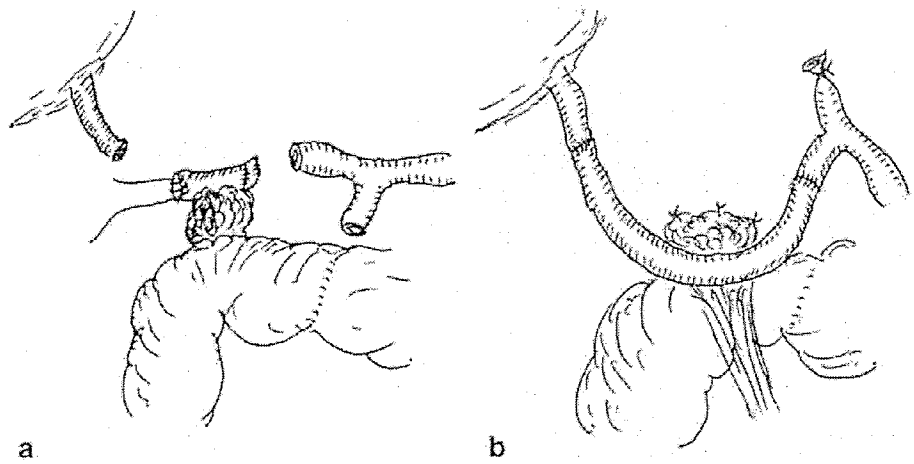


Fig. 2a,b Case 1: schema of the reconstruction for hepatic artery pseudoaneurysm. a Arteries of pre- and post-aneurysm were cut and closed by continuous suturing. The gastroduodenal artery was dissected and divided for the anastomosis. b Autologous IMA was interposed between the donor's hepatic artery and the recipient's gastroduodenal artery. The aneurysm was covered by the omentum

Further examination revealed that continuous intimal dissection had developed in the arterial wall and extended to the root of the common hepatic artery. Therefore, we looked for the stump of the splenic artery and confirmed that its intima was intact and had sufficient arterial outflow. The previous anastomotic portion of the hepatic artery, in which no thrombosis was detected, was resected. An arterial graft of autologous IMA was retrieved as in the first case and interposed between the donor's hepatic artery and the recipient's splenic artery. The hepatic arterial flow recovered completely after surgery and was maintained until the patient died of sudden massive bleeding from an esophageal ulcer 11 months after transplantation.

## Discussion

Pseudoaneurysm of the hepatic artery is a rare but life-threatening complication in liver transplantation. It has an incidence of less than 1%, and major clinical signs are gastrointestinal or abdominal bleeding [4]. The main cause of the pseudoaneurysm is a bacterial infection due to bile leaks, pancreatitis or other infections such as fungal infections. In case 1, no sign of infection was detected and the cause was unknown, though it might have been due to a technical problem in arterial anastomosis. Another possibility is that the deep duodenal ulcer that first developed could have injured the arterial wall and might have promoted the aneurysm. It has been proposed that the most appropriate treatment for pseudoaneurysm is embolization by angiography or ligation of the hepatic artery [5, 6]. However, ligation carries the prospect of extremely high morbidity and mortality, especially early after transplantation. Therefore, treatment by excision and immediate re-vascularization has been recommended by Bonham et al [7]. In the present case, it was not very difficult to approach the hepatic artery near the aneurysm because there were no findings of infection. The pre- and post-aneurysm artery was dissected successfully, and the arterial graft of autologous IMA was interposed. In this case, because there was no infection, reconstruction of the artery seemed to be better than intervention therapy, even if the pseudoaneurysm itself could not be taken out.

Intimal dissection of the hepatic artery is also a rare complication in LDLT, and has seldom been reported [8]. This author personally experienced two cases, among 300 cases of LDLT (data were not published), in which the intimal dissection of the donor's hepatic artery occurred immediately after the anastomosis of the hepatic artery from an unknown cause. Once it occurred, it was almost impossible to restore the flow by any means, as the dissection continued to the artery branches inside the liver graft. In contrast, we have sometimes encountered an intimal deformity of the recipient's hepatic artery like a honeycomb in chronic liver disease. In such cases, the recipient's artery was repeatedly cut towards the root of celiac trunk until the normal wall appeared. In case 2, the anastomosis in the first operation might have been dealt with in another way if the orifice of the recipient's artery and the outflow had been observed more carefully or if pre-transplantation angiography had been performed to detect splanchnic artery stenosis [9].

