

VIRAL HEPATITIS

Hepatitis C virus (HCV) genotypes in 373 Italian children with HCV infection: changing distribution and correlation with clinical features and outcome

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Background and aim: Little is known of hepatitis C virus (HCV) genotypes in HCV infected children. This retrospective, multicentre study investigated genotype distribution and correlation with clinical features and outcome in a large series of Italian children.

Methods: Between 1990 and 2002, 373 HCV RNA positive children, consecutively recruited in 15 centres, were assayed for genotypes by a commercial line probe assay.

Results: The following genotype distribution pattern was recorded: genotype 1b=41%; 1a=20%; 2=17%; 3=14.5%; 4=5%; other=2.5%. The prevalence of genotypes 1b and 2 decreased significantly ($p<0.001$) among children born from 1990 onwards compared with older children (46% v 70%) while the rate of genotypes 3 and 4 increased significantly (from 8% to 30%). Children infected with genotype 3 had the highest alanine aminotransferase levels and the highest rate of spontaneous viraemia clearance within the first three years of life (32% v 3% in children with genotype 1; $p<0.001$). Of 96 children enrolled in interferon trials during the survey, 22% definitely lost HCV RNA, including 57% of those with genotypes 2 and 3.

Conclusion: HCV genotypes 1 and 2 are still prevalent among infected adolescents and young adults in Italy but rates of infection with genotypes 3 and 4 are rapidly increasing among children. These changes could modify the clinical pattern of hepatitis C in forthcoming years as children infected with genotype 3 have the best chance of spontaneous viraemia clearance early in life, and respond to interferon in a high proportion of cases.

Hepatitis C virus (HCV) exhibits marked sequence heterogeneity. Six major genotypes, labelled 1–6, and numerous subtypes have been described,¹ with different geographical distributions.^{2–6} Genotypes 1b and 2 are prevalent in Southern Europe; 1a in the USA and Northern Europe; 3 in the Indian subcontinent and among drug abusers in Europe; and type 4 in Africa and the Middle East. Several studies in adults have specifically evaluated the putative role of genotypes in the progression of liver disease and in the response to interferon (IFN) therapy. Infection with genotype 1b has been associated with a greater risk of progression to cirrhosis^{7–9} and hepatocellular carcinoma but these data are controversial.^{10–13} It has been suggested that the severity of liver disease could reflect a longer duration of infection rather than a more aggressive form of hepatitis.^{14 15} On the other hand, a correlation has been found between hepatic steatosis and infection with genotype 3 that could be responsible for liver damage through a direct effect on hepatic lipid homeostasis.¹⁶ There is, instead, general agreement on the role of genotypes as predictors of response to IFN, alone or combined with ribavirin, the highest response rates being reported in patients infected with genotypes 2 and 3.^{17 18}

Little is known of HCV genotypes in children,^{19–21} essentially because children are less commonly infected than adults. In Italy, the estimated prevalence of anti-HCV in the paediatric population was 0.3% in the early 1990s,²² thus most paediatric studies were limited by the small sample size. Nevertheless, paediatric HCV infection could be an interesting model to evaluate the role of genotypes due to the

peculiar modes of infection, minor relevance of environmental cofactors of liver damage, and clinical features of liver disease,^{23–26} usually asymptomatic and with mild liver lesions,²⁷ but with a variable biochemical expression. In this multicentre retrospective study, we describe the epidemiological and clinical features associated with different HCV genotypes in a large series of HCV infected children without underlying systemic diseases, consecutively observed in 15 Italian centres between 1990 and 2002.

PATIENTS AND METHODS

Design of the study

This multicentre retrospective study was designed to investigate HCV genotypes and related epidemiological and clinical features in a series of anti-HCV and HCV RNA positive children consecutively observed in 15 paediatric and infectious disease departments between 1990 and 2002. These institutions participated in the "Italian Observatory for Hepatitis C in Children", set up in 1998 by the Hepatologic Group of the Italian Society of Paediatric Gastroenterology and Hepatology with the purpose of censuring children with HCV infection.

Each of the referral centres fulfilled the following requirements: referral area unchanged over the survey; routine investigation of HCV RNA in anti-HCV positive children; HCV genotypes investigated in at least two thirds of viraemic

Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; IFN, interferon; HAI, histological activity index; IVDA, intravenous drug abuse

patients; and availability of baseline and follow up clinical information based on patient records. None of the centres managed selected hepatological populations and only two had conducted a study of mother-infant HCV transmission lasting one or two years in the past decade. Eleven centres were located in Northern, three in Central, and one in Southern Italy.

