Age and Gender Distribution of Hepatitis C Virus Genotypes in the Metropolitan Area of Naples

Arnolfo Petruzzello^a Nicola Coppola^b Anna Maria Diodato^a
Vincenzo Iervolino^a Rosa Azzaro^a Gaetano Di Costanzo^a
Catia Addolorata Di Macchia^a Tommaso Di Meo^a Giovanna Loquercio^a
Giuseppe Pasquale^b Carmela Cacciapuoti^a

^aLaboratory of Virology and Molecular Biology 'V. Tridente', Transfusion Service, Department of Haematology, Istituto Nazionale Tumori, Fondazione G. Pascale, and ^bDepartment of Public Medicine, Section of Infectious Diseases, Seconda Università di Napoli, Naples, Italy

Key Words

Hepatitis C virus · Hepatitis C virus diagnosis · Hepatitis C virus prevalence · Genotypes · Risk factors · Hepatitis C virus genotyping

Abstract

Objective: To assess any time-related variations in the distribution of hepatitis C virus (HCV) genotypes in the metropolitan area of Naples, Italy. **Methods:** HCV genotypes were analysed in 255 HCV RNA-positive patients with chronic HCV infection, observed from 2009 to 2011, and compared with data pertaining to a sample of 176 HCV RNA-positive patients observed from 2006 to 2008. **Results:** In both periods of analysis, genotype 1b was predominant (51.8 and 48.3%, respectively), followed by genotype 2 (27.9 and 31.7%, respectively). These HCV genotypes were particularly prevalent in older patients, whereas genotypes 3a and 1a were observed more frequently in the younger population. Genotype 1b was particularly common in females compared to males in both study periods (39.3% of 89 males vs. 64.3% of

87 females in the 3-year period 2006–2008, $p < 0.001$; 41.5% of 118 males vs. 54.0% of 137 females in the period 2009–2011, $p < 0.05$). The prevalence of patients with genotype 1b in the age range 51–60 years was higher in the 2006–2008 period than in 2009–2011 (76.9 vs. 37.7%; $p < 0.0005$) and lower in the over 60 year olds (55.1 vs. 59.6%; $p = 0.5$). **Conclusion:** Genotype 1b, historically the most prevalent in Italy, is still predominant; however, when comparing the two time periods, a cohort effect evidencing the increasing prevalence of genotype 1b among elderly patients was revealed.

Copyright © 2013 S. Karger AG, Basel

Introduction

The hepatitis C virus (HCV) is distributed worldwide and displays a considerable genetic heterogeneity among isolates. This led to its classification into 6 major genotypes, differing from each other by 30% in terms of nucleotide sequence, and several subtypes with a difference of less than 20% of their sequence [1–5].

The distribution of HCV genotypes varies significantly with geography [6–8]; genotypes 1, 2 and 3 are found throughout the world whereas the other genotypes are relatively restricted to certain geographic regions of Africa, Asia and the Middle East [9–11]. In particular, genotype 4 is prevalent in north and central Africa and in the Middle East [12–14], genotype 5 prevails in South Africa [15] and in some areas of central France [16], and genotype 6 is frequent in the Far East, especially in China [17–19]. A clear correlation between age and genotype incidence has also been found, i.e. a greater prevalence of 1b and 2a/2c in older patients and 1a and 3a in younger individuals [20–22], suggesting the involvement of intravenous drug use.

In some countries, a change in prevalence from HCV genotype 1 to genotypes 3 and 4 over the last decade has been reported; this is presumably ascribable to the eradication of transfusion-associated infections, continuous increase in intravenous drug use, increase in immigration patterns and the spread of unsafe sexual practices in sexually active age groups [23]. In Italy, around half of cases are of the 1b type, with 2a/2c being the next prevalent subtype. Whether or not a north-south distribution gradient exists is as yet unconfirmed; however, in the country as a whole, the incidence of genotype 3a appears to be increasing and certainly merits investigation [24, 25].

Hence, the aim of this study was to provide up-to-date information on the prevalence of the various HCV genotypes and any age- or gender-related correlations in a group of HCV-positive patients from the Naples metropolitan area.

