A High Prevalence of Serum GB Virus C/Hepatitis G Virus RNA in Children with and without Liver Disease

Robert Halasz, Björn Fischler, Antal Nemeth, Suzana Lundholm, and Matti Sällberg

The role of GB virus C/hepatitis G virus (GBV-C/HGV) in adult and pediatric liver disease is unclear. We detected serum GBV-C/HGV RNA by reverse transcriptase polymerase chain reaction in 1 (3%) of 38 cholestatic infants, in 4 (4%) of 95 children without liver disease, and in none of 30 children with autoimmune hepatitis. One cholestatic infant had antibodies, presumably maternal, to GBV-C/HGV. Sequence analysis of a nonstructural 3 region fragment suggested that mother-to-infant transmission was the route of infection for the cholestatic infant. The four infected children without liver disease had normal liver function test results and lacked risk factors for bloodborne infections. Thus, the detection of GBV-C/HGV RNA among children with and without liver disease suggests that chronic GBV-C/HGV infections may be established early in life, possibly by mother-to-infant transmission. This may explain in part the high prevalence of serum GBV-C/HGV RNA and antibodies in healthy adults.

The recently described GB virus C/hepatitis G virus (GBV-C/HGV) [1, 2] appears to be more prevalent in healthy adults than is the closely related hepatitis C virus [3–5]. Knowledge about the transmission routes of GBV-C/HGV is limited, and the importance of this virus in adult as well as pediatric liver disease has been debated [3, 4, 6–8].

We and others have recently reported on mother-to-infant transmission of GBV-C/HGV [9, 10]. However, the clinical importance of GBV-C/HGV infections at this early age is not known. In infants <6 months of age, cholestasis is one of the most common expressions of liver disease. This is generally referred to as neonatal cholestasis [11]. Despite a long list of possible causes, the etiology of neonatal cholestasis remains unknown in >50% of cases [11]. This is problematic, since the mortality and morbidity is high. In previous studies, congenital or perinatal viral infections other than those with GBV-C/HGV have been linked to neonatal cholestasis [12–14].

The aim of the present study was to investigate the prevalence and possible routes of transmission of GBV-C/HGV in children with or without liver disease.

Patients

Three different groups of patients were studied. The first group included 38 infants (22 boys and 16 girls; median age, 70 days; range, 30–180 days; table 1) with clinical and biochemical signs of cholestasis. They were referred to our tertiary unit for pediatric hepatology and investigated for known genetic, metabolic, and infectious causes of neonatal cholestasis [11]. Fourteen of these 38 infants had extrahepatic biliary atresia; the other 24 had intrahepatic forms of cholestasis. Of these, nine had defined genetic and/or metabolic diseases causing cholestasis, including α1-antitrypsin deficiency in two, progressive familial intrahepatic cholestasis in two, inborn error of fatty acid metabolism in two, Alagille syndrome (syndromic paucity of intrahepatic bile ducts) in one, Aagenaes syndrome (chronic intrahepatic cholestasis with lymphoedema) in one, and severe combined immunodeficiency in one patient. For the other 15 patients, no certain cause of the cholestasis was found. However, six of them had signs of ongoing cytomegalovirus (CMV) infection, that is, IgM to CMV was detected in serum and/or CMV was isolated in urine [14].

The second group consisted of 95 children, mainly toddlers (42 boys and 53 girls; median age, 2.2 years; range, 0.6–9.2 years; table 1), who underwent small bowel biopsy and venous blood sampling for clinical investigation of gastrointestinal malabsorption. None of these children had any known liver disease.

The third group included 30 patients, mainly teenagers (8 boys and 22 girls; median age, 15.1 years; range, 2–20 years; table 1), with chronic autoimmune hepatitis (AIH), all of whom were investigated and treated at our department. Known causes of chronic hepatitis, including hepatitis B virus and hepatitis C virus infection, were excluded in all patients.

Serum samples from all patients were stored at −20°C.

Methods

GBV-C/HGV RNA was detected in stored sera by reverse transcriptase PCR, with primers from the 5′-noncoding region...
Table 1. Age distribution and numbers of sera positive for GBV-C/HGV RNA and antibody to GBV-C/HGV envelope 2 glycoprotein in 38 children with neonatal cholestasis, 95 children without liver disease, and 30 persons with autoimmune hepatitis.

