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Jansen, P.L.M.; Bosma, P.J.; Bakker, C.; Lems, S.P.M.; Slooff, M.J.H.; Haagsma, E.B.

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Persistent unconjugated hyperbilirubinemia after liver transplantation due to an abnormal bilirubin UDP-glucuronosyltransferase gene promoter sequence in the donor

Peter L. M. Jansen, Piter J. Bosma, Conny Bakker, Simon P. M. Lems, Maarten J. H. Slooff and Elizabeth B. Haagsma

Division of Hepatology and Gastroenterology, Transplantation Immunology, and Surgery, University Hospital Groningen and Division of Gastroenterology, Academic Medical Center, Amsterdam, The Netherlands

Background/Aims: Gilbert's syndrome is genetically characterized by an extra TA element in the TATAA-box of the promoter region upstream of the bilirubin UDP-glucuronosyltransferase (UGT1A) coding region (Bosma et al. N Engl J Med 1995; 333: 1171-5). Persistent unconjugated hyperbilirubinemia is occasionally observed in liver transplant recipients with an otherwise normal liver function. We postulate that these patients could have received a liver from a donor with the Gilbert's syndrome genotype. Therefore, we investigated the UGT1A-gene TATAA-box in DNA from liver graft donors of jaundiced and non-jaundiced recipients.

Methods: DNA was obtained from stored donor lymphocytes and the number of TA elements in the TATAA-box of the UGT1A-gene promoter region was analyzed by polymerase chain-reaction.

Results: We observed two liver transplant recipients with persistent unconjugated hyperbilirubinemia. They received liver grafts from donors who were homozygous for an abnormal A(TA)7TAA-box in the UGT1A-gene. Four of 10 non-jaundiced recipients received livers from donors who were homozygous for the normal A(TA)6TAA-box and six received livers from donors who were heterozygous with a normal A(TA)6TAA-box on one allele and a prolonged A(TA)7TAA-box on the other allele.

Conclusions: This study shows that liver graft recipients with persistent unconjugated hyperbilirubinemia may have received a liver from a donor with an abnormal TATAA-box in the bilirubin UGT1A-gene promoter region.

Key words: Gilbert's syndrome; Hyperbilirubinemia; Jaundice; Liver transplantation; TATAA box; UDP-glucuronosyltransferase.

Recent studies have revealed that Gilbert's syndrome appears to result from an abnormality in the gene for bilirubin UDP-glucuronosyltransferase (bilirubin-UGT1A): the TATAA-box in the promoter region of the UGT1A (UGT1*1) exon contains seven TA repeats instead of the usual six (1,2). Patients with Gilbert's syndrome are homozygous for this A(TA)7TAA promoter sequence. Among the population homozygosity for the A(TA)7TAA promoter sequence abnormality occurs with a frequency of 10-16% (1,2). Clinically manifest Gilbert's syndrome with a stable elevated serum unconjugated bilirubin level, or with unconjugated hyperbilirubinemia after a 24-h fast, occurs with a frequency varying between 2 and 12% (3-7).

Among the 100 adult patients who received a liver transplant in our center between 1989 and 1994 we observed two patients with mild persistent unconjugated hyperbilirubinemia and an otherwise normal liver function. In this study we analyzed the bilirubin UGT1A-gene TATAA-box in DNA extracted from stored liver graft donor-lymphocytes and compared this with the bilirubin UGT1A-gene of the donors of 10 transplanted patients without jaundice.
**Materials and Methods**

**Patients**

Patient A is a 53-year-old male with liver cirrhosis in connection with autoimmune hepatitis. He received an ABO-matched liver transplant in 1994. The post-transplantation course was unremarkable. The medication consists of cyclosporin (2×125 mg), azathioprine (1×125 mg) and prednisolone (1×10 mg). Three months after transplantation, all laboratory functions had normalized except for a persistently high unconjugated serum bilirubin level. Relevant recent laboratory values are (normal values within brackets): total bilirubin 54 μmol/l (<17 μmol/l), direct-reacting bilirubin 5 μmol/l, ASAT 18 U/l (<40 U/l), ALAT 28 U/l (<30 U/l), lactate dehydrogenase 240 U/l (<235 U/l), alkaline phosphatase 49 U/l (<120 U/l), and hemoglobin 8.8 mmol/l (8.7–10.6 mmol/l).

Patient B is a 48-year-old male with primary sclerosing cholangitis and inactive ulcerative colitis. He received an ABO-matched liver transplant with a duct-to-duct anastomosis in 1994. In this patient a high unconjugated serum bilirubin level persisted when 3 months after transplantation the transaminase, alkaline phosphatase and gamma-glutamyltransferase activities had normalized. Recent laboratory values are: total bilirubin 93 μmol/l, direct-reacting bilirubin 7 μmol/l, ASAT 18 U/l, ALAT 23 U/l, lactate dehydrogenase 147 U/l, alkaline phosphatase 41 U/l and hemoglobin 8.7 mmol/l. This patient is on cyclosporin (2×100 mg), azathioprine (1×125 mg) and prednisolone (1×10 mg).