Materials and Methods

Study Population and Sample Collection

The population analysed in this study comprised 5,550 consecutively recruited outpatients resident in the metropolitan area of Naples, Italy, and referred to the National Tumour Institute 'Fondazione G. Pascale' Transfusion Service Virology Clinic in Naples between January 2009 and June 2011.

In the study cohort, 2,830 patients (51.0%) were females and 2,720 (49.0%) males; the mean age of female patients was 47.5 years (range 18–77), and that of males was 50.5 years (range 18–83). Data on risk factors for HCV were only available for some patients and were therefore not considered in this study. Patients with autoimmune disorders were excluded to prevent interference with serological testing.

Of the 5,550 enrolled subjects, 436 (7.8%) were found to be positive for anti-HCV. Two hundred and fifty-five (58.0%) members of this population were also positive for HCV RNA and were therefore included in this study.

Of the 181 patients excluded from the study, 73 (40.3%) were found to be HCV RNA negative, while in 108 (59.7%), serum was insufficient for HCV RNA determination. No significant gender or age differences were found between the study group and the excluded patients.

Viral genotyping was performed on the 255 included subjects, a total of 118 males (46.0%) and 137 females (54.0%); both gender groups featured a mean age of 52.5 years (range 21–84).

In order to analyse possible changes in the distribution of HCV genotypes in the metropolitan area of Naples, another group of 176 consecutive anti-HCV-positive/HCV RNA-positive samples, collected between January 2006 and December 2008, also from subjects living in the metropolitan area of Naples and referred to the same clinic, were assessed as above. There were no differences in the pattern of sampling or testing used in the two considered periods.

Viral genotyping was performed on sample subjects, a total of 89 males (50.6%) and 87 females (49.4%); both gender groups featured a mean age of 53.5 years (range 25–82).

Serology and Molecular Analysis

All the plasma samples pertaining to both study groups were analysed for the presence of anti-HCV antibodies by means of the Vitros ECI test (OrthoClinical Diagnostics), used according to the manufacturers' instructions.

The OrthoClinical Vitros ECI test employs chemiluminescent technology in which a light signal is created by a positive antibody-antigen reaction in direct proportion to the amount of antibody present. Results are reported as signal to cutoff. Results greater than or equal to 1.00 are considered reactive for HCV antibodies. The specificity and sensitivity of the test are, respectively, 99.97 and 100%.

Only repeatedly anti-HCV-reactive samples were subsequently tested for the presence of HCV RNA via the COBAS AmpliPrep/COBAS Taqman HCV 48 (Roche Diagnostics System Inc.), which exploits a polymerase chain reaction in real time. The linear range of quantification of the test was 1.50×10^1 to 6.90×10^7 HCV RNA IU/ml, using the accuracy acceptance criterion of $\pm 0.3 \log^{10}$. The specificity of the test was 100%, and its detection limit was 15 IU/ml.

HCV genotyping was performed using the Versant HCV Genotype Assay 2.0 LiPA test (Siemens Healthcare Diagnostics), which involves the amplification and hybridization of viral genome fragments, the latter by means of genotype-specific probes adsorbed onto nitrocellulose strips. The various steps were performed by Auto-LiPA, a fully automated system for complete genotyping. The specificity and sensitivity of this test are, respectively, 96 and 99.4%, and its detection limit is 15 IU/ml.

Statistical Analysis

Statistical analysis of the data was performed using SPSS for Windows, version 7.5. Frequency tables were analysed using χ^2 tests, with Pearson correlations being used to assess the significance of the correlation between the categorical variables. Differences in the mean ages for the genotypes and subtypes were analysed using Student's *t* test. In all tests, *p* values <0.05 were regarded as statistically significant.

The odds estimation of genotype distribution in relation to gender and age in the two periods was performed by multiple logistic regression analysis.

Table 1. Gender distribution of HCV subtypes in the metropolitan area of Naples in 2006–2008

Genotype	Frequency	Gender	
		male	female
1	113 (64.3)	50 (56.2)	63 (72.4)
1a	14 (7.9)	7 (7.9)	7 (8.1)
1b	91 (51.8)	35 (39.3)	56 (64.3)
1*	8 (4.6)	8 (9.0)	0 (0)
2	49 (27.9)	27 (30.3)	22 (25.3)
2a/2c	38 (21.7)	18 (20.2)	20 (23.0)
2*	11 (6.2)	9 (10.1)	2 (2.3)
3a	12 (6.8)	12 (13.5)	0 (0)
4*	2 (1.0)	0 (0)	2 (2.3)
Total	176	89	87

Values represent numbers with percentages in parentheses. Asterisks indicate other cases not further subclassified. $p < 0.001$ (genotypes vs. gender).

