Hepatitis G virus (HGV) infection is more common than hepatitis C virus (HCV) infection and is frequently found in healthy individuals. Although parenteral spread of HGV is well recognized, other routes of transmission probably occur as well. In a prospective study of mother-to-infant transmission of hepatitis viruses, 69 pregnant women with antibodies to HCV and their 81 newborn children were included. Serum levels of HCV RNA and HGV RNA were detected by polymerase chain reaction (PCR) assays, and antibodies to HCV and HGV envelope protein E2 were detected by enzyme-linked immunosorbent assay. Fifty-nine of the mothers had HCV viremia, whereas 16 had HGV viremia. HCV transmission from viremic mothers occurred in 2.8%–4.2% of the cases, whereas HGV transmission from viremic mothers occurred in 75.0%–80.0% of the cases ($P < .001$). Sequencing of the PCR products of HGV from the mother-infant serum pairs showed minor differences in most cases but sequence homology in each pair. Although the rate of perinatal HGV transmission highly exceeded that of perinatal HCV transmission, HGV did not seem to induce hepatitis in the children.

Hepatitis G virus (HGV) and GB virus type C, two strain variants of the same virus, have recently been found in serum samples from patients with hepatitis [1, 2]. (For simplification, we will use the term HGV in this article.) Despite the history of discovery of HGV, the significance of this virus in liver disease is still controversial. HGV has been associated with acute and chronic hepatitis of unknown origin, but HGV infection is also often found in individuals lacking signs of liver disease [1–6]. The prevalence of HGV viremia among blood donors (1.5%–2%) exceeds that of hepatitis C virus (HCV) viremia [2, 4, 7–10], and HGV viremia is also frequently found in patients infected with HCV [8, 9]. Although parenteral risk factors are common in HGV-infected individuals, many have no apparent risk behavior, and the mode of virus acquisition in these subjects remains obscure. However, mother-to-infant transmission of HCV does occur, generally in <5%–10% of cases [11–14] if the mother is not coinfected with HIV. As for HGV, recent reports indicate that perinatal transmission occurs as well [15–18].

In this study of mothers with antibody to HCV but not to HIV and their newborn children, we analyzed the prevalences of HGV viremia and HCV viremia among mothers and their newborns and tested the mothers for antibody to HGV. For the viremic children and their mothers, partial sequencing of the HGV genome was done. The risk for transmission of the two viruses was estimated and compared.

**Patients and Methods**

The women in this study were all positive for antibody to HCV by standard screening and immunoblot confirmation assays; they were included in an ongoing (since 1982) prospective clinical investigation on the perinatal transmission of hepatits virus agents in the Göteborg region of Sweden. Before 1991, only women with histologically confirmed chronic non-A, non-B hepatitis who were routinely followed up at our department were recruited for the study. Since 1991, the ongoing investigation included all pregnant women with known positivity for antibody to HCV who were previously followed up at our department or referred to us after antenatal screening for antibody to HCV, regardless of whether chronic hepatitis was histologically confirmed.

In this study, the findings for a total of 69 women and their 81 newborn children are reported. These women represent ~85% of those included in the ongoing investigation; the other 15% were excluded from this study, since no blood samples were obtained from the children. None of the women were positive for antibody to HIV or hepatitis B surface antigen. Fourteen of the women were previously described in a study of HCV transmission [11].

**Mothers.** Of the 69 women, 48 (69.6%) were former intravenous drug users, 6 (8.7%) had received blood transfusions,
5 (7.2%) had been sex partners to intravenous drug addicts with known or unknown HCV infection status, and 10 (14.5%) anamnestically had no risk factors for hepatitis C. In 76 of the pregnancies, at least one previously unthawed serum sample (−20°C) was obtained during gestation or within 1 month after delivery; these samples were analyzed for HCV viremia and HGV viremia by using PCR assays and for antibodies to HGV envelope protein E2. In five pregnancies, only serum samples obtained before and >1 month after gestation were available, but since analyses of the paired serum samples gave identical results, these pregnancies were also included.