<table>
<thead>
<tr>
<th>Group, age group (y)</th>
<th>No. of children</th>
<th>No. (%) with GBV-C/HGV RNA</th>
<th>No. (%) with antibody to GBV-C/HGV envelope 2 glycoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with neonatal cholestasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–0.5</td>
<td>38</td>
<td>1 (3)</td>
<td>1 (4)*</td>
</tr>
<tr>
<td>Children without liver disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5–1</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–2</td>
<td>33</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>2–4</td>
<td>29</td>
<td>2 (7)</td>
<td>0</td>
</tr>
<tr>
<td>4–10</td>
<td>19</td>
<td>1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Persons with autoimmune hepatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–13</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13–17</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17–20</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE. GBV-C/HGV = GB virus C/hepatitis G virus.
* Only 26 sera were analyzed for antibody to GBV-C/HGV envelope 2 glycoprotein; GBV-C/HGV RNA was not detected in the antibody-positive serum sample.

as described earlier [9]. Positive samples were then confirmed with primers from the nonstructural 3 (NS3) region [9], and the amplified product was sequenced by use of the Cy 5 Auto Read sequencing kit and an ALF Express DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). Phylogenetic analysis of the NS3 region sequences was done with the GeneWorks 2.3 software package (Intelligenetics, Mountain View, CA) and the unweighted pair group method with arithmetic mean.

Antibodies to GBV-C/HGV envelope 2 glycoprotein were analyzed in stored sera from all patients, except for 12 infants in the group with neonatal cholestasis, by use of a commercially available EIA (Anti-HGenv; Boehringer Mannheim, Mannheim, Germany). The assay was done according to the manufacturer’s instructions. Only samples that were reproducibly reactive were considered positive. Samples with reactivities that could not be reproduced and those with single borderline reactivity were regarded as negative.

Results

GBV-C/HGV RNA was detected in the serum from 1 (3%) of the 38 children with neonatal cholestasis, from 4 (4%) of the 95 patients without liver disease, and from none of the 30 patients with AIH.

The GBV-C/HGV RNA–positive patient with neonatal cholestasis was delivered vaginally after a normal pregnancy and was breast-fed. The boy had not received any blood products. He presented at 3 months of age with jaundice and biochemical markers of cholestasis, including extremely low levels of prothrombin, which required parenteral treatment with vitamin K. Apart from GBV-C/HGV RNA, virological examination revealed signs of ongoing CMV infection. He had good excretion on hepatobiliary scintigraphy, a normal α1-antitrypsin level, a normal sweat test, and no sign of inborn errors of metabolism. A liver biopsy revealed findings compatible with giant cell hepatitis. He was treated with fat-soluble vitamins, and his cholestasis resolved. On final check-up at 15 months of age, he was healthy, with normal biochemical markers and normal growth and development.

GBV-C/HGV RNA was also detected in a stored serum sample from his mother, derived at the time of the investigation of the boy’s neonatal cholestasis. The mother had no history of intravenous drug use, nor had she received any blood products. Phylogenetic analysis was done for the GBV-C/HGV NS3 region to test the genetic relatedness between the infected child and the mother. In the NS3 region dendrograms, the GBV-C/HGV sequences from the infected child consistently grouped with the GBV-C/HGV sequences derived from his mother (figure 1).

In the group of children without liver disease, three of the four GBV-C/HGV RNA–positive patients (three boys and one girl) had celiac disease. This was confirmed by small-bowel biopsies, which is the internationally agreed-upon diagnostic criterion for celiac disease. The fourth child was suspected of having celiac disease; however, only one small bowel biopsy was done. The first three patients were analyzed at the time of their third biopsy (at 3.6, 3.9, and 4.1 years of age, respectively), and the fourth at the time of his first and only biopsy (at the age of 1.2 years). All four were breast-fed; none of them had received any blood products. Liver function tests were repeatedly normal in all four. Phylogenetic analysis confirmed that the amplified fragments were of GBV-C/HGV origin. No close genetic relation between the GBV-C/HGV sequences from the five children was observed (figure 1).

Antibodies to GBV-C/HGV envelope 2 glycoprotein was reproducibly detected in the serum from only one patient, belonging to the group with neonatal cholestasis. The same serum sample was negative for GBV-C/HGV RNA by PCR. The patient was diagnosed as having extrahepatic biliary atresia. The serum sample was drawn at 2 months of age, before any surgical procedure, and he had not received any blood products. Later a portoenterostomy was done, and he became anicteric at 5 months of age. On follow-up at 4.5 years of age, he had compensated chronic liver disease.

Discussion

The significance of GBV-C/HGV in liver disease has been questioned [3, 4]. However, a recent report showed an association between GBV-C/HGV and elevated biochemical markers for cholestatic liver disease in adults [15]. Since the etiology...
of neonatal cholestasis is often unknown, the importance of a newly described viral agent such as GBV-C/HGV needs to be examined. In the present study, 1 of 38 patients with neonatal cholestasis was GBV-C/HGV RNA–positive. This rate of seropositivity was similar to that of children without liver disease, suggesting that GBV-C/HGV is not an important etiologic factor for the development of neonatal cholestasis. For comparison, in a previous study of patients with neonatal cholestasis, we found no association with hepatitis B virus or hepatitis C virus infections [14], and similar results were reported by others [16, 17].

