

## Molecular Epidemiology of Hepatitis C Virus Infection in an Area of Hyperendemicity in Southern Italy: a Population-Based Study

ALBERTO R. OSELLA,<sup>1\*</sup> LAURA SONZOGNI,<sup>2</sup> ALDO CAVALLINI,<sup>3</sup> LUCIANA FOTI,<sup>2</sup> VITO GUERRA,<sup>1</sup> ALFREDO DI LEO,<sup>3</sup> MARIO U. MONDELLI,<sup>4</sup> GIOVANNI MISCIAGNA,<sup>1</sup> AND ENRICO M. SILINI<sup>2,5</sup>

Laboratory of Epidemiology and Biostatistics<sup>1</sup> and Laboratory of Biochemistry,<sup>3</sup> IRCCS “Saverio De Bellis,” 70013 Castellana Grotte (BA), Associazione Studio Avanzato Epatiti Virali (A.S.A.E.V.), 24040 Bonate Sotto (BG),<sup>2</sup> Departments of Pathology<sup>5</sup> and Infectious Diseases,<sup>4</sup> University and IRCCS “S. Matteo,” 27100 Pavia, Italy

Received 23 November 1998/Returned for modification 4 March 1999/Accepted 16 April 1999

**In a cohort of subjects from Italy, anti-hepatitis C virus (HCV) and HCV RNA [HCV(+) subgroup] prevalences were 24.6 and 79.6%, respectively. HCV types 1b and 2a/c accounted for 95% of infections. Adjusted alanine aminotransferase levels were higher in males than in females and in RNA-positive subjects than in RNA-negative subjects regardless of HCV type. Genotype distribution was unrelated to demographic variables.**

Population-based surveys performed in different areas of Italy have shown a high prevalence of antibodies to hepatitis C virus (HCV), which can reach 40% among older subjects of southern regions (3, 8, 11, 23). It is controversial whether this burden of infections is paralleled by high disease prevalence, as most anti-HCV-positive subjects have normal alanine aminotransferase (ALT) levels and no evidence of liver damage (3, 8, 23). Several variables can influence the expression of HCV-related liver disease: alcohol abuse (7, 12), associated liver disorders (21), immunogenetic factors (1, 9), and levels of replication and genetic variability of HCV (17, 19). Virus genotypes, in particular, have raised considerable interest (20), given the association of type 1b with resistance to interferon treatment and advanced stages of disease (6).

This paper describes the molecular epidemiology of HCV infection in a large population-based cohort of anti-HCV-positive subjects enrolled in Castellana, a small municipality in Apulia characterized by a high prevalence of anti-HCV antibody among older subjects and a moderate incidence. For this investigation serum HCV RNA and HCV genotypes were studied for over 400 unselected individuals.

The cohort was assembled in 1985 by systematic sampling from electoral registers and followed up in 1992 (11). Physical, ultrasound, and laboratory examinations were performed on both occasions, and serum samples were collected. A structured interview on sociodemographic status, health history, behavioral variables, and alcohol intake was also conducted. Variables considered in the analysis were (i) type of job, (ii) history of hepatitis, (iii) ALT elevation (at least twice the reference values), (iv) daily alcohol intake, and (v) past surgery. The study was originally designed to investigate the prevalence of gallstones (10); therefore, no systematic effort was made to record risk factors for parenterally transmitted infections. While HCV sexual transmission is presumably low and drug abuse is irrelevant in our cohort, transfusional risk might have contributed significantly to HCV spread, as transmission through transfusion has been previously reported in 6.2 to 12.5% of anti-HCV-positive subjects (3, 8, 23). To overcome this limitation, we de-

rived a proxy variable, past surgery, from the history, which expresses the parenteral transmission risk linked to medical procedures. Follow-up sera from 2,158 subjects were considered for the study, as samples collected from the cohort in 1985 had not been appropriately processed for molecular testing.