Patients

To be included in the study, anti-HCV and HCV RNA positive patients had to fulfil the following criteria: age 1–16 years; no coinfection with human immunodeficiency virus or with hepatitis B virus; and no concomitant metabolic or autoimmune disorders or underlying systemic diseases, including previous malignancy, uraemia, thalassaemia, and haemophilia.

In untreated patients, sustained viraemia clearance was defined as disappearance of HCV RNA documented at least twice over a two year period. In patients treated with a 6–12 month course of recombinant interferon alpha, sustained response indicated clearance of viraemia occurring within six months after stopping treatment and persisting thereafter with associated normalisation of alanine aminotransferase (ALT) levels. Finally, the diagnosis of past infection relied on sustained ALT normality in anti-HCV positive children with undetectable viraemia at baseline, confirmed at least once over a two year period.

Children were observed in the outpatient clinic every 4–6 months during the first two years of life and then every 6–12 months for physical examination and serological tests. For each patient an anonymous schedule was completed, including age and sex, putative source of infection, mode of observation, biochemical and virological data, liver histology when available, treatment, outcome, and complications. The data were collected in the coordinator centre in Padua and the study was approved by an independent ethics committee.

Methods

Anti-HCV was determined by second and third generation commercial ELISA assays. HCV RNA was investigated in fresh and well preserved stored sera by the polymerase chain reaction, using either homemade or commercial qualitative assays. No attempts were made to collect sera in a unique laboratory, but genotype determination was performed using the same line probe hybridisation assay (LIPA HCV; Innogenetics, Zwijndrecht, Belgium). Genotypes were classified according to Simmonds and colleagues.¹

Liver biopsy was performed in selected cases, before starting treatment or for staging purposes, by the Menghini technique, after informed consent was obtained from parents or guardians. Some of the biopsies had been reviewed and reported on by a unique pathologist (MG) in previous studies^{26, 27} using Ishak's scoring system²⁸ to evaluate the histological activity index (HAI) and entity of fibrosis. The final label was assigned as follows: minimal hepatitis when

HAI \leq 3; mild hepatitis for HAI 4–6; moderate hepatitis for HAI 7–12; and severe hepatitis for HAI $>$ 12.

Statistical analysis

The association between categorical variables and genotypes was assessed using the χ^2 and Fisher's exact test. The difference between the means of continuous variables in genotype subgroups was tested by ANOVA and the T3 Dunnett post hoc comparison. Mantel-Haenszel statistics were used for multitable comparison of HCV RNA clearance rate by genotypes in treated and untreated patients. The odds estimation of risk factors for HCV RNA clearance in untreated patients was performed by multiple logistic regression analysis.

RESULTS

During the observation period, 522 consecutive anti-HCV positive children were tested once or more for HCV RNA with the following results:

- 24 (4.5%) children were HCV RNA negative: two were negative at baseline and could not be seen thereafter while 21 (tested three times on average) remained negative and with normal ALT levels over a median follow up period of eight years. Sources of infection were blood transfusions (58%), maternal infection (25%), or unknown (17%); mean age at presentation was 73 (46) months.
- 498 children were HCV RNA positive: of them, 125 (24%) were not investigated for HCV genotypes for various reasons such as short term follow up before implementation of genotyping, early occurrence of viraemia clearance, and lack of well preserved serum samples for retrospective investigation. The remaining 373 (71.5%) HCV RNA positive children were tested for HCV genotypes and included in the study. There were no statistically significant differences between these two groups of viraemic children in terms of age, sex, duration of infection, and biochemical pattern (data not shown).

Genotype distribution and epidemiological aspects

The distribution of genotypes in the 373 children, and the related epidemiological and biochemical features, are summarised in tables 1 and 2. Duration of infection at the first observation in the referral centre ranged from one month to 12 years (median three months). As anti-HCV screening was introduced into blood banks from 1990, we also compared the prevalence of the different genotypes in children born before 1990 with that of children born (and thus certainly infected) in or after 1990. Table 2 shows the relationship between the principal genotypes and the putative source of infection.

