Genetic Variation in Interleukin 28B with Respect to Vertical Transmission of Hepatitis C Virus and Spontaneous Clearance in HCV-Infected Children

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The vertical transmission of hepatitis C virus (HCV-VT) is a major route of HCV infection in children, but the risk factors remain incompletely understood. This study analyzed the role of interleukin 28B (IL28B) in HCV-VT and in the spontaneous clearance of HCV among infected infants. Between 1991 and 2009, 145 mothers were recruited for this study: 100 were HCV-RNA+ve / human immunodeficiency virus negative (HIV−ve), with 128 children, and 33 were HCV-RNA−ve/HCV antibody+ve, with 43 children. The infants were tested for HCV-RNA at birth and at regular intervals until the age of 6 years. IL28B (single nucleotide polymorphism rs12979860) was determined in the mothers and children. HCV-VT was assumed when children presented HCV-RNA+ve in two subsequent blood samples. HCV-VT-infected infants were categorized as: (1) transient viremia with posterior HCV-RNA−ve and without serum-conversion; (2) persistent infection with serum-conversion. Of the 31 mothers with CC polymorphism, 19 (61%) were HCV-RNA+ve, whereas among the 68 mothers with non-CC polymorphism, 56 (82%) were HCV-RNA+ve. In all, 26 of 128 (20%) infants born to the HCV-RNA+ve mothers acquired HCV infection, but only 9 (7%) were chronically infected. The rate of HCV-VT was higher among the mothers with higher HCV viremia. No HCV-VT was detected in the HCV-RNA−ve women. Neither the mothers' nor the childrens' IL-28 status was associated with an increased risk of HCV-VT. The factors influencing viral clearance among the infected children were genotype non-1 and genotype CC of IL28B. In logistic regression, child CC polymorphism was the only predictor of HCV-clearance in HCV genotype-1. Conclusion: High maternal viral load is the only predictive factor of HCV-VT. IL28B plays no role in HCV-VT, but IL28B CC child polymorphism is associated independently with the spontaneous clearance of HCV genotype-1 among infected children. (HEPATOLOGY 2011;53:1830-1838)

Infection with hepatitis C virus (HCV) is a worldwide health problem, with more than 170 million individuals infected. In industrialized countries, HCV is the most common cause of chronic liver disease in children. Since 1992, HCV vertical transmission (HCV-VT) from an infected mother to her newborn infant has constituted the predominant acquisition mode of HCV infection and, despite better understanding of the risk factors involved in the perinatal transmission of HCV, to date little is known about the underlying transmission mechanisms and timing.[1,2] The natural history of HCV infection in children is not yet well defined; most children are asymptomatic despite common ongoing viremia and

Abbreviations: ALT, alanine transaminase; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus ribonucleic acid; HCV-RNA+ve, HCV-RNA positive; HCV-RNA−ve, HCV-RNA negative; HCV-VT, hepatitis C virus vertical transmission; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IL28B, interleukin 28B (interferon, lambda 3); PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

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alanine transaminase (ALT) levels that are variable but could reach levels compatible with acute hepatitis[1] and remain so for decades.[3] Risk factors for mother-to-child transmission of HCV have been shown to include the presence of a high concentration of HCV RNA in maternal blood and human immunodeficiency virus (HIV) coinfection.[4] Vertical transmission is almost always restricted to women with HCV-RNA detectable in peripheral blood by polymerase chain reaction (PCR). Nevertheless, all children born to women with anti-HCV antibodies should be tested for HCV. The relationship between HCV-VT and maternal HCV genotype remains unclear because few studies have investigated the role of HCV genotype as a risk factor for HCV-VT. It has been reported that high ALT levels during the first year of life and genotype 3 infections are associated with a higher chance of sustained clearance of HCV-RNA and biochemical remission.[5,6] However, other authors have indicated that there is no relationship between HCV-VT and maternal HCV genotype.[7] Moreover, the importance of birth mode (vaginal or cesarean) and type of feeding (breast feeding or replacement) has been investigated, in view of their possible influence on transmission, but the results achieved are conflicting and more data are required to clarify the role of these factors in HCV-VT.[8,9]

