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

Peter L. M. Jansen[1], Piter J. Bosma[4], Conny Bakker[4], Simon P. M. Lems[2], Maarten J. H. Slooff[3] and Elizabeth B. Haagsma[1]

[1]Division of Hepatology and Gastroenterology, [2]Transplantation Immunology, and [3]Surgery, University Hospital, Groningen and [4]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 promotor 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 promotor 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)$_7$TAA-box in the *UGT1A*-gene. Four of 10 non-jaundiced recipients received livers from donors who were homozygous for the normal A(TA)$_6$TAA-box and six received livers from donors who were heterozygous with a normal A(TA)$_6$TAA-box on one allele and a prolonged A(TA)$_7$ TAA-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 promotor 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 promotor 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)$_7$-TAA promotor sequence. Among the population

Correspondence: Peter L. M. Jansen, MD, Division of Hepatology and Gastroenterology, University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel: 31(50)3612620. Fax 31(50)3619306. e-mail: p.l.m.jansen@med.rug.nl.

homozygosity for the A(TA)$_7$TAA promotor 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 gammaglutamyltransferase 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 promotor 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'GAGGTTCTGGAAGTACTTTGC3', and an antisense primer, 5'CCAAGCATGCTCAGCCAG3'. 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 $MgCl_2$, 1.5 mmol/l. Both strands of the amplified segment were sequenced with two internal primers (1).

TABLE 1

Diagnosis, age, sex and time of transplantation of jaundiced and non-jaundiced transplant recipients

Recipient				Donor		
Age at Tx	Sex	Diagnosis	Bilirubin after Tx (μmol/l)	TATAA element		Bilirubin (μmol/l)
				allele a	allele b	
46	f	alcoholic liver cirrhosis	10±3	6	6	21
59	f	primary biliary cirrhosis	10±2	6	6	10
22	m	cryptogenic liver cirrhosis	12±3	6	6	13
46	f	primary biliary cirrhosis	15±3	6	6	4
30	f	Wilson's disease	17±5	6	7	–
47	f	cryptogenic liver cirrhosis	11±3	6	7	1
53	m	hepatitis C	14±4	6	7	22
23	f	primary sclerosing cholangitis	9±3	6	7	8
40	f	primary sclerosing cholangitis	6±1	6	7	6
41	f	alcoholic liver cirrhosis	16±5	6	7	10
51	m	autoimmune hepatitis	53±8*	7	7	22
46	m	primary sclerosing cholangitis	82±19*	7	7	37

Bilirubin after Tx represents the mean serum bilirubin levels±SD of 10–12 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* promotor 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)₆-TAA sequence on one allele and an abnormal A(TA)₇-TAA sequence on the other allele. The last column
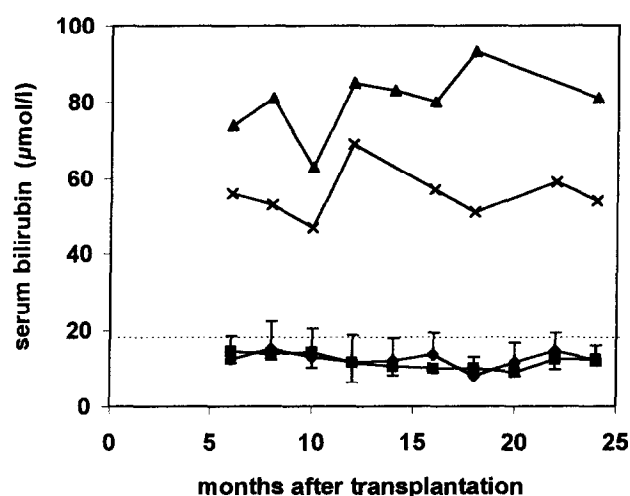

Fig. 1. Hyperbilirubinemia in liver transplant recipients. Average serum bilirubin levels of four patients who received liver grafts from homozygous normal donors with an A(TA)₆TAA/A(TA)₆TAA sequence in the bilirubin UGT1A-*gene promotor region (diamonds) are shown, compared with bilirubin levels of six patients who received liver grafts from heterozygous A(TA)₆TAA/A(TA)₇TAA donors (■-■-■). The data points represent means±SD. In addition, the serum bilirubin values of two patients who received a liver graft from a homozygous donor with the abnormal A(TA)₇TAA/TA(TA)₇TAA genotype are shown (patient A, ×-×-×; patient B, ▲-▲-▲). Serum bilirubin levels (μmol/l) are shown on the ordinate and the time after liver transplantation (months) on the abscissa. The stippled line shows the normal value (<17 μmol/l).*

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/yA(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)₇TAA instead of an A(TA)₆TAA sequence in the bilirubin *UGT1A*-gene promotor 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 promotor 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 TA-TAA-box in the *UGT1A*-gene promotor 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 promotor 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 promotor 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 promotor region with an abnormal TATAA-box is less efficient in binding regulatory proteins which control gene transcription. Thus persons with an A(TA)₇-TAA/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)$_7$-TAA/A(TA)$_7$TAA promotor sequence abnormality have an elevated serum bilirubin level after a 24-h fast. This suggests that, in addition to the promotor 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)$_7$TAA/A(TA)$_7$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.