Several arterial grafts for reconstruction of the hepatic artery in liver transplantation have been proposed. In the present study, the main branch of the IMA was dissected, and approximately 3.0 cm of the artery from the root to the first branch was retrieved. The blood flow in the descending colon was not compromised after retrieval of the IMA branch, and no complications related to this procedure were observed. This procedure is recommended because the approach is very easy and the

diameter of the IMA graft is similar to the hepatic artery.

All of the arterial anastomoses in the present study were performed by microsurgical techniques. Microsurgery in LDLT was introduced for small artery anastomosis, such as left lateral lobe transplantation in pediatric cases, and, as a consequence, the incidence of hepatic artery thrombosis has become very low [10]. Recently, anastomosis using a surgical loupe has been revived in LDLT of right lobe grafts because of the relatively larger size of the arteries involved. It might have been possible to suture the arteries in the present cases using a surgical loupe. However, we still believe that anastomosis of the hepatic artery can be performed quickly and safely by experts using microsurgery, and that it is extremely beneficial to avoid critical complications of the hepatic artery in LDLT.

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# Magnet Compression Anastomosis for Bile Duct Stenosis After Duct-to-Duct Biliary Reconstruction in Living Donor Liver Transplantation

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A 44-year-old woman who had undergone living donor liver transplantation for fulminant hepatic failure presented obstructive jaundice 1 year after transplantation. A right lobe from her husband had been used for the original graft. Intraoperative cholangiography of the donor showed the bile duct of posterior inferior segment (B6) branching from the bile duct of anterior segment (Fig. 1). The bile duct of the donor was transected in the very short segment of the common trunk of the posterior and anterior branches of the right lobe. The orifice of the bile duct of the graft was single, but the shape of it was like the nose of a pig. This single orifice was anastomosed to the stump of the recipient's common hepatic duct. A biliary stent tube (4-French-sized) was inserted into only the bile duct of the posterior segment. Cold- and warm-ischemia time was 42 and 45 minutes, respectively. She initially recovered uneventfully in the early period after liver transplantation. The external stent tube was removed 3 months after the transplantation.

Laboratory data at 11 months after the transplantation showed slight elevation of transaminases (aspartate aminotransferase: 80 IU/L, alanine aminotransferase: 100 IU/L) and total bilirubin (1.4 mg/dL). One month later, ultrasonography showed the dilated intrahepatic duct. Endoscopic retrograde cholangiography and percutaneous transhepatic cholangiography disclosed the complete obstruction of the anterior branch (Fig. 2). The dilated duct was drained by the percutaneous transhepatic cholangiography drainage tube. Balloon dilatation was attempted though the percutaneous transhepatic cholangiography drainage tube, but it was

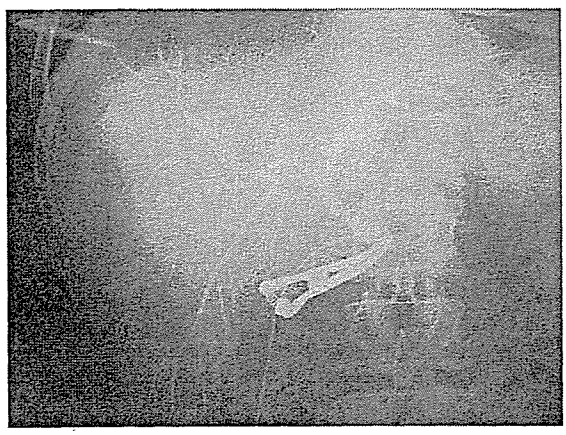

**Figure 1. Intraoperative cholangiogram of the donor in the initial living donor liver transplantation. Bile duct of posterior inferior segment (B6) branching from the bile duct of the anterior segment**

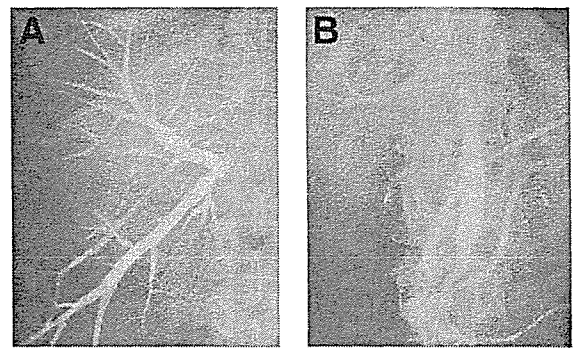


**Figure 2. (A) Percutaneous transhepatic cholangiography showed complete biliary obstruction of the anterior branch. (B) Endoscopic retrograde cholangiography could not show the anterior branch.**