Children. After birth, the intention was to follow up the children until the age of 18–24 months by means of obtaining blood samples at regular intervals for determination of antibody to HCV (after 1990) and alanine aminotransferase (ALT) levels and for long-term storage of serum samples (−20°C). Samples were not obtained from all children at regular intervals. For this study, we selected one sample from each child at around the age of 1 year (median, 12 months; range, 1–67 months). If this sample was positive for HCV RNA or HGV RNA or if the mother was HGV RNA–positive, at least one additional earlier sample (if available) was analyzed for confirmation and to determine the onset of viremia. Antibody to HCV in the children was analyzed at all times when samples were obtained. Further testing for seroconversion to HGV envelope protein E2 was done on the last sample from children who never developed HGV viremia despite having a mother with HGV viremia.

PCR analysis for HCV RNA and HGV RNA. Viral RNA was extracted from serum as previously described [19] with use of water controls between every sample. HCV RNA was detected by using a nested in-house PCR assay as previously described [19]. The sensitivity of this assay for RNA quantitated by branched DNA is around 100 copies/mL. PCR analysis for HGV RNA was performed in a similar way with use of primers against the 5′-noncoding region of HGV as previously described in detail [10].

Primers in the first round of the PCR assay for HGV RNA were 5′-CGGCCAAAAGGTGGTGGATG (sense strand) and 5′-CACTGGTCTTTGTCAACTCG (antisense strand), and in the second round they were 5′-GGTGATGACAGGGTTGGTAG to be intravenous drug users; however, the difference in the proportion of intravenous drug users was not significant (33 [76.7%] of 43 vs. 15 [57.7%] of 26, respectively; \( P < .30 \)). Primers in the first round of the PCR assay for HGV RNA were 5′- CGGCCAAAAGGTGGTGGATG (sense strand) and GCCTATTGGTCAAGAGAGACAT (antisense strand) and in the second round they were 5′-GGTGATGACAGGGTTGGTAG (sense strand) and GCCTATTGGTCAAGAGAGACAT (antisense strand). Products were visualized by means of ultraviolet light on agarose gels after ethidium bromide staining.

Sequencing of PCR-amplified products of HGV. HGV PCR fragments were cut out from gels, and the DNA was purified and subjected to cycle sequencing with the inner amplification primers by means of the ABI PRISM TM dye terminator cycle sequencing kit with AmpliTaq DNA polymerase FS (Perkin-Elmer, Foster City, CA). Products were then analyzed on an ABI 373 Sequenator (Perkin-Elmer), edited, and aligned by using the Factura and Sequence Navigator programs (version 1.0.1; Perkin-Elmer).

Serological testing. A third-generation ELISA (anti-HCV Murex; Murex Diagnostics, Dartford, United Kingdom) was used to test for antibody to HCV. Antibody to HGV envelope protein E2 was analyzed with a recently developed ELISA (anti-HGenv; Boehringer Mannheim, Mannheim, Germany) [20].

Statistical analysis. The risk (i.e., the probability) of perinatal transmission, including 95% confidence intervals, was calculated exactly with use of the binomial distribution. Fisher’s exact test (double-sided) was used to compare proportions.

Results

Clinical and virological findings for the women. Fifty-four (78.3%) of the 69 women had biochemical evidence of chronic hepatitis (i.e., elevated [above the upper limit of normal] ALT levels on at least two occasions >6 months apart, before or after pregnancy). Thirty-two of these women underwent liver biopsy (not during pregnancy); this procedure showed chronic hepatitis in 30 women, while two had normal liver histology. Repeated testing for 15 women (21.7%) revealed normal ALT levels; none of them underwent liver biopsy.

