To study mother-to-infant transmission of GB virus C/hepatitis G virus (GBV-C/HGV), blood samples of infants born to carrier mothers were collected beginning 3 months after birth and were tested for GBV-C/HGV RNA until 1 year of age. Of 2046 mothers, 2.1% were positive for GBV-C/HGV RNA, and 25 of their infants were followed for a median of 12 months. Thirteen infants (52%) were viremic, and infection became persistent in all. Maternal GBV-C/HGV RNA levels of this group were >10^7 copies/mL. Nucleotide sequence comparison in 5 viremic mother-infant pairs revealed a homology of 93%–98.2%, and none delivered by elective cesarean section. In comparison, of the 12 uninfected infants’ mothers, 10 had lower GBV-C/HGV RNA levels (mean, 5×10^4 copies/mL), and the remaining 2 high-titered mothers had elective cesarean section. Thus, high-titered maternal viremia and mode of delivery are closely associated with the mother-to-infant transmission of GBV-C/HGV to infants, and the infection usually becomes persistent.

Mother-to-infant transmission of blood-borne viruses, such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus, is well documented [1–6]. This mode of transmission plays a key role in maintaining HBV infection from generation to generation [1, 2, 7, 8]. Recently, the flaviviridae GB virus C (GBV-C) and hepatitis G virus (HGV) have been identified independently from plasma samples of patients with chronic hepatitis [9, 10] and found to be variants of the same virus [11]. It is transfusion-transmissible [10, 12, 13] but can also be found in patients with community-acquired hepatitis [14] and is distributed worldwide, with a higher prevalence in certain risk groups [9–15].

Although perinatal transmission of GBV-C/HGV has been reported to occur in some instances [16–18], the case number is small, and thus the prevalence, risk factors, and significance remain unclear. We therefore investigated the frequency of mother-to-infant transmission of GBV-C/HGV in a larger series and elucidated the possible factors associated with this infection in early infancy.

Materials and Methods

Subjects. Serum samples of 2046 consecutive pregnant women collected from May 1995 to May 1996 and stored at −20°C were retrieved and screened for GBV-C/HGV RNA. Blood samples were taken from the pregnant women who regularly attended the prenatal clinic at National Taiwan University Hospital during the first or early second trimester for routine examinations that include assays for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and anti-HCV [19]. Mothers were defined as carriers of GBV-C/HGV when serum specimens were positive for viral RNA at least twice within an interval of ≥3 months. Blood samples were collected from infants born to carrier mothers and were tested for GBV-C/HGV RNA and alanine aminotransferase (ALT) starting from 3 months after birth until 1 year of age (usually every 2–3 months). The infected neonates and blood sampling times are shown in figure 1. The duration of follow-up was comparable between infected and uninfected infants.

The mode of delivery was classified into elective cesarean section, emergent cesarean section, and normal spontaneous delivery [20].

Serologic assays. Serum HBsAg and HBeAg were tested by EIA, and anti-HCV was assayed by a second-generation HCV EIA 2.0 (Abbott, Abbott Park, IL). The serum ALT level was determined by an autoanalyzer (Hitachi, Tokyo). An abnormal serum ALT level was defined as >40 IU/L.

Detection and quantitation of serum GBV-C/HGV RNA. Serum GBV-C/HGV RNA was detected by reverse transcription–polymerase chain reaction (RT-PCR) with primers from the 5′-untranslated region of the viral genome [21], and the positive maternal serum samples were further quantified. To screen the GBV-C/HGV genome, serum specimens from 10 pregnant women were pooled and then subjected to RT-PCR assay [12, 21]. In case of a positive result, the pool was broken down, and the individual sample was assayed by the same RT-PCR. To avoid false-positive results, the precautions described by Kwok and Higuchi [22] were strictly followed, and the same experiments were done in three different research laboratories of our medical center.