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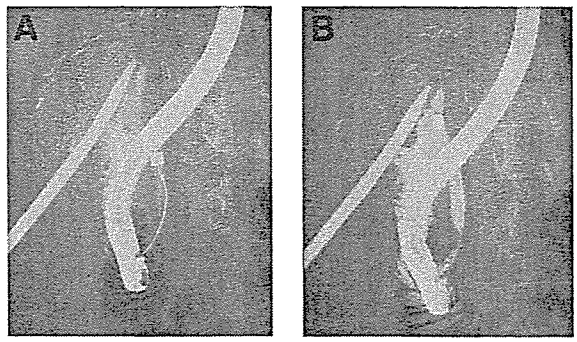


**Figure 3. Placing of the parent magnet. (A) A parent magnet (diameter 4 mm) attached to a guide with covered tube was placed endoscopically through the papilla Vateri that was enlarged enough with endoscopic sphincterectomy at supine position. (B) The parent magnet was placed at the stricture point.**

not possible because the guidewire could not pass the stenosis. Relaparotomy with choledochojejunostomy was considered, but the patient strongly preferred the nonsurgical procedure. That is why the magnet compression anastomosis was applied.

## Magnet Compression Anastomosis

As preparation for the procedure, the percutaneous transhepatic cholangiography drainage tube had been gradually dilated by 2-French-size every week from 8 French to 18 French. The papilla Vateri had been also enlarged by the endoscopic sphincterectomy, A minor tranquilizer (diazepam 10 mg) was given to the patient prior to the procedure. The

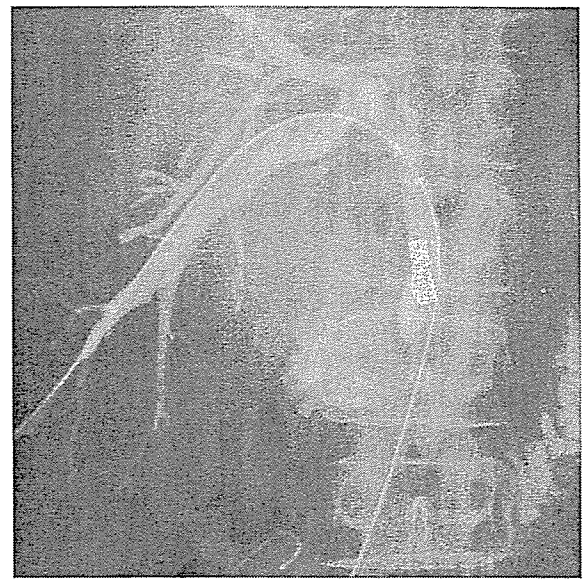

**Figure 5. Reanastomosis was established by day 42 after magnets were inserted.**

parent magnet (diameter 4 mm) attached to a guide with a covered tube was inserted into the common bile duct and placed at the common bile duct side of the stricture (Fig. 3). A 16-French-sized sheath tube was inserted through the dilated percutaneous transhepatic cholangiography drainage fistula, and the daughter magnet attached to a guide wire was inserted to the intrahepatic duct (Fig. 4A-B). The 2 magnets were immediately attracted toward each other, sandwiching the stricture (Fig. 4C) (Yamauchi