Anti-HCV serology was established by second-generation enzyme-linked immunosorbent assay and confirmed by using RIBA 2.0 (Ortho Diagnostic Systems, Raritan, N.J.) for 2,116 available sera. Anti-HCV antibody prevalence was 24.6% (521 subjects, including 287 males); the mean age  $\pm$  standard deviation of anti-HCV antibody-positive patients was  $58.9 \pm 9.3$  years (males,  $59.6 \pm 8.9$  years; females,  $58.1 \pm 9.8$  years). Four hundred seventeen anti-HCV-positive sera of 521 sera were available for molecular testing. Serum HCV RNA was extracted by using the QIAamp Viral RNA kit (QIAGEN Inc., Hilden, Germany) and tested by nested PCR by using primers from the 5' untranslated region of HCV. Genotyping was performed by the method of Okamoto with subsequent modifications (17).

Serum HCV RNA was detected in 332 (79.6%) of 417 available anti-HCV-positive samples, indicating that active replication was present in the majority of subjects. This figure should be considered conservative, since the analysis was performed at a single time point and on sera stored frozen for 5 years. The HCV type distribution was as follows: 1 serum of type 1a (0.3%), 119 sera of type 1b (35.8%), 196 sera of type 2a (59.0%), 1 serum of type 2a/2b (0.3%), 1 serum of type 2a/c (0.3%) and 14 untypeable sera (4.2%).

Analysis of covariance was performed to estimate the effect of viral factors on ALT levels controlled for sex, age, and daily alcohol intake. Four subject categories, i.e., type 1 infected, type 2 infected, untypeable, and HCV RNA negative, were considered (Table 1). Mean ALT levels were higher in HCV RNA-positive subjects than in RNA-negative subjects regardless of HCV type ( $P < 0.05$ ) and in males than in females ( $P < 0.05$ ). The prevalence of ALT elevation among HCV-infected subjects was 35% overall and 43% among those with HCV RNA positivity. This is in contrast to findings reported by another group of authors, who, studying a cohort of subjects from southern Italy with similar socioeconomic status, found ALT elevation of any degree in 4.1% of the subjects (8). However, our findings are consistent with those acquired in other population-based studies (3) and with the accepted view

\* Corresponding author. Mailing address: Laboratory of Epidemiology and Biostatistics, IRCCS “Saverio de Bellis,” Via F. Valente, 4, 70013 Castellana Grotte (BA), Italy. Phone: 39 080 4960248. Fax: 39 080 4961394. E-mail: irccsbiblio@mail.media.it.

TABLE 1. Age- and daily alcohol intake-adjusted ALT (natural log transformed) levels of subjects classified by HCV type and sex

Sex	Mean ALT level (U/liter) of subjects with HCV type <sup>a</sup> :				Total
	1a/1b	2a, 2a/2b, 2a/c	Untypeable	RNA(-)	
Male <sup>b</sup>	3.38 ± 0.08	3.31 ± 0.07	3.31 ± 0.24	2.86 ± 0.10	3.21 ± 0.07 <sup>c</sup>
Female <sup>d</sup>	3.18 ± 0.09	3.23 ± 0.07	2.43 ± 0.28	2.82 ± 0.10	2.91 ± 0.08
Total <sup>e</sup>	3.28 ± 0.06	3.27 ± 0.05	2.87 ± 0.18	2.84 ± 0.07	

<sup>a</sup> Analysis of covariance (ANCOVA) revealed no significant interaction of sex and HCV genotype for mean ALT levels.

<sup>b</sup>  $P < 0.05$ , RNA(-) compared with 1a/1b and with 2a, 2a/2b, 2a/c.

<sup>c</sup>  $P < 0.05$ , compared with total for females.

<sup>d</sup>  $P < 0.05$ , RNA(-) compared with 1a/1b and with 2a, 2a/2b, 2a/c.

<sup>e</sup>  $P < 0.05$ , RNA(-) compared with 1a/1b and with 2a, 2a/2b, 2a/c.

of the natural history of HCV infection (2). Normal ALT levels were more frequent for subjects with HCV type 2a/c infections than for those with type 1b infections (58.1 and 52.5%, respectively), although this difference did not reach the statistical significance reported by other groups who found an excess of HCV type 2a/c infections in selected HCV carriers with persistently normal ALT levels (15–17). A detailed assessment of disease severity was not performed during the survey, which included ultrasonography of the upper abdominal region as the only means of assessment; nonetheless, it is likely that a significant proportion of HCV-infected subjects had liver disease, since liver-related disease accounts for 40% of the mortality among anti-HCV-positive subjects in Castellana (14).