The HCV risk factors traditionally considered (HIV coinfection, HCV viral load) do not properly describe the possibility of HCV-VT or that of HCV chronic infection. It has been suggested that the role of the immune defense system could better account for the pathogenesis of HCV infection.[10,11] Thus, the relevance of the genetic background has been taken into consideration, with special attention being focused on the human leukocyte antigen (HLA) system, because of its central role in immune response. Bosi et al.[10] showed that HLA DR13 might modulate the immune response to HCV, exerting a protective role against the development of vertical infection. Other studies have reported that HLA-DRB1*0701, HLA-DRB1*10, and DRB1*1401 alleles in the child play a predisposing role for transmission, whereas HLA-DRB1*1104, DRB1*1302 alleles in the child and the HLA-DRB1*04 in the mother are apparently protective.[11,12] These findings highlight the importance of the genetic background in the vertical transmission of HCV and the need for more knowledge of genetic factors and HCV-VT. Recent studies indicate that there is a relationship between Rs12979860 CC interleukin 28B (IL28B) genotype and HCV treatment response in adults.[13-15] However, the CC IL28B genotype influences in HCV-VT and the spontaneous clearance of HCV among infected children have been little investigated. We hypothesize that maternal and/or neonatal IL28B immunogenetic factors may affect both HCV-VT and its chronic infection.

The aim of the present study was to identify the role of the IL28B genotype and of other risk factors for HCV-VT, and to determine the predictors of spontaneous clearance among children infected with HCV. There was found to be a significant association between IL28B Rs12979860 CC child genotype and the likelihood of the spontaneous clearance of HCV among infants born to HCV-infected mothers. On the other hand, high maternal viral load was the only variable predictive of HCV-VT. The findings of this study could enhance our understanding of both the pathogenesis of vertical HCV infection and of the spontaneous clearance of HCV infection among children, as well as enabling a better identification of cases at higher risk, which would be useful for the development of prevention strategies.

Materials and Methods

Subjects. A prospective cohort study was conducted at Hospital Universitario San Cecilio in Granada (Spain) from 1991 until 2009. In all, 112 consecutive HCV-RNA-positive mothers with their 142 children and 33 HCV-RNA-negative/HCV antibody-positive mothers with their 43 children were enrolled and followed up for at least 6 years. All patients included in this study were Caucasian. These mothers were routinely tested for HCV during prenatal care. The background data for the 179 pregnancies of 145 mothers are given in Fig. 1. The diagnosis of HCV-VT was based on detectable HCV-RNA in the peripheral blood by PCR. HCV-VT was defined as children who presented HCV-RNA-positive in at least two subsequent blood samples. The study groups for HCV-VT were: (1) transient viremia, infants who exhibited HCV-RNA+ve in at least two subsequent blood samples with posterior HCV-RNA−ve and without serum-conversion; (2) chronic or persistent infection group, defined

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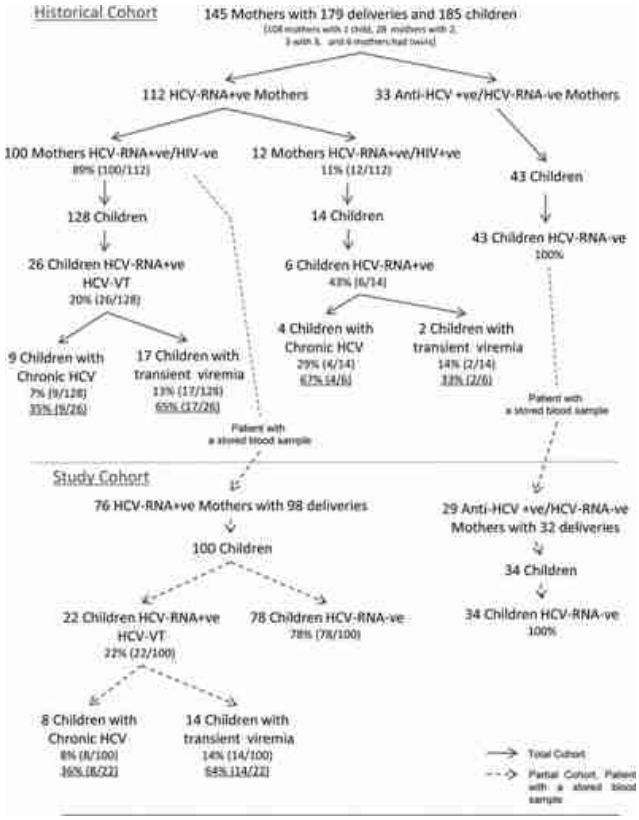