To quantify viral RNA levels, a competitive RT-PCR assay was developed by mixing one-tenth of sample serum RNA with serial
dilutions of competitor GBV-C/HGV RNA that differs from the RNA of interest by an insertion of 37 bp. The mixtures were cotranscribed by random hexamers and coamplified by nested PCRs with two primer pairs. The outer primers consisted of primer GB-C2-s1 of genomic polarity (5*-AGCACAACCTAGAGATGTCATGG-3*, 4054±4435 of GBV-C/HGV RNA) and primer GB-C4-a1 of antigenomic polarity (5*-GACAGTTCAATCATC-3*, 4055±4474 of GBV-C/HGV RNA). The inner primers consisted of primer GB-C-s1 of genomic polarity (5*-GACAGTTCAATCATC-3*, 4257±4276 of GBV-C/HGV RNA) and primer GB-C4-a1 of antigenomic polarity. Both PCRs were done under the same conditions described before [21]. This competitive RT-PCR could detect 10^6 RNA copies/mL of serum. To prepare the RNA competitor, consisting of the NS3 region of GBV-C/HGV genome with a 37-mer insert, we constructed a recombinant plasmid. We first PCR-amplified a cDNA of 400 bp (nt 4054–4435 of GBV-C/HGV RNA) and cloned it into the Smal site of the pGEM3Z vector. Because of sequence variation, the original Smal site in the plasmid was destroyed. Meanwhile, two partially complementary nucleotides, AFP819F (5*-TGCTGTTAATTAATTGGCAAAATGTCCATT-3*) and AFP819R (3*-ATTAAATACCCGTGATACCCATT-5*), were synthesized and put together to form a partially double-stranded oligomer. After Klenow fragment treatment, the blunt-ended, 37-bp-long insert was then prepared and ligated into the Smal site in the middle of the HGV cDNA fragment (nt 4381), followed by in vitro transcription (MEGAscript T7 kit; Ambion, Austin, TX). Before use in competitive PCR, the in vitro transcription RNA products were treated with RNase-free DNase (Promega, Madison, WI) to remove the DNA template. The lengths of the PCR products of the wild type GBV-C/HGV RNA obtained with the first and second primer sets were 380 and 180 bp, respectively, while PCR products of the competitor RNA with the same primer sets were 417 and 217 bp, respectively. After ethidium bromide staining of the PCR products on electrophoresis, at the point at which the amounts of the wild type GBV-C/HGV product (380 bp) and the competitor product (417 bp) nearly equaled, the serum concentration should equal the known original concentration of the competitor RNA (2.79 X 10^-10 pg/copy).
Table 1. Clinical data, serum GBV-C/HGV RNA levels, and mode of delivery in mothers of GBV-C/HGV–infected infants and whose infants were not infected.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age* (years)</th>
<th>Parity*</th>
<th>GBV-C/HGV-RNA level, copies/mL</th>
<th>Mode of delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers of infected infants</td>
<td>33.3 ± 5.9</td>
<td>0.9 ± 0.8</td>
<td>All &gt;1 x 10^7</td>
<td>6 NSD</td>
</tr>
<tr>
<td>(n = 13)</td>
<td></td>
<td></td>
<td>(1 x 10^7–1 x 10^9)</td>
<td>7 emergent CS</td>
</tr>
<tr>
<td>Mothers of uninfected infants</td>
<td>29.8 ± 4.0</td>
<td>0.8 ± 0.9</td>
<td>10 ± 1 x 10^8</td>
<td>7 NSD</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td></td>
<td>2^1 &gt;1 x 10^7</td>
<td>5 elective CS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;1 x 10^5–5 x 10^6)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. NSD, normal spontaneous delivery; CS, cesarean section. Data in parentheses are ranges.

* Data are mean ± 1 SD.

² Both received elective cesarean section.

These lines of evidence at the molecular level strongly suggested that the neonates were indeed infected by their mothers.

On the other hand, there were 12 infants not infected with GBV-C/HGV (table 1). All 12 mothers were negative for HBsAg except 1, who was positive for both HBsAg and HBeAg. In addition, only her infant had elevated serum ALT levels twice (70 and 207 IU/L), whereas the remaining 11 infants had normal ALT levels throughout the follow-up period. There was another mother infected with both HCV and GBV-C/HGV, but the infant was not infected by either virus. The serum GBV-C/HGV RNA levels of these 12 mothers were lower; 8 had levels <5 x 10^4 copies/mL, 2 had 5 x 10^4 copies/mL, and 1 each had 5 x 10^7 and 5 x 10^8 copies/mL. Seven of the 12 had normal spontaneous delivery, while the remaining 5 received elective cesarean section. There were 2 mothers who had higher GBV-C/HGV RNA levels (>1 x 10^7 copies/mL) in this group; however, both delivered by elective cesarean section and their infants did not contract GBV-C/HGV infection. Taken together, these data show that babies born to mothers with high-titered viremia had higher risk of contracting GBV-C/HGV from their mothers (87% vs. 0, P < .001) (table 1).

