italian patients who were infected with hepatitis C virus (HCV), by stratified analysis.

Stratification variable	Controls with HCV genotype		Case patients with HCV genotype		Unadjusted OR	Mantel-Haenszel–adjusted OR
	2a/c	1b	2a/c	1b		
Cohort of birth[a]						
Before 1930	37	13	7	16	6.5 (2.2–19.3)	
1930–1939	24	20	1	13	15.6 (1.9–129.8)	
1940–1949	18	11	1	5	8.2 (0.8–79.5)	
1950 or after	5	1	0	0	Undefined	8.4 (3.5–20.5)
Age at diagnosis, years[a]						
<45	9	1	1	3	27 (1.3–578.3)	
45–59	25	29	4	17	3.7 (1.1–12.3)	
⩾60	50	15	4	14	11.7 (3.3–40.8)	7.0 (3.1–15.9)
Sex[a]						
Male	45	23	5	18	7.0 (2.3–21.4)	
Female	39	22	4	16	7.1 (2.1–23.9)	7.1(3.1–16.0)
Daily alcohol intake[a]						
None	17	8	4	20	10.6 (2.7–41.5)	
Moderate	52	29	2	7	6.3 (1.2–32.2)	
Excessive	15	8	3	7	4.4 (0.9–21.7)	6.9 (2.9–16.7)
Job[a]						
Farmer	14	10	2	6	4.2 (0.7–25.3)	
Salaried position	23	13	3	16	9.4 (2.3–38.6)	
Pensioner	43	19	4	10	5.6 (1.6–20.3)	
Other	4	3	0	2	Undefined	6.8 (2.9–15.5)

NOTE. Data are no. of patients, unless otherwise indicated.

[a] OR homogeneity, $P > .25$.

by steady, daily consumption of wine with meals. Therefore, in addition to overall levels of alcohol intake, modalities of consumption and the typical Mediterranean diet might be important with regard to the modulation of liver damage due to alcohol [28]. Moreover, in Italy, moderate drinkers have a longer life expectancy than occasional and heavy drinkers [29].

Table 5. Adjusted ORs for cirrhosis among Italian patients who were infected with hepatitis C virus (HCV), by multiple logistic regression.

Variable	OR[a]	95% CI
HCV genotype 1b	7.49[b]	3.15–17.81
Female	0.76	0.31–1.86
Age, years		
<45	1	—
45–59	0.47	0.11–1.98
⩾60	0.52	0.13–2.17
Alcohol intake[c]	0.99	0.97–1.01

[a] Mutually adjusted.

[b] $P < .01$.

[c] Continuous variable.

We are aware that definite evidence regarding the differential pathogenicity of HCV genotypes will surely require other prospective observations, but the epidemiological setting of this study is favorable for addressing this issue; it is also noteworthy, considering that patient recruitment started before antiviral therapies were widely applied and may therefore reflect the true natural history of this infection. Our results reinforce the conclusion that biological differences may underlay the genetic diversity of HCV phylogenetic variants. Biological studies are needed to support the conclusions drawn from epidemiological and clinical observations.

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