Fig. 1. Infant outcomes according to maternal HCV status. The data for the 179 pregnancies of 145 mothers are shown. In the HCV-VT and chronic infection study, the risk factors were identified among the HIV-negative mothers using a stored blood sample (Partial Cohort).

as children with persistent HCV-RNA+ve with HCV serum-conversion (detectable anti-HCV). The HCV-RNA+ve in at least two samples criterion was established to minimize the risk of false positives. When the infants presented an initial HCV-RNA+ve test, a further analysis was performed in a new blood sample, a few days later, in order to confirm the first positive and to determine the viral genotype. No false positives were recorded in this study and all infants were HCV-RNA+ve in the second test. Risk factors for HCV-VT, transient viremia, and chronic infection were determined among the HIV-negative mothers using a stored blood sample (Fig. 1; 76 HCV-RNA-positive mothers and 29 HCV-RNA-negative/HCV antibody-positive mothers with their children). The risk factors for HCV-VT, transient viremia, and chronic infection were considered, and the values for HCV viral load, genotype, delivery mode, duration of ruptured membranes, ALT levels, breast-feeding, and the duration of breastfeeding were obtained. The infants were examined by pediatricians and tested for HCV-RNA at birth and at 2, 4, 6, 8, 10, 12, 18, and 24 months; and thereafter at 3, 4, 5, and 6 years. Informed written consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration, as reflected in the *a priori* approval granted by the Ethics Committee.

Virologic Assays. HCV genotyping was determined by reverse hybridization (Inno-LIPA II HCV Innogenetics SA Ghent, Belgium). The viral load (cutoff <15 IU/mL, HCV Ampliprep TaqMan, Roche Molecular System) was determined quantitatively during delivery.

IL28B Genotyping. Rs12979860 genotyping was performed by means of a Taqman 5′ allelic discrimination assay (Custom Assay Service). The primers used were forward GCCTGTCGTGTACTGAACCA and reverse GCGCGGAGTGCAATTCAAC. The Taqman probes from the reverse strand were TGGTTCGCGC CTTC labeled with VIC and CTGGTTCACGCC TTC labeled with FAM. Single nucleotide polymorphism (SNP) amplification assays were used according to the manufacturer's instructions. The PCR reaction was carried out in a total volume of 10 μL with the following amplification protocol: preincubation at 50°C for 2 minutes and at 95°C for 10 minutes, followed by 40 cycles of 95°C, 15 seconds; 60°C, 1 minute. The genotype of each sample was automatically attributed by the SDS 2.2.1 software for allelic discrimination (Applied Biosystems, Foster City, CA).

Statistical Analysis. The dependent variables were vertical transmission and the degree of HCV chronic infection among the infants. Bivariate analysis was conducted using the χ^2 test and Fisher's exact test, and the degree of association between HCV-VT/chronic infection and the independent variables was determined by calculating the corresponding odds ratio (OR) and its 95% confidence interval (95% CI) by means of simple logistic regression. Quantitative variables are expressed as the means ± SEM (standard error of the mean). For differences in the quantitative variables, the paired/unpaired Student's *t* test or the Mann-Whitney *U* test was used. Multivariate logistic regression was conducted for the simultaneous analysis of more than one statistical variable and to determine the interaction among the different variables. The following covariates were included in the multivariable model: ALT level, viral genotype, viral load, delivery mode, breast-feeding, and IL28B. A *P*-value < 0.05 was considered statistically significant. All statistical calculations were performed using SPSS software v. 15.0 for Windows.

Results

General Cohort

Of the 145 mothers recruited (Historical Cohort), 112 were HCV-RNA-positive (77%) and 33 were