Table 2. Gender distribution of HCV subtypes in the metropolitan area of Naples in 2009–2011

Genotype	Frequency	Gender	
		male	female
1	159 (62.4)	67 (56.8)	92 (67.1)
1a	12 (4.7)	6 (5.1)	6 (4.4)
1b	123 (48.3)	49 (41.5)	74 (54.0)
1*	24 (9.4)	12 (10.2)	12 (8.7)
2	81 (31.7)	39 (33.0)	42 (30.7)
2a/2c	60 (23.5)	27 (22.8)	33 (24.1)
2*	21 (8.2)	12 (10.2)	9 (6.6)
3a	12 (4.7)	12 (10.2)	0 (0)
4*	3 (1.2)	0 (0)	3 (2.2)
Total	255	118	137

Values represent numbers with percentages in parentheses. Asterisks indicate other cases not further subclassified. $p < 0.005$ (genotypes vs. gender).

Results

The distribution of the genotypes among the patients in the two periods of analysis is reported in tables 1 and 2. All type 3 cases were found to be subtype 3a, and no genotypes 5 and 6 were detected. Genotype 1b was the most common in both periods, but its prevalence decreased from 2006–2008 to 2009–2011, being 51.8 and 48.3%, respectively. This was accompanied by a parallel increase in the rate of genotype 2 (including subtype 2a/2c and non-subtypable genotype 2) from 27.9 to 31.7%. In our study, subtypes 1* and 2* (not further classified) represent the third and fourth largest groups, respectively, in the 3-year period 2009–2011 (9.4 and 8.2%, respectively). Subtype 3a decreased from 6.8 to 4.7%, and genotype 1a also decreased, from 7.9 to 4.7%. Although the exact classification of not-further-subtypable genotype 1 (1*) is not clear, its frequency (4.6 and 9.4%, respectively, in the 2006–2008 and 2009–2011 populations) could have significantly changed the distribution of this genotype during the last 6 years. Genotype 4 was the most stable of all over time, with an overall prevalence of 1.1%.

We then analysed the gender-related differences observed between the subtypes (tables 1, 2). Genotype 1b was more frequently observed in females than in males in both periods analysed (39.3% of 89 males vs. 64.3% of 87 females in the 3-year period 2006–2008, $p < 0.001$; 41.5% of 118 males vs. 54.0% of 137 females in the period 2009–2011, $p < 0.05$). Conversely, genotype 3a was more fre-

quent in males than in females (13.5 vs. 0% in the period 2006–2008, $p < 0.0005$; 10.2 vs. 0% in the period 2009–2011, $p < 0.0005$), possibly due to a higher prevalence of intravenous drug addiction among men. No significant gender differences were observed for genotypes 1a and 2 (including subtype 2a/2c and non-subtypable genotype 2), while genotype 4 was more frequent in females than in males (2.3 vs. 0% in the period 2006–2008 and 2.2 vs. 0% in the period 2009–2011). Nevertheless, these differences were not found to be significant upon statistical analysis.

Infected patients showed a different distribution of risk factors for HCV acquisition in the main genotype subgroups (tables 3, 4). Dental therapy, blood transfusion and surgery were the most frequent risk factors for HCV acquisition in genotypes 1 and 2, whereas intravenous drug abuse and tattooing were prevalent in genotype 3a. No data were available for genotype 4.

An increase in the prevalence of dental therapy as a major risk factor for genotype 1 was observed from 2006–2008 to 2009–2011, being 28.4 and 45.3%, respectively. This was accompanied by a parallel decrease in the rate of transmission via blood transfusion from 16.9 to 7.5%.