Biopsies obtained 1 year after transplantation showed a normal histology in both patients. Serum bilirubin levels were measured with standard laboratory tests, based on the diazo reaction (8). Direct-reacting bilirubin was less than 10% in both patients.

The control patients, two males and eight females, were randomly chosen from patients who had no laboratory abnormalities and who were transplanted between 1989 and 1994. Like the two index patients, these controls had a normal liver histology 1 year after transplantation. Eight patients receive prednisolone (1×10 mg), azathioprine (1×100–125 mg) and cyclosporin (200–400 mg per day), one patient receives FK 506 (10 mg per day), and one patient receives no other immunosuppressant.

**Nucleotide sequencing**

Genomic DNA was extracted from stored donor lymphocytes and the promoter region of the bilirubin UGT1A-gene was amplified by the polymerase chain reaction (PCR) as described (1). The segment of DNA, 5' to the coding region (nucleotide −227 to nucleotide −132), was amplified with a sense primer, 5'GAGGTTCAGGAAATCTTTG3', and an antisense primer, 5'CCAAGCATGCTACAGCCG3'. PCR was performed for 30 cycles consisting of denaturation at 95°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 30 s, with MgCl2, 1.5 mmol/l. Both strands of the amplified segment were sequenced with two internal primers (1).

**TABLE 1**

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Age at Tx</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Bilirubin after Tx (μmol/l)</th>
<th>TATAA element</th>
<th>Bilirubin (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bilirubin after Tx (μmol/l)</td>
<td>TATAA element</td>
<td>Bilirubin (μmol/l)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>f</td>
<td></td>
<td>alcoholic liver cirrhosis</td>
<td>10±3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>59</td>
<td>f</td>
<td></td>
<td>primary biliary cirrhosis</td>
<td>10±2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>22</td>
<td>m</td>
<td></td>
<td>cryptogenic liver cirrhosis</td>
<td>12±3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>46</td>
<td>f</td>
<td></td>
<td>primary biliary cirrhosis</td>
<td>15±3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>30</td>
<td>f</td>
<td></td>
<td>Wilson's disease</td>
<td>17±5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>47</td>
<td>f</td>
<td></td>
<td>cryptogenic liver cirrhosis</td>
<td>11±3</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>53</td>
<td>m</td>
<td></td>
<td>hepatitis C</td>
<td>14±4</td>
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<tr>
<td>23</td>
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<td>9±3</td>
<td>7</td>
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<td>40</td>
<td>f</td>
<td></td>
<td>primary sclerosing cholangitis</td>
<td>6±1</td>
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<tr>
<td>41</td>
<td>f</td>
<td></td>
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<td>16±5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>51</td>
<td>m</td>
<td></td>
<td>autoimmune hepatitis</td>
<td>53±8*</td>
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<tr>
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<td>m</td>
<td></td>
<td>primary sclerosing cholangitis</td>
<td>82±19*</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Bilirubin after Tx represents the mean serum bilirubin levels ± SD of 10–17 separate values obtained in a period 0.5–2 years after transplantation. These patients were transplanted 2–7 years (3.5±1.4 years) ago. They all have normal liver functions (e.g. serum alkaline phosphatase and transaminase activities are within normal limits). The UGT1A-gene TATAA-box in DNA extracted from donor lymphocytes was analyzed and the number of TA elements of both alleles is shown. The serum bilirubin levels of the donors at the time of organ donation is shown in the right-hand column (direct-reacting fractions were within normal limits).
Results
The age, sex and diagnosis of the liver transplant recipients are given in Table 1. The serum bilirubin values of the recipients represent means of 10-12 separate values obtained 0.5–2.0 years after transplantation. The direct serum bilirubin levels, the alkaline phosphatase and transaminase activities of these patients were within normal limits. The two right-hand columns show data of the liver graft donors. Analysis of genomic DNA of donor lymphocytes revealed that the two patients with an abnormally elevated serum bilirubin level after transplantation (indicated by *) received a liver from donors with an abnormal A(TA)_7TAA sequence in the bilirubin-UGT1A promotior region. They are both homozygous for this abnormal TATAA-box.

Four of 10 non-jaundiced recipients received liver grafts from donors who were homozygous for the normal A(TA)_6TAA sequence and six received grafts from donors who were heterozygous with a normal A(TA)_6TAA sequence on one allele and an abnormal A(TA)_7TAA sequence on the other allele. The last column shows serum bilirubin levels of the donor at the time of liver donation.