Table 1. Characteristics of HCV-RNA-Positive Infants and Their Parents

Case	Genotype Mother/ Child	Epidemiology	Viral Load (UI/mL)	BF (days)	Type of Birth	HCV-RNA+ve* (month)	Highest ALT† (U/L)	Seronegative‡ (month)	IL28B Child/ Mother	Gender	Gestational Age	HCV-RNA Father
1	1	Drugs	>600,000	0	Cesarean	2 (Chronic)	145	No	CT/CT	Male	30	+
2	1	Sporadic	>600,000	75	Noncesarean	2 (Chronic)	108	No	TT/TT	Female	36	-
3	1	Sporadic	>600,000	90	Noncesarean	2 (Chronic)	140	No	CT/CC	Female	39	-
4	1	Transfusion	>600,000	75	Cesarean	3 (Chronic)	78	No	CT/CT	Male	38	-
5	1	Sporadic	>600,000	0	Noncesarean	4 (Chronic)	88	No	CT/TT	Male	39	-
6	1	Sporadic	>600,000	360	Noncesarean	3 (Chronic)	220	No	CC/CC	Male	39	-
7	1	Drugs	>600,000	0	Noncesarean	2 (Chronic)	86	No	TT/CT	Male	33	+
8	1	Transfusion	>600,000	0	Noncesarean	2 (Chronic)	145	No	CT/CC	Female	39	-
9	1	Sporadic	>600,000	15	Noncesarean	4 (3)	199	12	CC/CC	Female	40	-
10	1	Sporadic	>600,000	60	Noncesarean	1 (2)	19	14	CC/CC	Male	40	-
11	1	Sporadic	>600,000	50	Noncesarean	12 (2)	26	8	CT/TT	Male	39	-
12	1	Sporadic	<600,000	240	Noncesarean	2 (2)	34	8	CC/CC	Female	40	-
13	1	Transfusion	>600,000	6	Cesarean	1 (2)	25	12	CC/CC	Female	41	-
14	1	Transfusion	>600,000	120	Noncesarean	2 (3)	50	18	CC/CT	Male	39	-
15	1	Transfusion	<600,000	40	Noncesarean	2 (3)	69	12	CT/CT	Male	41	-
16	3	Drugs	<600,000	30	Cesarean	2 (2)	33	12	TT/CT	Male	38	-
17	3	Drugs	>600,000	90	Noncesarean	1(3)	68	12	CC/CC	Female	41	-
18	3	Drugs	>600,000	30	Noncesarean	3 (3)	53	12	CC/CC	Male	40	-
19	3	Transfusion	>600,000	0	Noncesarean	8 (2)	39	12	CC/CT	Female	28	-
20	3	Sporadic	>600,000	0	Noncesarean	2 (3)	40	10	CT/CC	Male	40	-
21	3	Drugs	>600,000	0	Noncesarean	4 (3)	44	18	CT/CT	Male	38	+
22	4	Drugs	>600,000	0	Noncesarean	4 (2)	32	12	TT/CT	Male	40	+

BF= Breast-feeding.

*Month with first VHC-RNA positive; the number in parentheses shows the number of tests with HCV-RNA positive; Chronic, indicates that these infants presented HCV-RNA positive permanently.

†The normal range of values for ALT is from 5 to 40 U/L.

‡Month in which the HCV antibodies (mother antibodies) were not detected in the infant.

HCV-RNA-negative/HCV antibody-positive (23%, Fig. 1). In total, 185 infants were born to these mothers. The HCV-RNA-positive mothers had 142 children and 43 were recorded in the HCV-RNA-negative/HCV antibody-positive group. The rate of HCV-VT was 20% (26/128) in the infants born to HCV-RNA+ve/HIV−ve noncoinfected mothers and 43% (6/14) in those born to HIV+ve-coinfected mothers (OR = 3.6; 95% CI: 1.4-6.6; $P = 0.009$). The rate of infants with persistent infection (chronic infants) was 7% (9/128) in infants born to HCV-RNA+ve/HIV−ve mothers and 35% (9/26) with respect to the HCV-VT infants. Moreover, the virus cleared in 17 children (17/26, 65%). On the other hand, the rate was 29% (4/14) in infants born to HIV+ve-coinfected mothers and 67% (4/6) with respect to the HCV-VT infants (OR = 5.3; 95% CI: 2.2-14.5; $P = 0.0001$). In this case, the virus cleared in two infants (2/6, 33%). The genotype in each of the infants was consistent with that of their mothers. None had received a blood transfusion or presented other risk factors. The characteristics of the HCV-RNA+ve infants and their parents are described in Table 1. No vertical transmission was noted among the HCV-RNA−ve women.