The GBV-C/HGV RNA–positive infant with neonatal cholestasis also had ongoing CMV infection, which in itself might have been sufficient to cause his cholestasis [14]. However, the possibility that both viral infections were linked to the development of neonatal cholestasis cannot be ruled out. Since genetically similar GBV-C/HGV RNA was found in both the mother and the cholestatic infant, vertical or early horizontal transmission seems the most probable route of infection. For yet-unknown reasons, mother-to-infant transmission of this virus is thought to occur more frequently than with the related hepatitis C virus [9, 10]. As we and others have shown, vertical infection with GBV-C/HGV often results in asymptomatic infection, with persistent viremia [9, 10]. However, in analogy with children with vertically transmitted hepatitis B virus or hepatitis C virus infections, one cannot exclude that perinatally acquired GBV-C/HGV infection could cause liver damage in adulthood. Additionally, in the serum from one of the cholestatic infants, antibodies to GBV-C/HGV envelope 2 glycoprotein were detected at 2 months of age. Because of the early age, we assume that these were passively transferred maternal antibodies.

As in adults, the rate of GBV-C/HGV RNA seropositivity in children is high in selected groups at risk for bloodborne infections [18]. The situation in healthy children outside the risk groups is largely unknown. This is of general interest, considering the high incidence in healthy adults [3–5]. Recently, Chen et al. [18] reported that in a group of healthy children, aged 0.5–12 years, 1% were GBV-C/HGV RNA–positive. The rate of 4.2% found in the present study is even higher, and results such as these suggest that many seropositive adults might have been infected with GBV-C/HGV at birth or during early childhood.

The route of infection for the four children in the group without liver disease remains uncertain. All four had undergone small bowel biopsy, and this procedure might theoretically pose an increased risk of viral transmission. However, the GBV-C/HGV isolates from each of the four children were genetically heterogeneous, and thus transmission within this small group seems less probable. Since no sera were available from the mothers of these four children, the possibility of vertical transmission could not be addressed.

The advantage of testing patients without liver disease who were evaluated for malabsorption was that venous blood sampling was part of the routine clinical investigation. Blood sampling in healthy children is generally not accepted for ethical reasons. It should be noted that the etiology of celiac disease is still unknown; for example, adenovirus infection has been implicated in the pathogenesis of this enteropathy [19]. Whether GBV-C/HGV has a tropism for or is in some way pathogenic for the gut remains to be investigated.

The patients with AIH were analyzed for the presence of GBV-C/HGV for two reasons. The first was that the etiology of AIH still is not known, and theoretically GBV-C/HGV could be a triggering factor for that disease. The second reason was that we sought a group of older children, mostly teenagers, to compare the rate of GBV-C/HGV RNA–positive patients in...
different age groups. For the first question we, as others [8, 20], found no link between AIH and the appearance of GBV-C/HGV RNA in the serum. For the second issue, the prevalence in this age group at least was not higher than in the group of younger children without liver disease. This could suggest that most infected children acquire the infection early in life.

The high prevalence of GBV-C/HGV RNA and antibodies among seemingly healthy adults does not seem to be explained entirely by viral transmission through known parenteral routes, for example, contaminated blood products or intravenous drug use [5, 21]. One could speculate whether nonparenteral routes, such as close social contacts, could cause chronic GBV-C/HGV infections. However, we previously noted that a firstborn female twin whose twin brother and mother had chronic GBV-C/HGV infections did not develop chronic GBV-C/HGV infection during the 42 months of follow-up [9]. In contrast, vertical transmission of the virus to the secondborn male twin gave rise to chronic viremia. It is of interest that we could not find clear evidence for an increase in antibodies to GBV-C/HGV envelope 2 glycoprotein with increasing age of the tested children. This would be consistent with the observation that clearance of neonatal GBV-C/HGV infection at an early age is rare [22]. If so, the presence of GBV-C/HGV antibodies in adults may be secondary either to a later clearance of neonatal GBV-C/HGV infection or to horizontal transmission at an older age (or both). However, these hypotheses need to be confirmed.

We conclude that GBV-C/HGV is not commonly associated with neonatal cholestasis or with AIH. Furthermore, early infections with GBV-C/HGV, possibly transmitted from the mother and resulting in a chronic viremia, may account for the high rate of GBV-C/HGV RNA positivity in persons without liver disease, both children and adults.

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References