Correlations of HCV types with demographic and epidemiological variables were explored by using contingency tables and by grouping patients as described above. HCV types 1b and 2a/c were equally distributed among the variables analyzed, indicating that they had been present in our community for at least 60 to 70 years and had circulated together, likely through similar routes of transmission (4, 8, 22). This contention is strengthened by the high prevalence of anti-HCV in the population (11), the old mean age of infected subjects, the homogeneous socioeconomic structure of the community, which lacks relevant immigration, and the absence of infections by “new” HCV types, such as types 1a and 3a, which were not introduced into Italy, through drug abuse, until the 1960s (13, 18). Both type 1b and type 2a/c are endemic in Italy and show remarkably similar prevalence rates in southern regions other than Apulia.

In light of these considerations, the Castellana cohort provides an excellent model for the study of the differential pathogenicity of HCV types in the general population allowing for minimized biases due to differences in infection duration and cohort effects on genotype distribution. In this regard, it is interesting that in Apulia, the HCV type distribution among patients with chronic liver disease differs significantly from that observed in our cohort, most patients (55%) being infected with genotype 1b, consistent with the presumed higher degree of pathogenicity of this genotype (5).

This work was supported in part by grant 030RFM93/01 from the Italian Ministry of Health to the IRCCS Policlinico S. Matteo di Pavia. We thank Maria Bianco (Laboratory Technician).

#### REFERENCES

- Alric, L., M. Fort, J. Izopet, J.-P. Charlet, J. Selves, J. Puel, J. P. Pascal, M. Duffaut, and M. Abbal. 1997. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. *Gastroenterology* **113**:1675–1681.
- Alter, M. J., H. S. Margolis, K. Krawczynski, F. N. Judson, J. K. Miller,