Risk Factors in HCV Vertical Transmission. In the HCV-VT and chronic infection study, risk factors were identified among the HIV-negative mothers using a stored blood sample (Study Cohort; Fig. 1). The characteristics of the HCV-RNA+ve infants and their parents are described in Table 1. The rate of HCV-VT was higher for infants born to mothers with high HCV viremia (>600,000 IU/mL) than for infants born to mothers with low HCV viremia (<600,000; Table 2; $P = 0.02$). Neither gender, nor weight, nor viral genotype (genotype 1 versus genotype non-1), nor type of birth (cesarean versus noncesarean), nor breast-feeding were associated with increased risk of HCV-VT. None of the infected infants were HCV-RNA-positive at birth and the mean age at the first HCV-RNA-positive result was 3.81 ± 0.91 months. The infected children presented a lower birth weight (nonsignificant) than that of the noninfected children. 37% of the noninfected children presented ALT levels > 40 U/L whereas 68% of the infected infants had high levels of ALT (>40 U/L, $P = 0.016$).

Risk Factors with Respect to HCV Chronic Infection in Infants. The study of risk factors for chronic infection was performed in HIV-negative mothers using a stored blood sample (Study Cohort, Fig. 1).

Table 2. Selected Risk Factors of HCV Transmission to Infants Related to Infection status, for HCV-RNA positive women

Risk Factors/Infection Status n=100	Infected n=22 (22%)	Noninfected n=78 (78%)	*P*-Value
Gender			
Male (58)	13 (22)	45 (78)	ns
Female (42)	9 (21)	33 (79)	
Weight (g)* (100)	2871 ± 217	3000 ± 93	ns
Viral Genotype			
Geno. 1 (74)	15 (20)	59 (80)	ns
Geno. non-1 (26)	7 (27)	19 (73)	
Type of birth			
Cesarean (20)	4 (20)	16 (80)	ns
Noncesarean (80)	18 (23)	62 (77)	
Breast-fed Infants			
Yes (67)	14 (21)	53 (79)	ns
No (32)	8 (25)	24 (75)	
Breast-feeding days*(99)	87 ± 24	77 ± 11	ns
Mother's HCV Viral Load *(IU/mL)*			
>600,000 (56)	19 (34)	37 (66)	0.02
≤600,000 (42)	3 (7)	39 (93)	

*Mean ± the standard error of the mean (SEM).

Fourteen of the 22 HCV-VT-infected infants (64%) cleared the HCV virus spontaneously (transient viremia group) and eight infants (36%) had persistent infection (chronic group). The rate of HCV chronic infection was higher among the infants with viral genotype 1 than among those with genotype non-1 (Table 3; $P = 0.02$). In fact, no chronic infection was noted in the infants with genotype non-1 (n = 7, of whom six had genotype 3 and one had genotype 4), whereas only 1/9 infants with genotype non-1 in the general cohort had persistent infection at the end of the study (this infant was a boy whose mother was genotype 3 but HIV-positive). Neither gender, nor weight, nor the mother's HCV viral load, nor the type of birth (cesarean versus noncesarean), nor breast-feeding were associated with increased risk of HCV chronic infection among these infants. Among the HCV chronic group of infants, the first HCV-RNA-positive result was recorded at a mean age of 2.33 ± 0.3 months, whereas the corresponding value for the transient viremia group was 4.15 ± 1.1 months (nonsignificant). Furthermore, the chronic HCV infants had a lower birth weight than did the transient viremia children (nonsignificant). In all, 50% of the infants with transient viremia presented ALT levels >40 U/L, whereas all the chronic infants presented ALT levels above 40 U/L ($P = 0.02$).

Study of IL28B and Its Association with HCV-RNA+/−ve Mothers. This study was performed among the HIV-negative mothers using a stored blood sample (n = 105, Study Cohort; Fig. 1. In six mothers it was not possible to determine the IL28B polymorphism). Of the 31 mothers with IL28B CC polymorphism, 19 were HCV-RNA-positive (61%), whereas among the 68 mothers with non-CC polymorphism (CT or TT polymorphism), 56 were HCV-RNA-positive (82%). Accordingly, the mothers with non-CC IL28B polymorphism had a greater probability of being HCV-RNA-positive than did those with CC polymorphism (OR = 2.95; 95% CI: 1.1-7.7; $P = 0.026$). On the other hand, the HCV viral load was not associated with IL28B polymorphism. Thus, 52% of the mothers with CC IL28B polymorphism presented a high viral load (>600,000 IU/mL), as did 54% of the mothers with IL28B non-CC polymorphism.