- Genotype 1b was the single most frequent type (41%); more than half of the children with this pattern had been infected before 1990 (the oldest patient was born in 1974),

Table 1 Distribution of hepatitis C virus (HCV) genotypes and demographic features in 373 children

	HCV genotype (n (%))					
	1a (73 (20))	1b (153 (41))	2 (63 (17))	3 (54 (14.5))	4 (20 (5))	Other (10 (2.5))
Age (months) (mean (SD))‡	64.4 (49.8)	81.6 (58.6)	92.0 (56.3)	34.8 (34.1)	23.5 (21.0)	Not done
Born before 1990 (181)††† (n (%))	33 (18)	89 (49)	38 (21)	9 (5)	6 (3.5)	6 (3.5)
Born in/after 1990 (192)††† (n (%))	40 (21)	64 (33.5)	25 (13)	45 (23.5)	14 (7)	4 (2)
Male (184)* (n (%))	40 (22)	86 (47)	24 (13)	28 (15)	3 (1.5)	3 (1.5)
Female (189)* (n (%))	33 (18)	67 (36)	39 (20)	26 (14)	17 (9)	7 (3%)

Statistical significance: *p=0.07 for genotype distribution in male and female patients; †††p<0.0001 for genotype distribution in children born before and after 1990; ‡genotype 1b versus 3, p<0.001; genotype 1b versus 4, p<0.013; genotype 2 versus 3, p<0.0001; genotype 2 versus 4, p<0.006.

Table 2 Relationship between the principal genotypes and the putative source of infection

	Genotype					p Value
	1a (73)	1b (153)	2 (63)	3 (54)	4 (20)	
Transfusions (104) (n (%))	19 (18)	59 (57)	23 (22)	3 (3)	0	<0.001*
Surgery, other procedures (16) (n (%))	3 (19)	5 (31)	8 (50)	0	0	nd
Infected mother (198) (n (%))	42 (21)	68 (35)	24 (12)	44 (22)	20 (10)	<0.001
Drug abuser (81) (n (%))	23 (28.5)	14 (17.5)	5 (6)	26 (32)	13 (16)	
Other/unknown (45) (n (%))	9 (20)	21 (46.5)	8 (18)	7 (15.5)	0	nd

*Genotypes 3 and 4 pooled for analysis.

and more than half of the transfused children were infected with this genotype.

- Genotype 1a was more frequently associated with maternal infection and almost half of the mothers admitted intravenous drug abuse (IVDA); 45% of children were born before 1990 and the oldest patient was born in 1982.
- Genotype 2 was preferably seen in older children with percutaneous or unknown exposure; rarely the mother was known to be IVDA. The oldest case was born in 1974.
- Genotype 3 was typically observed in children of infected mothers, half of whom were IVDA. The large majority of patients came to observation early in life and 83% were seen after 1990. The oldest child was born in 1981.
- Genotype 4 was invariably associated with maternal infection, often in the drug abuse setting; infection was diagnosed early in life, with increasing prevalence in the last decade. The oldest child was born in 1980. Interestingly, most patients were female, but the number of children is too small to interpret this correlation.
- A group of 10 children had mixed 1/2 or 1/3 genotypes (eight cases tested early in the 1990s) or an undetermined pattern

Overall, 76% of children whose mother admitted IVDA had genotype 1a, 3, or 4 versus 37% of children whose mother had no history of IVDA. As regards geographic distribution, the prevalence of genotypes 1a, 3, and 4 was significantly ($p < 0.01$) greater among children from Northern and Central regions (133/310 = 43%) than among those from Southern Italy (14/63 = 22%) who were more often infected with genotypes 1b and 2.

Genotypes and features of liver disease at presentation

Infection was asymptomatic in 82% of cases and presented with mild non-specific symptoms in the remaining patients. Table 3 shows baseline ALT levels in children with different genotypes. The highest values were found in children infected with genotype 3 and the lowest in those infected with genotype 2.

No attempts were made to correlate genotypes with liver histology in this study, essentially because liver biopsy had been obtained, either at enrolment or during follow up, usually before starting IFN treatment, in a selected population of 127 HCV RNA positive children with abnormal ALT

levels. Only 12 children had genotype 3 or 4. Moreover, 84 of these biopsies had been previously revised and reported by the same pathologist in previous studies.^{26, 27} The histological pattern described in this subgroup was relatively homogeneous, with 78% of cases classified as minimal-mild hepatitis, 20% as moderate hepatitis, 1% as severe hepatitis, and 1% as cirrhosis.