In order to compare the prevalence of HCV genotypes according to age during these two periods, the patients were categorized into 5 age groups, as shown in tables 5 and 6. The distribution varied significantly according to age in both analysis periods. In both time frames, genotype 1b was more common in the over-50 age group than

Table 3. Route of transmission for HCV infection by genotype subgroup in the metropolitan area of Naples in 2006–2008

Route of transmission	Genotype			
	1	2	3a	4
Unknown	31 (27.4)	11 (22.5)	1 (8.3)	2 (100)
Dental therapy	32 (28.4)	20 (40.8)	0 (0)	0 (0)
Surgery	17 (15.0)	10 (20.4)	0 (0)	0 (0)
Blood transfusion	19 (16.9)	5 (10.2)	0 (0)	0 (0)
Intravenous drug use	6 (5.3)	0 (0)	7 (58.4)	0 (0)
Piercing/tattoo	3 (2.6)	0 (0)	3 (25.0)	0 (0)
Needle stick injuries	5 (4.4)	3 (6.1)	1 (8.3)	0 (0)
Total	113 (100)	49 (100)	12 (100)	2 (100)

Values represent numbers with percentages in parentheses. If more than one risk factor was present, the one most likely to have caused infection was assigned.

Table 4. Route of transmission for HCV infection by genotype subgroup in the metropolitan area of Naples in 2009–2011

Route of transmission	Genotype			
	1	2	3a	4
Unknown	42 (26.5)	25 (30.8)	1 (8.3)	3 (100)
Dental therapy	72 (45.3)	32 (39.6)	0 (0)	0 (0)
Surgery	20 (12.6)	10 (12.3)	0 (0)	0 (0)
Blood transfusion	12 (7.5)	4 (4.9)	0 (0)	0 (0)
Intravenous drug use	7 (4.4)	1 (1.2)	8 (66.7)	0 (0)
Piercing/tattoo	1 (0.6)	0 (0)	3 (25.0)	0 (0)
Needle stick injuries	5 (3.1)	9 (11.1)	0 (0)	0 (0)
Total	159 (100)	81 (100)	12 (100)	3 (100)

Values represent numbers with percentages in parentheses. If more than one risk factor was present, the one most likely to have caused infection was assigned.

Table 5. Age distribution of HCV genotypes in the metropolitan area of Naples in 2006–2008

Genotype	>60 years	51–60 years	41–50 years	31–40 years	<30 years	Total
1a	3 (3.4)	3 (7.7)	4 (14.3)	4 (26.6)	1 (20)	14
1b	49 (55.1)	30 (76.9)	8 (28.6)	2 (13.4)	2 (40)	91
1*	5 (5.6)	0 (0)	1 (3.6)	2 (13.4)	0 (0)	8
2a/2c	27 (30.3)	4 (10.3)	5 (17.8)	3 (20)	0 (0)	38
2*	5 (5.6)	2 (5.1)	2 (7.1)	1 (6.6)	0 (0)	11
3a	0 (0)	0 (0)	7 (25.0)	3 (20)	2 (40)	12
4*	0 (0)	0 (0)	1 (3.6)	0 (0)	0 (0)	2
Total	89	39	28	15	5	176

Values represent numbers with percentages in parentheses. Asterisks indicate other cases not further subclassified.

Table 6. Age distribution of HCV genotypes in the metropolitan area of Naples in 2009–2011

Genotype	>60 years	51–60 years	41–50 years	31–40 years	<30 years	Total
1a	0 (0)	12 (22.6)	0 (0)	0 (0)	0 (0)	12
1b	93 (59.6)	20 (37.7)	5 (29.4)	3 (16.6)	2 (18.2)	123
1*	6 (3.8)	6 (11.3)	3 (17.6)	9 (50.2)	0 (0)	24
2a/2c	48 (30.8)	6 (11.3)	0 (0)	3 (16.6)	3 (27.3)	60
2*	9 (5.8)	3 (5.7)	3 (17.6)	0 (0)	6 (54.5)	21
3a	0 (0)	3 (5.7)	6 (35.4)	3 (16.6)	0 (0)	12
4*	0 (0)	3 (5.7)	0 (0)	0 (0)	0 (0)	3
Total	156	53	17	18	11	255

Values represent numbers with percentages in parentheses. Asterisks indicate other cases not further subclassified.