In table 1 the women are divided into four groups according to the findings of PCR analysis. Fifty-nine women (85.5%) had HCV viremia. None of the 10 HCV RNA–negative mothers (including one woman with HGV viremia) had elevated ALT levels before or after delivery, and none of them underwent liver biopsy. At least one additional serum sample from six of these 10 women was analyzed for HCV RNA with the same negative result. Thus, these 10 women probably had had a self-limited HCV infection and were not chronically infected with HCV. Sixteen mothers (23.2%) had HGV viremia, and 27 (39.1%) had antibody to HGV envelope protein E2. None of these women had both markers of HGV infection.

There was no significant difference in the frequency of chronic hepatitis (defined as an ALT level above the upper limit of normal on at least two occasions) among women with isolated HCV infection and those who were coinfected with HCV and HGV (90.9% vs. 93.3%, respectively; \( P > .30 \)). Those women with markers of HGV exposure (HGV RNA or antibody to HGV envelope protein E2) seemed more likely to be intravenous drug users; however, the difference in the proportion of intravenous drug users was not significant (33 [76.7%] of 43 vs. 15 [57.7%] of 26, respectively; \( P = .16 \)).

HCV transmission. HCV RNA was not detected in any of the 10 children born to HCV RNA–negative women. HGV viremia was detected in three (4.2%) of the 71 children born to mothers with HCV viremia. One child (L1) developed chronic HCV infection that was verified by biopsy at 21 months of age; this child was the only one who actively produced antibody to HCV (figure 1). Cord blood from one child was HCV RNA–positive only, but samples from this child that were obtained during further follow-up were repeatedly HCV RNA–negative; ALT levels were consistently normal, and seroconversion to HCV did not occur. Thus, contamination of cord blood with maternal blood at the time that samples were
obtained was likely. The first available sample from a third child at 6 months of age was HCV RNA–positive, but samples obtained thereafter were repeatedly negative. As in the previous child, ALT levels were normal, and antibody to HCV disappeared.

Thus, of the three children, one was probably falsely positive because of contamination of cord blood by maternal blood, and one probably had temporary viremia that spontaneously resolved. Of the 71 children born to HCV RNA–positive women, 67 were followed up for >12 months, and all of these children lost antibody to HCV with the exception of the child who developed chronic hepatitis C. The remaining four children were followed up for <12 months, and the last serum samples from these children were still positive for antibody to HCV.

![Figure 1: Detection of hepatitis G virus (HGV) viremia (present, +; absent, −) in 20 children with HGV-infected mothers (* denotes child with coinfection with HGV and hepatitis C virus).](cid9928b4511265495f344c18e654f527110d9151)
The estimated risk for HCV transmission from viremic mothers was 4.2% (three of 71; 95% CI, 0.9%–11.9%) or 2.8% (95% CI, 0.3%–9.8%) if the child with only positive cord blood was not included because of contamination by maternal blood.

**HGV transmission.** Of the 20 children born to HGV RNA–positive mothers, 16 (80.0%) were found to be HGV RNA–positive (figure 1). Of the 10 children with HGV viremia from whom blood samples were available during the first month of life, four were viremic at 1 month of age and during repeated testing thereafter (figure 1). Cord blood samples from two children were negative, but samples from these children were positive at 10 and 26 months. Three children were negative for HGV at 1 month of age, but specimens from these children were positive at 18, 6, and 13 months. A cord blood sample from one child was positive, but samples were negative at 13 and 27 months; further testing did not reveal antibody to HGV envelope protein E2.

None of the HGV RNA–negative children born to viremic mothers developed antibody to HGV envelope protein E2 during follow-up. All 16 children with HGV viremia had no clinical or biochemical signs of hepatitis.

The estimated risk for HGV transmission was 80.0% (95% CI, 56.3%–97.5%) or 75.0% (15 of 20; 95% CI, 50.9%–94.0%) if the child with only positive cord blood was categorized as uninfected. This risk was significantly higher than the risk for HCV transmission: 80.0% vs. 4.2%, respectively (P<.001), or 75.0% vs. 2.8%, respectively (P<.001), if cord blood positivity was disregarded.

