Parent-to-offspring transmission of hepatitis G virus (HGV [or GBV-C]) was studied in sera of 42 mothers at high risk for bloodborne infections and from their 45 infants (3 twin pairs). Seven (17%) of the mothers had HGV RNA in serum by a polymerase chain reaction assay. One of the 8 (12.5%) infants born to HGV-infected mothers became positive for HGV at 3 months of age. He remained HGV-infected throughout the study (42 months), with no signs of liver disease. His twin sister remained HGV-negative despite the presence of serum and salivary HGV in both the mother and the brother. Analysis of HGV sequences from the infected mother and the infected child confirmed a genetic link between the virus of the mother and the infected child. Thus, mother-to-infant transmission of HGV, presumably occurring at partus, may cause persistent HGV viremia.
with PCR conditions similar to those previously reported [5]. The sequences of the outer primers were 5'-TCYTTGATGATGGA-CTGTC-3' (antisense, G9) [5] and 5'-TATGGCCATGGHATH-CCYCT-3' (sense, G8) [5]. Primer G9 was used to initiate cDNA synthesis. The seminested was performed with inner primers 5'- TCYTTACCCT-RTAATAGCC-3' [5] and G8. The expected size of this seminested PCR product was 140 bp.

To confirm an HGV origin for the amplified cDNA, sequence analysis was performed using the dieoxy chain termination method, and bands were separated and read using an automated sequencer according to the manufacturer’s instructions (ALF-Express; Pharmacia, Uppsala, Sweden). Phylogenetic analysis of both 5'-noncoding and NS3 region sequences was performed using the GeneWorks 2.3 software package (Intelligenetics, Mountain View, CA) and the unweighted pair group method with arithmetic mean. On the basis of a recent publication [11], this would suggest that many of these strains may belong to HGV genotypes analyzed using the unpaired t test.

Results

Presence of HGV infections. Of the 42 mothers, 7 were HGV RNA-positive (17%); of these, 6 were also positive for HCV RNA. Mean ALT level at delivery was 1.18 µkat/L (normal, <0.6 µkat/L) among the 6 mothers coinfected with HCV and HGV compared with 0.57 µkat/L in 27 mothers infected with HCV alone (P = .05, t test). Mean ages in those 2 groups were 31.0 (range, 27–34) and 31.7 (range, 24–41) years, respectively (not significant, t test).

Eight babies were born to these 7 mothers (1 twin pair): 3 were delivered by cesarean section, and 5 were delivered vaginally. Seven were breast-fed, and the eighth was formula-fed due to high maternal doses of legally prescribed methadone. Of 8 (12.5%) infants, the second-born twin was HGV RNA-negative at birth but was consistently positive from 3 to 42 months of age. In contrast, the first-born twin was negative for HGV RNA throughout the study. The mother of this twin pair was positive for both HCV and HGV RNA but negative for HIV. Both twins were delivered by planned cesarean section due to breech presentation, and there was no history of premature rupture of the membranes. The twins were breast-fed for 2 months. Neither one received any blood products. The HGV-infected boy had normal ALT and AST levels throughout. However, his GT was elevated more than three times the upper limit of normal value at birth but later normalized. He showed no clinical signs of liver disease, and his birth weight, growth, and development were all within the normal ranges. His twin sister was consistently negative for HGV RNA during the 3.5-year study period. Also, stored sera from 2 older brothers, ages 7 and 10 years, and from all other infants born to HGV RNA-positive mothers were negative for HGV RNA.

Detection of salivary HGV RNA. Saliva samples from the HGV-infected boy at the age of 3.5 years and from his mother at the same time were both found to contain HGV RNA.

Sequence analysis. From all samples from which HGV cDNA could be amplified, sequence analysis was performed for both the 5'-noncoding (figure 1) and NS3 (figure 2) regions. In all patients, the sequences were found to be of HGV origin, which confirmed the specificity of the PCRs used. Phylogenetic analyses were performed for both HGV regions to test the genetic relation between the HGV-infected child and his mother. In both the 5'-noncoding and NS3 region dendrograms, the HGV sequences from the infected child consistently grouped with the HGV sequences derived from his mother. Thus, we could identify a genetic link between the HGV identified in the mother and the child, substantiating a vertical route of HGV transmission. Also, of note, within the 5'-noncoding region, all strains seemed to have a closer relation to the HGV prototype strains than the GBV-C prototype strain (Figure 1).