Fig. 1. Distribution of HCV genotype 1b by age group in the two consecutive periods.

in those aged 50 and under; in fact, in the 2006–2008 period, 79 of 128 subjects (61.7%) who were older than 50 had genotype 1b, a higher percentage than that observed in the 48 patients less than 51 years of age (25%; $p < 0.0001$); in 2009–2011, of 209 over 50 year olds, 113 (54.1%) had genotype 1b, versus 10 of the 48 (21.7%) who were younger than 50 years of age ($p < 0.0001$; tables 5, 6). In contrast, genotype 3a was more frequently observed in younger patients (25% of the 48 patients who were less than 50 years old vs. none of 128 who were more than 51 years old in the period 2006–2008, $p < 0.0001$; 19.6% of the 46 patients who were less than 51 years old vs. 1.4% of the 209 who were more than 50 years old in the period 2009–2011, $p < 0.0001$; tables 5, 6). No difference was observed in either analysis period as regards the number of patients with HCV genotype 2 (including subtype 2a/2c and non-subtypable genotype 2) in different age groups.

By analysing the distribution of HCV genotypes in the two periods, we observed that the prevalence of patients with genotype 1b in patients aged 51–60 years was higher in 2006–2008 than in 2009–2011 (76.9 vs. 37.7%; $p < 0.0005$) and lower in the patients over 60 years old (55.1 vs. 59.6%; $p = 0.5$; fig. 1). A similar distribution was also evident for genotype 3a (fig. 2); in the period 2006–2008, this genotype was present only in the under-50 age group, while in the period 2009–2011 it was also seen in the 51–60 years age group (fig. 2). The number of patients infected with genotype 4 was small but appears to have increased in older patients over the last 5 years (tables 5, 6).

Fig. 2. Distribution of HCV genotype 3a by age group in the two consecutive periods.

The significance of the different distributions of HCV genotypes in the two periods was then analysed using multivariable logistic regression in which the prevalence of different genotypes was independently associated with gender and age. We demonstrated that all the differences in HCV genotype prevalence were significant and not related to variability of gender or age group (data not shown).

Discussion

The data from the present study seem to suggest that there has been no substantial change in the overall epidemiology of HCV genotypes in the metropolitan area of Naples over the last 5 years. Genotype 1b, historically the most prevalent, both in this area and throughout Italy, remains so (48.3%), followed by genotype 2a/2c (23.5%). Genotypes 1a and 3a, on the other hand, are considerably less common (both 4.7%) in the geographical area in question, as compared to the findings from studies performed in other regions of northern Italy (9.3 and 12.7%, respectively), even though the exact classification of ‘not further classified’ genotype 1 (1*), which some studies group together with subtype 1a [20], is still unclear.

The gender distribution shows a clear frequency among females and males of genotype 1b (54.0% of 134 females vs. 41.5% of 118 males; $p < 0.05$), while genotype 3a is completely absent in males (10.2 vs. 0%; $p < 0.0005$).

Considering the age group distribution, genotypes 1b and 2 (including subtype 2a/2c and non-subtypable gen-

otype 2) were seen considerably more frequently in older patients, while 3a was particularly common in their younger counterparts. This appears to confirm the reduced risk of transfusion-related transmission and the 'stratification' of genotypes associated with risk factors reported in the literature [20, 26].

Moreover, our data seem to confirm the existence of two transmission patterns of HCV genotypes in Italy as previously reported by other authors [24]; the first pattern is characterized by infections with subtypes 1b and 2a/2c, mainly in adults over 60 years of age, consistent with past health care-related practices, and the second by subtypes 3 and 4, mainly found in the 31–60 years age group, consistent with intravenous drug abuse and immigration. Many published studies have suggested direct links between genotypes 1b and 2a/2c and iatrogenic infections and/or blood transfusions, and between genotypes 1a and 3 and drug addiction [27]. Our study showed clearly that blood transfusions now play a minor role in causing new infections because of the strict screening procedures, whereas the risk of acquiring HCV through dental therapy seems to have increased in the last 3 years.

Moreover, a direct correlation between genotype and age, with a high frequency of genotypes 1b and 2a/2c in older patients and genotypes 1a and 3a in younger patients, has also been observed [23].