Genotypes and outcome

A total of 366 children were followed for at least six months (mean (SD) 5.04 (3.15) years) from first observation in the referral centre. Duration of follow up calculated from the putative time of exposure or from the first anti-HCV positive test was 7.0 (4.6) years. During observation, 96 patients were treated with IFN as participants in some therapeutic trial and 270 remained untreated.

Outcome in untreated cases

No relevant clinical events related to HCV infection occurred during observation and all patients remained anti-HCV positive. Table 4 shows the biochemical and virological outcome during follow up.

Spontaneous sustained HCV RNA clearance occurred in a minority of patients, in most instances within the third year of life. When only children aged three years or less at the time of diagnosis were considered, it appeared that viraemia clearance was significantly associated with genotype 3 compared with genotype 1. In a multivariable logistic regression analysis, genotype 3 and young age at diagnosis were independently associated with spontaneous sustained viraemia clearance (table 5). Loss of viraemia was associated with persistent ALT normalisation. Of note is the fact that persistent ALT normality or sustained normalisation in the presence of viraemia was frequent in children with genotype 2.

Outcome in treated patients

Treated children were older than two years and had ALT levels ≥ 1.5 times normal. In most liver biopsy had shown mild liver disease. IFN had been administered by subcutaneous injection at a dose of 3–5 MU/m² thrice weekly for 6–12 months. Table 6 shows the response rate six months after stopping treatment, calculated following "intention to treat" criteria (all patients had received at least two injections). The efficacy of IFN was significantly greater in

Table 3 Alanine aminotransferase (ALT) levels at the baseline observation in 363 children with genotypes 1–4

ALT	Genotype					
	1a	1b	2	3	4	Other
Times \times normal (mean (SD))	2.6 (2.0)	2.5 (2.4)	2.2 (1.9)	3.4 (3.0)	2.9 (2.2)	3.2 (2.8)

ANOVA: $p = 0.051$.

Table 4 Outcome in 270 untreated children followed for at least six months, including 119 cases diagnosed within the third year of life

Genotype	No of cases	HCV RNA clearance		Viraemia with persistent ALT normality
		Total cases*	Within 3rd y of life†	
1a	49	1 (2%)	1/19 (5%)	5 (10.2%)
1b	107	2 (2%)	1/40 (2.5%)	20 (18.6%)
2	50	2 (4%)	1/15 (7%)	20 (40%)
3	41	9 (22%)	9/28 (32%)	5 (12.1%)
4	20	1 (5%)	1/17 (6%)	0
Other	3	0	0	0

Statistical analysis: *HCV RNA clearance distribution according to different genotypes: $p < 0.001$. †HCV RNA clearance distribution during the first three years of life according to different genotypes: $p < 0.001$; genotype 3 versus 1, $p < 0.001$.
HCV, hepatitis C virus; ALT, alanine aminotransferase.

Table 5 Multivariable logistic regression analysis: variables independently associated with hepatitis C virus RNA clearance in untreated patients

Variable	Regression coefficient	Standard error	p Value	Odds ratio (95% CI)
Age at diagnosis (months)	-0.036	0.016	0.027	0.964 (0.934-0.996)
ALT IU/l	0.065	0.070	0.350	1.067 (0.931-1.224)
Genotype 2 v 1	1.186	0.972	0.223	3.272 (0.487-21.983)
Genotype 3 v 1	1.741	0.769	0.024	5.703 (1.264-25.724)
Genotype 4 v 1	0.498	1.212	0.681	1.645 (0.153-17.711)
Genotype other v 1	-5.295	60.435	0.930	0.005 (0.000-9876543)

ALT, alanine aminotransferase.

children with genotypes 2 and 3. Comparison of treated and untreated children by genotypes using the Mantel Haenszel statistic showed a significantly higher rate of viraemia clearance ($p < 0.001$) in treated children.

DISCUSSION

The distribution of HCV genotypes in Italy has been extensively investigated in infected adults, both in selected groups and in the general population.²⁹⁻³³ Genotype 1b is prevalent, particularly among transfused patients; genotype 2 is frequent in community acquired cases, especially in Southern Italy, while the proportion of genotypes 1a, 3, and 4 depends on the prevalence of drug abuse in a given area. These patterns and their fluctuations are the result of important socio-sanitary events recorded in the past decades, such as: the disappearance of post transfusion hepatitis C; improvement in sanitation policies which reduced the risk of community acquired hepatitis³²; spread of IVDA which caused an epidemic of viral hepatitis among young adults, especially in the Northern urban areas between the mid 1970s and the mid 1980s³⁴; and unsafe sexual behaviours and frequent travel to highly endemic areas of the far East,³⁵ which promoted the circulation of genotypes different from 1b and 2. These events, either directly or indirectly, have influenced the distribution pattern of HCV genotypes in children. In fact, following the disappearance of post transfusion hepatitis, maternal infection has become the almost exclusive source of contagion,³⁶ and the pattern of HCV genotypes in childhood is expected to reflect the epidemiological trends of infection in childbearing aged women.