Study of IL28B and Its Association with the Vertical Transmission of HCV Genotype 1, Transient Viremia, and Chronic Infection. We evaluated the role of IL28B polymorphism on the vertical transmission of HCV genotype 1, transient viremia, and persistent infection in infants. Neither the mothers' nor the childrens' IL28B polymorphism was associated with an increased risk of HCV-VT (Table 4). On the other hand, the study of the role of the IL28B genotype in HCV transient viremia and chronic infection revealed that 83% of the children with Rs12979860 CC genotype presented spontaneous clearance (infants with transient viremia), whereas among the children with

Table 3. Selected Risk Factors of Chronic HCV Infection in Infants

Risk Factors/Infection Status n=22	Chronic n=8 (36%)	Transient Viremia n=14 (64%)	*P*-Value
Gender			
Male (9)	3 (33)	6 (67)	ns
Female (13)	5 (39)	8 (61)	
Weight (g)* (22)	2656 ± 375	3122 ± 155	ns
Viral Genotype			
Geno. 1 (n=15)	8 (53)	7 (47)	0.02
Geno. non-1 (n=7)	0 (0)	7 (100)	
Type of birth			
Cesarean (n=4)	2 (50)	2 (50)	ns
Noncesarean (n=18)	6 (33)	12 (67)	
Breast-fed Infants			
Yes (14)	4 (29)	10 (71)	ns
No (n=8)	4 (50)	4 (50)	
Breast-feeding days* (22)	150±70	68±21	ns
HCV-RNA+ begin (month)* (22)	2.33 ± 0.3	4.15 ± 1.1	Ns
Mother's HCV Viral Load (IU/mL)			
>600,000 (19)	8 (42)	11 (58)	ns
≤600,000 (3)	0 (0)	3 (100)	
Child's ALT			
>40 U/L (15)	8 (53)	7 (47)	0.02
≤40 U/L (7)	0 (0)	7 (100)	

*Mean ± the standard error of the mean (SEM).

Table 4. Role of IL28B in HCV Vertical Transmission and Chronic HCV Infection in Viral Genotype 1 Infants

HCV Vertical Transmission			
Risk Factors/Infection Status n=74	Infected n=15 (20%)	Noninfected n=59 (80%)	*P*-Value
Mother's IL-28B status			
CC (19)	7 (37)	12 (63)	ns
Non-CC (55)	8 (15)	47 (85)	
Child's IL-28B status			
CC (25)	6 (24)	19 (76)	ns
Non-CC (46)	9 (20)	37 (80)	
HCV Chronification			
Risk Factors/Infection Status n=15	Chronic n=8 (53%)	Transient Viremia n=7 (47%)	*P*-Value
Mother's IL-28B status			
CC (7)	3 (43)	4 (57)	ns
Non-CC (n=8)	5 (63)	3 (37)	
Child's IL-28B status			
CC (n=6)	1 (17)	5 (83)	0.04
Non-CC (n=9)	7 (78)	2 (22)	

non-CC genotype (CT or TT polymorphism), only 22% had transient viremia ($P = 0.04$). Moreover, the mother's IL28B genotype was not associated with spontaneous clearance (transient viremia) and therefore was not associated either with HCV persistent infection in infants (Table 4).

Multivariate Logistic Regression

HCV Vertical Transmission. The multivariate analysis showed that a high HCV viral load (>600,000 IU/mL; OR: 7.3; 95% CI: 1.8-29.4; $P = 0.005$) and ALT values among infants exceeding 40 U/L (OR: 5.3; 95% CI: 1.5-18.8; $P = 0.01$) were independently associated with HCV-VT (Fig. 2). These factors remained independently associated with HCV-VT when HCV genotype 1 was selected (HCV viral load >600,000 versus ≤600,000 IU/mL; OR: 10.2; 95% CI: 1.73-58; $P = 0.01$ and children's ALT levels >40 versus ≤40 U/L, OR: 9.1; 95% CI: 1.7-50; $P = 0.01$).

HCV Chronic Infection. The multivariate analysis showed IL28B Rs12979860 CC genotype in infants to be the only factor independently associated with HCV clearance and therefore with transient viremia (Fig. 2; OR: 17.5; 95% CI: 1.2-250; $P = 0.035$).