Discussion

We found among 2046 pregnant women a total of 42 (2.1%) viremic for GBV-C/HGV, a prevalence higher than that of HCV infection in the same population on Taiwan [19]. Furthermore, 52% (95% confidence interval, 32.4%–71.6%) of the viremic mothers transmitted GBV-C/HGV to their offspring. The maternal transmission was documented by serial follow-up in the infants as well as by nucleotide sequence comparison of the E2 region of the viral genome. Sequence homology of this region ranged from 93%–98.2% between the 5 mother-infant pairs we analyzed, and phylogenetic analysis further showed a close genetic relatedness of the virus in each pair (figure 2).

All 13 GBV-C/HGV–infected infants were still viremic at age ≥9 months; they were thus likely to become chronic carriers of this virus. The very high carrier rate in GBV-C/HGV–infected infants, resembling that of perinatal transmission of HBV, differed from the chronicity rate of 38% in transfusion-acquired GBV-C/HGV–infected adults [12]. The reason remains unclear; however, by analogy to the situation in HBV infection, the immature immunity in the neonates might play
some role. Although all 13 GBV-C/HGV–infected infants became persistently viremic during our follow-up, elevation of serum ALT level was noted only transiently and mildly in 3 cases (only once in each) and remained normal thereafter. We are not sure that the ALT elevations were indeed caused by GBV-C/HGV. Even if this is true, our observations imply that the hepatic damage caused by this virus in infants may be mild and brief, as has been reported in adults [12–14]. As to the elevated serum ALT levels in the only uninfected infant of an HBV carrier mother, the infant had received hepatitis B immunophylaxis after birth [7] and the infant was negative for HBsAg, so the cause of hepatitis remained unknown. Another infant, whose mother was positive for both anti-HCV and GBV-C/HGV RNA, did not contract HCV or GBV-C/HGV infection; this may be due to the elective cesarean delivery in this case.

The efficiency of mother-to-infant transmission of blood-borne viruses generally correlates with the level of maternal viremia [2–6]. However, a high level of maternal viremia alone is not sufficient, provided that the maternal blood does not leak into fetal or newborn circulation [20, 24]. This is consistent with the findings in a large study of perinatal HCV infection [4], in which not all high-titered carrier mothers transmit HCV to their babies. On the basis of our previous studies [3, 20], the mode of delivery is also important in the occurrence of perinatal transmission of bloodborne viruses. In the present study, we found that all mothers of the 13 GBV-C/HGV–infected infants had higher serum GBV-C/HGV RNA levels (>1 × 10^5 copies/mL), and none had elective cesarean delivery, which induces the least microtransfusion from mother to fetus [20]. By contrast, 2 mothers, who had equally high levels of viremia but received elective cesarean section, did not transmit GBV-C/HGV to their infants. In total, among 15 mothers with high-titered GBV-C/HGV viremia, only the 2 infants delivered by elective cesarean section were spared the infection, whereas all of the remaining 13 infants who were born by normal spontaneous delivery or emergent cesarean section became infected. As to the infants of the remaining 10 low-titered carrier mothers (<1 × 10^5 copies/mL), none became viremic irrespective of the mode of delivery (table 1), suggesting the key role of maternal GBV-C/HGV level in causing mother-to-infant transmission.

Although we have demonstrated maternal transmission of GBV-C/HGV, whether this occurs vertically or perinatally cannot be determined in our study, because the first serum samples were taken at age ≥3 months. To explore the time when the transmission occurs, it will be necessary to collect earlier serum samples. Actually, GBV-C/HGV genome has been found to be absent in the cord blood of a neonate until 1 month after the baby was born to a carrier mother [18]. This indicates that the transmission of GBV-C/HGV occurs perinatally. This and the aforementioned key role of high-titered maternal viremia in contributing to the infection indicate that the situations of GBV-C/HGV and HBV infection are similar [1, 2]. In case GBV-C/HGV is proven to be pathogenic and important, it will then be necessary to interrupt perinatal transmission, as has been done in HBV infection [7, 8]. Nevertheless, this is premature at present, since we know very little about the pathogenicity and the immunology of GBV-C/HGV infections [11–15].

Because we used only viral RNA to define GBV-C/HGV infection, the incidence may be underestimated. This should also be studied by other means, such as assays for antibody to envelope protein E2 of GBV-C/HGV [25, 26]. Appearance of the E2 antibody is associated with viral clearance and is mutually exclusive of serum GBV-C/HGV RNA. In view of the high rate of chronicity once the infant contracts GBV-C/HGV infection from the mother, as shown in our study, detection of this antibody perhaps will add only limited information in this setting.

In summary, we found that high-titered maternal viremia and the mode of delivery are the determinants in maternal transmission of GBV-C/HGV. In addition, the infection in early infancy easily became persistent, but the majority of cases were not associated with hepatitis.

References