Our study was retrospective, was based on children observed in tertiary care centres, and the proportion of cases from Southern Italy was relatively low. These biases could reduce the accuracy of prevalence estimates but the large number of children consecutively enrolled in the same institutions with an unchanged referral area, enrolment based on serological screening rather than on symptomatic presentation, and substantial agreement of our data with

epidemiological trends in the adult population³¹ suggest that our patients could be representative of the paediatric population infected with HCV. Overall, the scenario of HCV infection in Italian children is one of dynamic events, currently represented by a declining prevalence of genotypes 1b and 2 (from 49% to 33%, and from 21% to 13%, respectively, before and after 1990) and by an increasing proportion of genotypes 3 and 4 (from 8% to 30%). Our results confirm that genotypes 3 and 4, and to a lesser extent 1a, are closely correlated with maternal drug abuse.

These epidemiological changes may have clinical and therapeutic implications. In fact, children infected with genotype 3 early in life seem to have the greatest chances of spontaneous sustained viraemia clearance. Both our results and those of Saracco and colleagues³¹ show that average ALT values in patients with genotype 3 are greater than those in patients with other genotypes, suggesting a more active cytolysis. Whether this behaviour is in keeping with recent studies claiming a direct cytopathic effect of genotype 3 on infected hepatocytes¹⁶ remains to be demonstrated. In a previous report²⁷ on liver histology in children with chronic HCV infection, we found a significant correlation between severity of intralobular focal necrosis and high ALT levels, thus supporting the hypothesis that infection with

Table 6 Prevalence of sustained response in the 96 patients treated with interferon (IFN) for 6-12 months

Genotype	No of cases	Median age (y) at start of IFN	Sustained response (n (%))
1a	24	7.5	3 (12)
1b	44	9	6 (14)
2	11	11.5	7 (64)
3	10	7	5 (50)
Other	7	8	0

Statistical analysis: distribution of sustained response in relation to different genotypes: $p < 0.001$. Genotype 2+3 versus type 1, $p < 0.001$.

genotype 3 may be more often responsible for active disease early in life.

In untreated children a multivariate analysis showed that HCV RNA clearance was independently associated with genotype 3 and with infection diagnosed early in life. Most children who cleared viraemia had been vertically infected and had lost HCV RNA within the third year of life, in agreement with previous reports,^{23–27} as if they were affected by a protracted acute hepatitis. On the other hand, the data in this study and our previous reports support the concept that sustained clearance of viraemia in a patient with longlasting infection is rare, independent of genotype. Of note is the observation that the oldest group (children with genotype 2) had the lowest average ALT values, and normal ALT levels were a frequent finding throughout the observation period. Similar values have been reported by Puoti and colleagues³⁸ who detected genotype 2 in 52% of HCV carriers with normal ALT levels. The reasons for this finding await further investigation.

Several studies in adults have suggested that the duration of infection might be more important than virus genotype in the progression of HCV associated liver disease.^{14–15} Actually, our data show that patients with a relatively short history of infection, such as children, develop mild liver lesions in the large majority of cases.

Finally, our data on IFN therapy confirm that rates of HCV RNA clearance are significantly higher in treated than in untreated patients, independent of genotype. Among treated children, those with types 2 and 3 had a significantly higher response rate to standard IFN than children with genotype 1. These issues are well established in adults but only inferred from the small available paediatric trials.

In conclusion, genotypes 1 and 2 are still largely prevalent among Italian adolescents and young adults with HCV infection, but genotypes 3 and 4, especially linked to maternal drug abuse, are becoming more and more frequent among children and young babies. These changes could modify the clinical pattern of hepatitis C in forthcoming years. In fact, children infected with genotype 3 have the best chances of spontaneous viraemia clearance after perinatal infection and show a remarkable response rate even to standard IFN alone; conversely, little is known of the outcome of infection with genotype 4, although preliminary results³⁹ would be consistent with a poor response to standard therapy.

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