Discussion

Vertical transmission of HCV represents the major cause of pediatric HCV infection today, and in industrialized countries it is the most common cause of chronic liver disease in children. About 10%-15% of those who are chronically infected might develop cirrhosis and eventually hepatocellular carcinoma.[16,17] HCV prevalence in pregnant women is similar to that of the general population and, in general, most HCV-infected pregnant women do not have obstetric complications. At present, there are no antiviral treatment recommendations for HCV-infected women during pregnancy, or guidelines for the prevention of vertical transmission.[18] Although persistent transmission of HCV from infected mothers to their infants is reported in 4%-8% of cases (chronic HCV children), transient HCV perinatal infection also occurs, with a prevalence of about 14%-17%.[19,20] Moreover, the maternal-infant transmission of HCV is more frequent than is generally reported, taking into account that spontaneous HCV-RNA clearance among children is more common than among adults and that in many studies the follow-up of infants is incomplete; moreover, in many cases only limited data, corresponding to the first years of life, are presented.[21] Interferon alpha (IFNα) is currently the approved drug for hepatitis C treatment for the pediatric population. Combination therapy with IFNα or pegylated IFNα plus ribavirin has recently been approved by the United States Food and Drug Administration (US FDA)-EMEA for children older than 3 years with chronic HCV infection, and clinical trials are in progress.[3,22] Although most children are asymptomatic and the associated liver damage appears to be less severe in children than in adults, they have a significantly poorer health status than community controls,[23] which suggests there is a need for the services currently available for adult HCV patients to be extended to support the families of children with HCV.

Conflicting data have been reported regarding the possible role of the level of maternal HCV viremia. Some studies have shown that a high concentration of serum HCV-RNA is associated with a higher risk of transmission, although no specific cutoff value predicting or excluding transmission has been defined.[11] However, other studies have found no such association, with a considerable overlap in concentrations of HCV-RNA between transmitting and nontransmitting mothers.[1,24] Moreover, maternal coinfection with HCV and HIV is associated with high maternal HCV-RNA and with a higher risk of transmission.[18,25] In the present study, we found that both the HCV-RNA concentration (over 600,000 IU/mL) and maternal coinfection with HIV were associated with a higher risk of HCV-VT. The infected infants were not HCV-RNA-positive at birth but all became so within 2-4 months. These data indicate that HCV maternal-fetal transmission

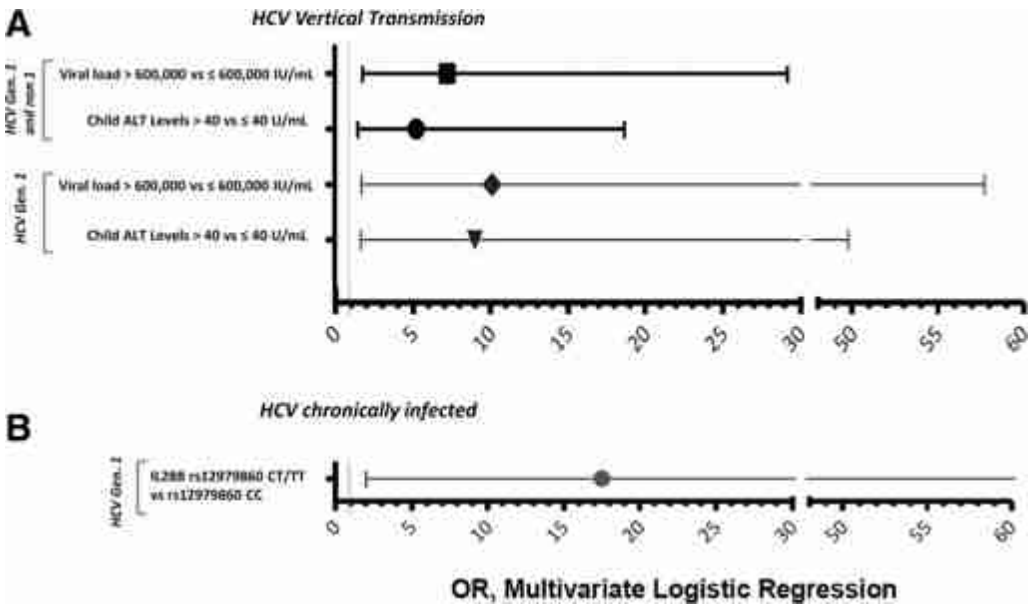

Fig. 2. Multivariate logistic regression: predictors of HCV-VT and HCV clearance. (A) HCV vertical transmission and (B) infants HCV chronically infected. The following covariates were included in the multivariate model: ALT level, viral genotype, viral load, delivery mode, breast-feeding, and IL28B polymorphism. OR is the probability of HCV-VT and HCV clearing for each predictor with respect to the reference group. These factors remained independently associated with HCV-VT when HCV genotype 1 was selected.