Sequencing of the amplified HGV fragments from mother–child serum pairs in cases of transmission showed HGV genotype 2a for nine serum pairs (A to I) and HGV genotype 2b for seven serum pairs (J to L) (figure 2). Isolates from serum pairs A, B, and C had identical viral sequences in the amplified region, otherwise the isolates from each serum pair showed isolate-specific substitutions that were often restricted to a single nucleotide. Sequences of the isolates from serum pairs A, D, E, F, G, H, I, J, K, and L have been deposited at GenBank (Bethesda, MD); their accession numbers are AF063854–AF063863, respectively.

Viral sequence homology was observed between isolates from the mother and the child in each serum pair. In serum pairs J and L, isolates from one of three children and from one of two children, respectively, had several isolate-specific substitutions but also manifested an additional single nucleotide substitution. This substitution remained unchanged after reamplification and during testing of alternative samples.

Twenty-seven (39.1%) of the 69 women were positive for antibody to HGV envelope protein E2, and HGV RNA was not detected in any of their 33 children. A further 26 women (37.7%) were negative for all markers of present or past HGV infection, and their 28 children were also HGV RNA–negative.

**Cotransmission of HGV and HCV.** HGV RNA was found in one of the three children with HCV viremia. This child was the same child (L1) who became chronically infected with HCV; thus, she developed a chronic coinfection with HCV and HGV (figure 1). The other two children with HCV viremia both had HGV RNA–negative mothers and did not become infected with HGV. The woman with isolated HGV viremia transmitted the infection to her child. Thus, HCV viremia was not a prerequisite for HGV transmission.

**Discussion**

The 75.0%–80.0% rate of perinatal HGV transmission that was found in our study is amazingly high, but this rate is in line with reports from other study groups [15–17]. In our comparison, HCV transmission was rare (2.8%–4.2% of cases); this finding is similar to those of several other studies of HCV-positive, HIV-negative mothers, most of which reported frequencies of <5% to 10% [11–14].

The prevalence of HGV viremia among healthy blood donors is around 1.6% in Sweden [10], a level correlating well with data reported from the United States [2, 4]. Since the ability of HGV to induce liver disease or other manifestations at present is considered to be low, general blood donor screening for HGV has not been implemented in any country. Blood donor screening is also complicated by the need for PCR assays to detect infectivity. Seropositivity for HGV detected with use of the recombinant envelope protein E2 as capture antigen is a marker of previous HGV infection; in this study, antibody to HGV envelope protein E2 was not found together with HGV RNA. In some instances (probably representing active virus clearance), these two markers could however be present simultaneously [20, 21].

Dual infection with HGV and HCV is common in individuals with parenteral risk factors, like drug addicts [8, 9]. This finding was confirmed in our study, where almost 70% of the women were drug users and altogether 43 women (62.3%) had markers of present or past HGV infection.

Despite the fact that HGV may be transmitted via parenteral routes, such as blood transfusion and intravenous drug abuse [2, 4, 8, 20, 22], our knowledge of HGV transmission still remains incomplete. Long-term carriage of HGV has been described in both children and adults [22, 23], and perinatal transmission may be an important source of infection in blood donors lacking parenteral risk factors.

Most of our children were probably infected at birth, or shortly afterward, since five of the nine viremic children analyzed early were negative for HGV RNA at birth (cord blood; two) or at 1 month of age (three) and then became viremic. A further four children were HGV-positive already at 1 month, but earlier samples were not available from these children. Since all children, at least initially, were breast-fed, breastfeeding could be an alternative mode of transmission.

Because of the limited number of patients and the low occurrence of HCV transmission, no conclusions concerning additive or synergistic effects on transmission from coinfect ed women...
Table 1

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Figure 2. Sequence comparisons of 209 nucleotides in the 5'-noncoding region of hepatitis G virus (HGV) isolates from 12 HGV-infected mothers and their 16 HGV-infected infants. Isolates were compared with one HGV prototype sequence (GenBank [Bethesda, MD] accession number U44402 [2]). Homologous nucleotides are indicated by hyphens (-), and deletions are indicated by underlines ( _ ).

References