Discussion

Mother-to-infant transmission of HGV RNA was detected in 1 of 8 infants, suggesting that HGV transmission can occur independent of HCV transmission in a mother infected by both viruses. The fact that the infected boy was negative at birth and positive from 3 months of age would indicate that the virus was transmitted either peri- or postnatally, rather than in utero. An HGV RNA-negative phase of <3 months following vertical HGV transmission is consistent with a recent case report in which the baby of a HGV RNA-positive mother was HGV-RNA seronegative at birth but positive at 4 and 6 weeks of age [12].

Unlike his twin sister, the boy in our study did not seem to be protected by the cesarean section, which can offer partial protection from the vertical transmission of other viruses [13]. The possibility of breast milk transmitting the virus was less likely in the present study since the uninfected twin sister ingested the same milk. It should also be noted that the boy did not cause infection of the twin sister, as determined by PCR, despite his saliva containing HGV RNA. Thus, in this particular case, close social contact did not provide a route for chronic HGV infection, even though 2 persons in the household had HGV in both serum and saliva. However, we cannot exclude the possibility that the twin sister, at some time point, experienced an acute or subclinical HGV infection that she subsequently cleared, since the HGV PCR only detects ongoing infection.

No clinical or biochemical signs of liver disease were noted in the HGV-infected boy despite persistent HGV viremia since he was 3 months of age. Since no liver biopsy was performed, low-level histologic damage can not be ruled out, in analogy with some studies of HCV RNA-positive patients with normal ALT values [14]. In contrast to the normal ALT levels observed in the HGV-infected boy, we did find that the doubly viremic mothers (HCV and HGV) had higher ALT values than those with only HCV viremia. This is potentially important and in

Statistical analysis. Differences in maternal ALT levels were analyzed using the unpaired t test.
Figure 1. Phylogenetic analysis using unweighted pair group method with arithmetic mean algorithm and alignment of 5'-noncoding region sequences from 7 HGV-infected mothers (MOM12 to MOM36) at partus and 2 samples from HGV-infected infant (CH33). Infant's 2 samples were taken at 9 (2-3; labeled *) and 18 (2-4; labeled **) months of age. Also analyzed are 5'-noncoding region sequences from 3 full-length HGV genomes (GBV-C, HGV44402, and HGV45966). Filled triangle indicates sample from mother of HGV-infected infant.
Figure 2. Phylogenetic analysis using unweighted pair group method with arithmetic mean algorithm and alignment of NS3 region sequences obtained from 7 HGV-infected mothers (MOM12 to MOM36) at partus and 2 samples from HGV-infected infant (CH33). Infant’s 2 samples were taken at 9 (2-3; labeled *) and 18 (2-4; labeled **) months of age. Also analyzed are NS3 region sequences from 3 full-length HGV genomes (GBV-C, HGV44402, and HGV45966) and from 1 HCV strain (HPCTBNS3). Filled triangle indicates sample from mother of HGV-infected infant.

disagreement with other studies [8] of patients with chronic double infections and deserves further study.

Of interest, we did see small changes in the HGV sequences from the child with respect to time at similar rates for both the 5'-NC and NS3 regions. One nonsynonymous mutation, causing a serine-to-proline change within the NS3 sequence, was observed in the last sample from the HGV-infected child. These observations suggest either a spontaneous mutation rate in the viral life cycle or mutations induced by host pressure, such as the immune system. The absence of liver damage may argue against immune-mediated pressure.

In summary, our study indicates that vertical transmission of HGV does occur, presumably at partus and not in utero, and may induce persistent viremia. This is supported by HGV
sequences not detected in the infected child until 3 months postpartus, the high sequence homology between the HGV from the mother and the infected child, a lack of other risk factors for the boy, and repeatedly positive serum samples from the boy during a 3-year period. Despite the persistent HGV viremia, no signs of liver damage were noted in the boy. Horizontal transmission between infants or toddlers and between parents and their children was not demonstrated, despite the finding of HGV in both the mothers’ and the boy’s serum and saliva. Thus, vertical, but possibly not horizontal, transmission of chronic HGV infections within a family may contribute to maintaining the thus far unexplained elevated prevalence of HGV RNA in the general population.

References