Fig. 1 shows that the serum bilirubin values in the two jaundiced patients, both recipients of livers from donors with the A(TA)_7TAA/A(TA)_7TAA genotype, are persistently elevated. The serum bilirubin values of the patients who received livers from donors which are either A(TA)_6TAA/A(TA)_6TAA homozygotes or A(TA)_6TAA/A(TA)_7TAA heterozygotes, are within normal limits.

Discussion
In two recent studies it was shown that patients with Gilbert's syndrome have an A(TA)_6TAA instead of an A(TA)_6TAA sequence in the bilirubin UGT1A-gene promotior region on both alleles (1,2). Patients with the much rarer Crigler-Najjar syndromes type 1 and 2 were found to have mutations in the coding region of the bilirubin UGT1A-gene on both alleles (9–11). A study performed in a family with two Crigler-Najjar type 2 patients, revealed that sibs with normal bilirubin levels are either homozygous normal or have a structural mutation in only one allele. Sibs with a Gilbert's syndrome phenotype (mild unconjugated hyperbilirubinemia) have abnormalities in both alleles: a structural UGT1A-gene mutation in one and the A(TA)_7TAA abnormality in the other allele (1,11). This contrasts with the study of Aono et al. (12). They describe persons, diagnosed as having Gilbert's syndrome, with a structural UGT1A-gene mutation on one allele only. However, these authors have not investigated the TATAA promotior sequence on the other allele. It may well be that, in addition to the structural UGT1A-gene mutation on one allele, their patients had an abnormal TATAA-box in the UGT1A-gene promotior region on the other allele.

Although other causes of Gilbert's syndrome have been described (13–18), a reduced hepatic bilirubin UDP-glucuronosyltransferase activity is the most frequently found abnormality (19). Direct evidence for the association between a prolonged TATAA-box in the promotior region of the UGT1A-gene and a decreased UDP-glucuronosyltransferase activity is not available yet but such a relation is very likely, since placing a promotior region with an A(TA)_7TAA sequence before a gene encoding for firefly luciferase indeed results in a decreased expression of this gene (1). A promotior region with an abnormal TATAA-box is less efficient in binding regulatory proteins which control gene transcription. Thus persons with an A(TA)_7TAA/A(TA)_7TAA genotype are likely to have a reduced hepatic bilirubin UGT1A activity. The frequency of this genotype in the general population is
10–16% (1,2). The frequency of clinically manifest Gilbert’s syndrome has been estimated to be in the range of 2–12% (3–7). It should be realized that the expression of Gilbert’s syndrome is highly variable and is influenced by factors such as food intake, cigarette smoking, alcohol consumption, medication and the fat content of the diet (20–23). The best test to bring a latent Gilbert’s syndrome to expression is a 24-h 400 calorie-restricted diet (21,22). Monaghan et al. (2) showed that most, but not all, persons with the A(TA)TAA/A(TA)TAA promoter sequence abnormality have an elevated serum bilirubin level after a 24-h fast. This suggests that, in addition to the promoter region abnormality, another factor may be necessary for the clinical expression of Gilbert’s syndrome. In liver transplant recipients who are homozygous for the TATAA-box abnormality, cyclosporin therapy could perhaps contribute to the expression of Gilbert’s syndrome. Cyclosporin is known to reduce the hepatobiliary secretion of bilirubin conjugates (24,25). In non-transplanted persons this “other factor” most likely is a slightly increased bilirubin production because this would overburden the decreased glucuronidation capacity of patients with Gilbert’s syndrome. This is in line with earlier reports showing a normalization of serum bilirubin levels in patients with a combination of hereditary spherocytosis and Gilbert’s syndrome after splenectomy (26).

Our study indicates that Gilbert’s syndrome can be transferred by a liver graft. It is possible that a liver from a donor with the A(TA)TAA/A(TA)TAA genotype causes clinically manifest Gilbert’s syndrome in the recipient. In the donor the Gilbert’s syndrome phenotype may or may not have been recognized. Both donors of the jaundiced liver graft recipients had slightly elevated serum bilirubin levels at the time of organ donation. This quite likely results from their Gilbert’s syndrome genotype. However, some of the donors of the non-jaundiced recipients also showed slightly elevated serum bilirubin levels. Donors at the time of organ donation usually are on ventilators in intensive care units. This is not the best time to judge whether or not a donor has Gilbert’s syndrome as there are too many complicating factors. It is also not really important because transplantation of a liver with the Gilbert’s syndrome genotype usually has few consequences for the recipient. However, occasionally an isolated unconjugated serum bilirubin level can cause confusion about the function of the liver graft and therefore one should be aware that Gilbert’s syndrome can be transferred from the donor to a recipient. On the other hand, an isolated unconjugated bilirubin serum level in the donor is no reason not to use that liver for organ donation.

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References