Comparative analysis of the two 3-year periods (2006–2008 and 2009–2011) revealed an interesting trend in genotype 1b. This has become more and more frequent among elderly patients in recent years, which is presumably ascribable to a cohort effect. It may be that these patients, having been infected several years ago, when genotype 1b was likely to be the only one present in the population and the rate of new infection was high, have maintained their old infection, among a pool of younger carriers infected with genotypes 3a and 1a. In fact, a high frequency of genotypes 3a and 1a in under-40-year-old subjects was observed in the period 2006–2008. This was

not confirmed in the 3-year period 2009–2011, when we observed a dramatic reduction in the prevalence of genotypes 3a and 1a in subjects under 30 years of age. Multivariate analysis showed that these differences in the prevalence of genotypes 3a and 1b in the two 3-year periods were independently associated with any variability in gender and/or age group.

Recently, the emergence of genotype 4 in some Mediterranean countries, especially Spain, Greece and Italy, has been documented [24, 28, 29]. The spread of genotype 4 has been correlated with intravenous drug use and active immigration from endemic areas in the Middle East and Africa. However, these findings seem not to be confirmed in our geographical area. Genotype 4 appears to have remained stable over the last 6 years, with a low average prevalence of 1.1%.

In conclusion, it could be hypothesized that 1b is the original 'resident' genotype, spread in the 1970s, especially through iatrogenic transmission. The introduction of anti-HCV screening tests in the 1990s may have dramatically reduced this risk, thereby generating the cohort effect we now observe. In the meantime, the spread of intravenous drug use could have led to a new wave of HCV infection among young people, with an increase of new 'emergent' genotypes (3a, 1a and 4) [30]. More recently, presumably due to the diffusion of different types of drugs not necessarily linked to intravenous practice, even this transmission chain has been curtailed.

Although HCV 1b still remains the prevailing genotype, a future evaluation of new 'emerging' subtypes will be necessary.

Acknowledgements

We gratefully acknowledge the technical assistance of Pasquale Barra, Giovanni Piscopo, Antonio Mattiello and Gennaro Cozzolino.

References

- Miyakawa Y, Okamoto H, Mayumi M: Classifying hepatitis C virus genotypes. *Mol Med Today* 1995;1:20–25.
- Okamoto H, Okada S, Sugiyama Y, Kurai K, Iizuka H, Machida A, Miyakawa Y, Mayumi M: Nucleotide sequence of genomic RNA of hepatitis C virus isolated from human carriers: comparison with reported isolates for conserved and divergent region. *J Gen Virol* 1991;72:2697–2704.
- Lauer GM, Walker BD: Hepatitis C virus infection. *N Engl J Med* 2001;345:41–52.
- Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby A, Barr BJ, Weiner AJ, Bradley DW, Kuo G, Houghton M: Genetic organization and diversity of the hepatitis C virus. *Proc Natl Acad Sci USA* 1991;88:2451–2455.
- Simmonds P: Variability of hepatitis C virus. *Hepatology* 1995;21:570–583.
- Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ: The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006;144:705–714.
- Esteban JI, Saulea S, Quer J: The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008;48:148–162.