- M. A. Gerber, R. E. Sampliner, E. L. Meeks, and M. J. Beach. 1992. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *N. Engl. J. Med.* **327**:1899–1905.
- Bellentani, S., C. Tiribelli, G. Saccoccio, M. Sodde, N. Fratti, C. De Martin, G. Cristianini, and the Dionysos Study Group. 1994. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. *Hepatology* **20**:1442–1449.
- Brechet, C. 1997. Hepatitis C virus 1b cirrhosis, and hepatocellular carcinoma. *Hepatology* **25**:772–774.
- Bruno, S., E. Silini, A. Crosignani, F. Borzio, G. Leandro, F. Bono, M. Asti, S. Rossi, A. Larghi, A. Cerino, M. Podda, and M. U. Mondelli. 1997. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. *Hepatology* **25**:754–758.
- Chemello, L., P. Bonetti, L. Cavaletto, F. Talato, V. Donadon, P. Casarin, F. Belussi, M. Frezza, F. Noventa, P. Pontisso, L. Benvegnù, C. Casarin, A. Alberti, and the Triveneto Viral Hepatitis Group. 1995. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. *Hepatology* **22**:700–706.
- Corrao, G., and S. Aricò. 1998. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. *Hepatology* **27**:914–919.
- Guadagnino, V., T. Stroffolini, M. Rapicetta, A. Costantino, L. A. Kondili, I. Menniti, B. Caroleo, C. Costa, G. Griffo, L. Loiacono, V. Pisani, A. Foca, and M. Piazza. 1997. Prevalence of risk factors and genotype distribution of hepatitis C virus infection in the general population. A community-based survey in southern Italy. *Hepatology* **26**:1006–1011.
- Kuzushita, N., N. Hayashi, T. Moribe, K. Katayama, T. Kanto, S. Nakatani, T. Kaneshige, T. Tatsumi, A. Ito, K. Mochizuki, Y. Sasaki, A. Kasahara, and M. Hori. 1998. Influence of HLA haplotypes on the clinical courses of individuals infected with hepatitis C virus. *Hepatology* **27**:240–244.
- Misciagna, G., C. Leoci, S. Elba, J. Petruzzi, V. Guerra, A. Mossa, M. R. Novello, A. Coviello, M. Capece-Minutolo, and I. Giorgio. 1994. The epidemiology of cholelithiasis in southern Italy. *Eur. J. Gastroenterol. Hepatol.* **6**:937–941.
- Osella, A. R., G. Misciagna, A. Leone, A. Di Leo, and G. Fiore. 1997. Epidemiology of hepatitis C virus infection in an area of southern Italy. *J. Hepatol.* **27**:30–35.
- Ostapowicz, G., K. J. Watson, S. A. Locarnini, and P. V. Desmond. 1998. Role of alcohol in the progression of liver disease caused by hepatitis C virus infection. *Hepatology* **27**:1730–1735.
- Pawlotsky, J.-M., L. Tsakiris, F. Roudot-Thoraval, C. Pellet, L. Stuyver, J. Duval, and D. Dhumeaux. 1995. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J. Infect. Dis.* **171**:1607–1610.
- Piccigallo, E., A. R. Osella, R. Cuppone, M. Chiloiro, A. Di Leo, and G. Misciagna. 1997. A population-based study of mortality among HCV-reactive individuals of southern Italy, abstr 10. In Program and abstracts of the Clinical Research Single Topic Conference. Chronic Viral Hepatitis: Emerging Issues and Research Direction. American Association for the Study of Liver Diseases (AASLD), Tyson's Corner, Va.
- Prati, D., C. Capelli, A. Zanella, F. Mozzi, P. Bosoni, M. Pappalettera, F. Zanuso, L. Vianello, E. Locatelli, C. De Fazio, G. Ronchi, E. Del Ninno, M. Colombo, and G. Sirchia. 1996. Influence of different hepatitis C virus genotypes on the course of asymptomatic hepatitis C virus infection. *Gastroenterology* **110**:178–183.
- Puoti, C., A. Magrini, T. Stati, P. Rigato, F. Montagnese, P. Rossi, L. Aldegheri, and S. Resta. 1997. Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal and abnormal alanine transaminase levels. *Hepatology* **26**:1393–1398.
- Silini, E. M., F. Bono, A. Cividini, A. Cerino, A. Maccabruni, C. Tinelli, S. Bruno, A. Bellobuono, and M. U. Mondelli. 1995. Molecular epidemiology of hepatitis C virus infection among intravenous drug users. *J. Hepatol.* **22**:691–695.
- Silini, E. M., F. Bono, and A. Cividini. 1995. Differential distribution of hepatitis C virus genotypes in patients with and without liver function abnormalities. *Hepatology* **21**:285–290.
- Silini, E. M., R. Bottelli, M. Asti, S. Bruno, M. E. Candusso, S. Brambilla, F. Bono, G. Iamoni, C. Tinelli, M. U. Mondelli, and G. Ideo. 1996. HCV genotypes and risk of hepatocellular carcinoma in cirrhosis: a case-control study. *Gastroenterology* **110**:199–205.
- Silini, E. M., and M. U. Mondelli. 1995. Significance of hepatitis C virus genotypes. *Viral Hepatitis Rev.* **1**:111–120.
- Smith, B. C., J. Gorve, M. A. Guzail, C. P. Day, A. K. Daly, A. D. Burt, and M. F. Bassendine. 1998. Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. *Hepatology* **27**:1695–1699.
- Smith, D. B., S. Pathirana, F. Davidson, E. Lawlor, J. Power, P.-L. Yap, and P. Simmonds. 1997. The origin of hepatitis C virus genotypes. *J. Gen. Virol.* **78**:321–328.
- Stroffolini, T., M. Menchinelli, G. Taliani, V. Dambruoso, G. Poliandri, A. Bozza, R. Lece, C. Clementi, I. Menniti, A. Compagnoni, and C. De Bac. 1995. High prevalence of hepatitis C virus infection in a small central Italian town: lack of evidence of parental exposure. *Ital. J. Gastroenterol.* **27**:235–238.