References

1. Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. N Engl J Med 1995; 333: 1171–5.
2. Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UDP- glucuronosyltransferase gene promotor and Gilbert's syndrome. Lancet 1996; 347: 578–81.
3. Owens D, Evans J. Population studies on Gilbert's syndrome. J Med Genet 1975; 12: 152–6.
4. Bailey A, Robinson D, Dawson AM. Does Gilbert's syndrome exist? Lancet 1977; i: 931–3.
5. Sieg A, Arab L, Schlierf G, Stiehl A, Kommerell B. Die Prävalenz des Gilbert-syndroms in Deutschland. Dtsch Med Wochenschr 1987; 112: 1206–8.
6. Fevery J. Pathogenesis of Gilbert's syndrome. Eur J Clin Invest 1981; 11: 417–8.
7. Watson KJR, Gollan JL. Gilbert's syndrome. Ballière's Clin Gastroenterol 1989; 3: 337–55.
8. Jendrassik L, Grof P. Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins. Biochem Z 1938; 297: 81–91.
9. Bosma PJ, Chowdhury NR, Goldhoorn BG, Hofker MH, Oude Elferink RPJ, Jansen PLM, et al. Sequence of exons and the flanking regions of human bilirubin UDP-glucuronosyltransferase gene complex and identification of a genetic mutation in a patient with Crigler-Najjar syndrome, type-I. Hepatology 1992; 15: 941–7.
10. Bosma PJ, Chowdhury JR, Huang TJ, Lahiri P, Oude Elferink RPJ, van Es HHG, et al. Mechanisms of inherited deficiencies of multiple UDP-glucuronosyltransferase isoforms in two patients with Crigler-Najjar syndrome, type I. FASEB J 1992; 6: 2859–63.
11. Bosma PJ, Goldhoorn B, Elferink RPJO, Sinaasappel M, Oostra BA, Jansen PLM. A mutation in bilirubin uridine 5'-diphosphate-glucuronosyltransferase isoform-1 causing Crigler-Najjar syndrome type-II. Gastroenterology 1993; 105: 216–20.
12. Aono S, Adachi Y, Uyama E, Yamada Y, Keino H, Nanno T, et al. Analysis of genes for bilirubin UDP-glucuronosyltransferase in Gilbert's syndrome. Lancet 1995; 345: 958–9.
13. Berk PD, Blaschke TF, Waggoner JC. Defective bromosulphthalein clearance in patients with constitutional hepatic dyfunction (Gilbert's syndrome). Gastroenterology 1972; 63: 472–81.
14. Martin JF, Vierling JM, Wolkoff AW, Scharschmidt BF, Vergalla J, Waggoner JG, et al. Abnormal hepatic transport of indocyanine green in Gilbert's syndrome. Gastroenterology 1976; 70: 385–91.
15. Frezza M, Pozzato G, Quaranta C, Tiribelli C, Piccinini C. Abnormality in the hepatic transport of rifamycin-sv in Gilbert's syndrome. Ital J Gastroenterol 1980; 12: 1–5.

16. Okhubo H, Okuda K, Iida S. A constitutional unconjugated hyperbilirubinemia combined with indocyanine green intolerance: a new functional disorder? Hepatology 1981; 1: 319–24.

17. Berk PD, Bloomer JR, Howe RB, Berlin NI. Constitutional hepatic dysfunction (Gilbert's syndrome): a new definition based on kinetic studies with unconjugated radiobilirubin. Am J Med 1970; 49: 296–305.

18. Okuda K, Ohkubo H, Musha H, Iida S. Marked delay in indocyanine green plasma clearance with a near normal bromosulphthalein retention test: a constitutional abnormality? Gut 1976; 17: 588–94.

19. Black M, Billing BH. Hepatic bilirubin UDP-glucuronyltransferase activity in liver disease and Gilbert's syndrome. N Engl J Med 1969; 280: 1266–71.

20. Ideo G, De Franchis R, Del Ninno E, Dioguardi N. Ethanol increases liver uridine-diphosphate-glucuronosyltransferase. Experientia 1971; 27: 24–5.

21. Owens D, Sherlock S. Diagnosis of Gilbert's syndrome: role of reduced caloric intake test. Br Med J 1973; iii: 559–63.

22. Whitmer DI, Gollan JL. Mechanisms and significance of fasting and dietary hyperbilirubinemia. Semin Liver Dis 1983; 342–51.

23. Black M, Sherlock S. Treatment of Gilbert's syndrome with phenobarbitone. Lancet 1970; i: 1359–62.

24. Cadranel JF, Erlinger S, Desruenne M, et al. Chronic administration of cyclosporin A induces a decrease in hepatic excretory function in man. Dig Dis Sci 1992; 37: 1473–6.

25. Galan AI, Zapata AJ, Roman ID, Munoz ME, Muriel C, Gonzalez J, et al. Impairment of maximal bilirubin secretion by cyclosporin A in the rat. Arch Int Physiol Biochim Biophys 1991; 99: 373–6.

26. Berk PD, Berman MD, Blitzer BL, Chretien P, Martin JF, Scharschmidt BF, et al. Effect of splenectomy on bilirubin clearance in patients with hereditary spherocytosis. J Lab Clin Med 1981; 98: 37–44.