- 8 Rubio M, Rubio C, Nogués A, Manonelles A: Hepatitis C virus genotypes. Study of 302 patients coinfecting by the human immunodeficiency virus. *Med Clin (Barc)* 2001;116: 650–651.
- 9 Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S: Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* 2005;19:13–18.
- 10 Takahashi M, Yamada G, Miyamoto R, Doi T, Endo H, Tsuji T: Natural course of chronic hepatitis C. *Am J Gastroenterol* 1993;88: 240–243.
- 11 Rivas-Estilla AM, Cordero-Pérez P, Trujillo-Murillo K, Ramos-Jiménez J, Chen-López C, Garza-Rodríguez ML, Ramírez-Gutiérrez A, Muñoz-Espinosa L: Genotyping of hepatitis C virus (HCV) in infected patients from Northeast Mexico. *Ann Hepatol* 2008;7:144–147.
- 12 Pouillot R, Lachenal G, Pybus OG, Rousset D, Njouom R: Variable epidemic histories of hepatitis C virus genotype 2 infection in West Africa and Cameroon. *Infect Genet Evol* 2008;8:676–681.
- 13 Nguyen MH, Keeffe EB: Chronic hepatitis: genotypes 4 to 9. *Clin Liver Dis* 2005;9:411–425.
- 14 Hayashi K, Fukuda Y, Nakano I, Katano Y, Yoyoda H, Yokozaki S, Hayakawa T, Morita K, Nishimura D, Kato K, Urano T, Takamatsu K: Prevalence and characterization of hepatitis C virus genotyping in Japanese hepatitis carriers. *Hepatol Res* 2003;25:409–414.
- 15 Verbeek J, Maes P, Lemey P, Pybus OG, Wollants E, Song E, Nevens F, Fevery J, Delport W, Van der Merwe S, Van Ranst M: Investigating the origin and spread of hepatitis C virus genotype 5a. *J Virol* 2006;80:4220–4226.
- 16 Henquell C, Cartau C, Abergel A, Laurichesse H, Regagnon C, De Champs C, Bailly JL, Peique-Lafeuille H: High prevalence of hepatitis C virus type 5 in central France evidenced by a prospective study from 1996 to 2002. *J Clin Microbiol* 2004;42:3030–3035.
- 17 Fakeeh M, Zaki AM: Hepatitis C: prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. *Am J Trop Hyg* 1999; 61:889–892.
- 18 Cheung RC: Epidemiology of hepatitis C virus infection in American veterans. *Am J Gastroenterol* 2000;95:740–744.
- 19 Echevarría JM, León P, Pozo F, Avellón A: Follow-up of the prevalence of hepatitis C virus genotypes in Spain during a nine-year period (1996–2004). *Enferm Infecc Microbiol Clin* 2006;24:20–25.
- 20 Cenci M, Massi M, Alderisio M, De Soccio G, Recchia O: Prevalence of hepatitis C virus (HCV) genotypes and increase of type 4 in central Italy: an update and report of a new method of HCV genotyping. *Anticancer Res* 2007;27:1219–1222.
- 21 Osella AR, Misciagna G, Guerra V, Elba S, Buongiorno G, Cavallini A, Di Leo A, Sonzogni L, Mondelli MU, Silini EM: Hepatitis C virus genotypes and risk of cirrhosis in Southern Italy. *Clin Infect Dis* 2001;33:70–75.
- 22 Pawlotsky JM, Tsakiris L, Roudot-Thoraval F: Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J Infect Dis* 1995;171: 1607–1611.
- 23 Roffi L, Ricci A, Ogliari C, et al: HCV genotypes in Northern Italy: a survey of 1368 histologically proven chronic hepatitis C patients. *J Hepatol* 1998;29:701–705.
- 24 Ansaldi F, Bruzzone B, Salmaso S, Rota MR, Durando P, Gasparini R, Icardi G: Different seroprevalence and molecular epidemiology of hepatitis C virus in Italy. *J Med Virol* 2005; 76:327–332.
- 25 Dal Molin G, Ansaldi F, Biagi C, D'Agaro P, Comar M, Croce L, Tiribelli C, Campello C: Changing molecular epidemiology of hepatitis C virus infection in Northeast Italy. *J Med Virol* 2002;68:352–356.
- 26 Guadagnino V, Stroffolini T, Rapicetta M, et al: Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. *Hepatology* 1997;26: 1006–1011.
- 27 Vitale F, Villafrae MR, Viviano E, et al: Distribution of hepatitis C virus genotypes among intravenous drug users. A ten-year study in Palermo, Sicily. *New Microbiol* 1998; 21:335–339.
- 28 Fernandez-Arcàs N, Lopez-Siles J, Trapero S, Ferraro A, Ibanez A, Orihuela F, Maldonado J, Alonso A: High prevalence of hepatitis C virus subtypes 4c and 4d in Malaga (Spain): phylogenetic and epidemiological analyses. *J Med Virol* 2006;78:1429–1435.
- 29 Katsoulidou A, Sypsa V, Tassopoulos NC, Boletis J, Karafoulidou A, Ketikoglou I, Tsantoulas D, Vafiadi I, Hatzis G, Skoutelis A, Akriviadis E, Vasiliadis T, Kitis G, Magiorkinis G, Hatzakis A: Molecular epidemiology of hepatitis C virus (HCV) in Greece: temporal trends in HCV genotype-specific incidence and molecular characterization of genotype 4 isolates. *J Viral Hepat* 2006;13: 19–27.
- 30 Pizzillo P, Almaso PL, Ferraro D, Craxì A, Di Stefano R: HCV genotypes in Sicily: is there any evidence of a shift? *J Med Virol* 2009;8: 1040–1046.