did not occur during gestation and, therefore, that the infants were infected during birth. Most of the infected children were asymptomatic despite high levels of ALT, compatible with acute hepatitis. The infants that cleared the HCV virus recovered normal ALT levels. With respect to the type of birth, there was no significant decrease in HCV-VT among the mothers who gave birth by cesarean section versus those who did not. The data on the effect of cesarean section on the risk of HCV perinatal transmission are heterogeneous and high-quality studies of this question have not been reported. A recent meta-analysis including eight studies and 641 mother-infant pairs suggests that cesarean section does not decrease perinatal HCV transmission from HCV-RNA+ve/HIV−ve mothers to infants.[8] No relationship between HCV-VT and the maternal HCV genotype has been found. On the other hand, when we studied spontaneous clearance (children with transient viremia) versus chronic infection in infected infants, the HCV viral genotype was associated with a higher risk of chronic infection. Thus, the rate of HCV chronicity was higher for infants with viral genotype 1 than for those with genotype non-1, a finding that is in accordance with the results of Bortolotti et al.[6] The role of viral genotype and its association with HCV spontaneous clearance and chronic infection should be explored further.

The HCV-VT risk factors that have been most intensively studied to date are viral factors, maternal characteristics, and birth mode. However, immunogenetic influence has been poorly investigated and mainly confined to HLA-class II serological polymorphisms, because of their central role in the adaptive response. Nevertheless, it has been suggested that the role of the immune defense system, as well as the relevance of the genetic background, could better explain the pathogenesis of HCV infection, and these factors have been examined.[10,11] In adult patients, genetic variations in the IL28B gene, an innate cytokine, have been associated with the response to IFN-α/ribavirin therapy and spontaneous clearance in HCV genotype 1.[26-28] For this reason, we evaluated the role of IL28B polymorphism in HCV genotype 1 vertical transmission, transient viremia, and chronic infection in infants. This is the first study that attempts to describe both HCV-VT and the spontaneous clearance of HCV, taking into account the influence of IL28B polymorphism in mothers and children. The data obtained indicate that the IL28B genotype of mothers and children does not influence HCV-VT. Nevertheless, in the chronic infection study, 83% of the infants with the CC genotype exhibited spontaneous clearance (transient viremia) versus only 22% of the children with a non-CC genotype. On the other hand, the maternal IL28B genotype did not influence HCV chronic infection. Multivariate analysis identified the infant's Rs12979860 CC IL28B genotype as the only factor independently associated with the spontaneous

clearance of HCV. To the best of our knowledge, the present study is the first one to identify IL28B Rs12979860 polymorphism as a predictor of HCV spontaneous clearance in infants infected with HCV genotype 1 by vertical transmission. More information is now needed to understand the mechanisms that underlie this association, as well as the clinical impact of IL28B polymorphisms on HCV infection.

The multivariate analysis performed clearly shows the distinction between the risk factors in HCV-VT and in chronic infection. In HCV-VT, a high HCV viral load was independently associated with HCV-VT, thus confirming the bivariate analysis and the data previously published, by ourselves and by others. These data suggest that the maternal characteristics are more important in HCV-VT than are those of the infants. However, in the chronic HCV infection study, the multivariate analysis showed that the only factor independently associated with HCV clearance was the infants' IL28B genotype, which confirmed our hypothesis that in infected infants the host's immunogenic influence is crucial to the HCV viral response.

Finally, all retrospective analyses have inherent limitations, but we have tried to minimize their effects. The standard method of HCV determination changed during the patient inclusion period but this factor was controlled by using the same PCR technique on all the patients studied, using a stored blood sample. Furthermore, the standard care of HIV and HCV patients also changed during the patient inclusion period; however, in this study the risk factors among the HIV-negative mothers (Study Cohort) were identified. According to standard protocols for HCV pregnant women, no HCV treatment should be applied during the pregnancy, and thus the changes in standard care for HCV patients do not affect our study.

In view of the data presented, we believe it is necessary to make a clear distinction between the risk factors of HCV-VT and of chronic infection. We confirm that viral load and HIV coinfection are the only risk factors involved in HCV-VT. On the other hand, the viral genotype non-1 and the infant's IL28B CC Rs12979860 polymorphism are associated with HCV spontaneous clearance. Our data are the first to account for HCV virus clearance and may provide important information about protective immunity to HCV.

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