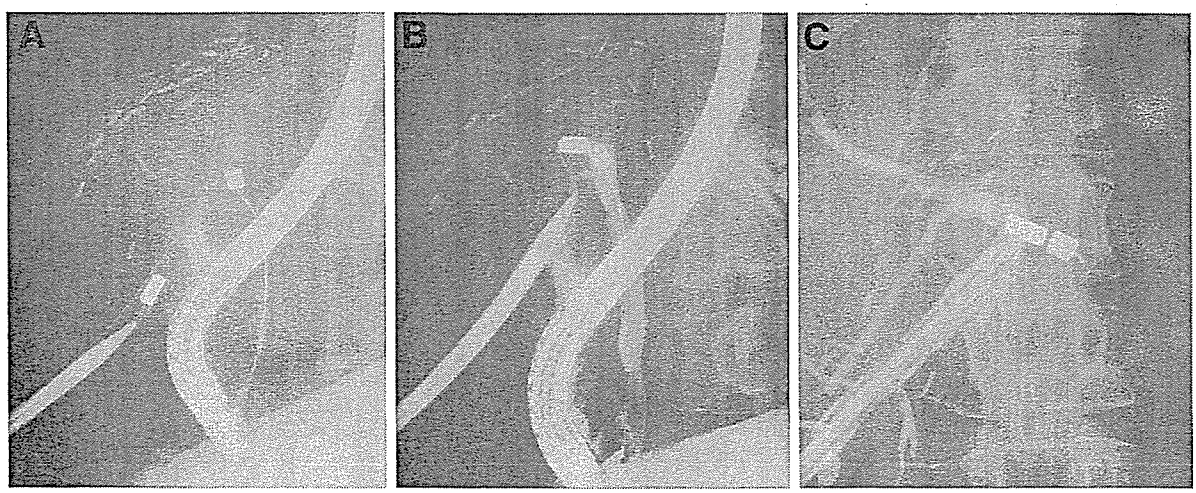


**Figure 4. Placing of the daughter magnet. (A) The daughter magnet attached to a guide wire was inserted through the sheath tube. (B) The daughter magnet was placed into the obstructed intrahepatic duct. (C) The 2 magnets were immediately attracted toward each other.**

procedure[1,2]). The sheath tube was removed and changed to the indwelling porous percutaneous transhepatic cholangiography drainage tube. Establishment of the reanastomosis was assured by day 42 with radiological examinations (Fig. 5). The indwelling drainage tube was pushed and was inserted down to the common bile duct through the anastomosed stoma, as the internal stent tube maintained the patency. The stent tube was removed 3 months later. There has been no recurrence of the stricture in the 15 months of follow-up after the creation of the new stoma.

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# Hepatitis B virus infection in lymphatic tissues in inactive hepatitis B carriers

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*Background/Aims*: **Hepatitis B virus (HBV) infection in extrahepatic tissues is controversial. To clarify whether episomal HBV can infect nonhepatic tissues, we investigated the molecular forms of HBV in the lymphatic cells of inactive HBV carriers who lacked viremia, thus avoiding contamination with HBV genomes originating from the viral particles present in the serum.**

*Methods*: **We assessed HBV genome, replicative forms, and viral integrants in the liver, serum, peripheral blood mononuclear cells (PBMC), and lymph nodes of 21 inactive HBV carriers who tested positive for antibodies against the HBV core antigen (anti-HBc).**

*Results*: **Of the 21 anti-HBc positive individuals, HBV-DNA was detected in liver samples of 15 (71.4%), in the lymph nodes of 11 (52.4%), and in PBMC of three (14.3%). However, none of the detected HBV genomes from lymphatic tissues included the replicative forms of HBV. In one case, integrated HBV was present in the lymphatic tissues and the host–viral junction was present in the intronic sequences of chromosome 17.**

*Conclusions*: **These data suggest that human lymphatic tissues cannot support viral replication in anti-HBc positive inactive HBV carriers, while retaining the viral genome as an integrated form.**



*Keywords*: **HBV; Latent infection; Occult HBV; PBMC; cccDNA; Lymph node**

## 1. Introduction

Hepatitis B virus (HBV) is a partially double-stranded DNA virus belonging to the Hepadnaviridae family [1,2]. Hepadnaviruses are characterized by their hepatotropic features and have a strong preference for infecting hepatocytes, although small amounts of hepadnaviral DNA is found in extrahepatic organs [2]. The existence of extrahepatic replication of HBV is controversial [3–15]. Several previous studies have suggested the presence of replicative intermediate forms of HBV in extrahepatic organs [4–6,14]. For example, viral mRNA and covalently closed circular DNA (cccDNA) were detected in peripheral blood mononuclear cells (PBMC) of highly viremic patients by PCR-based methods [14]. In contrast, others have demonstrated that human PBMC cannot be infected with HBV in vitro and in vivo [15]. Most of those studies tested the PBMC of HBV carriers who were positive for hepatitis B surface antigen (HBsAg) and/or HBV-DNA in the serum. However, in HBV carriers with circulating viral particles, the possibility that the detected viral genomes were attributed to viruses that had only adsorbed to the cells could not be completely excluded [15].

We recently demonstrated that occult HBV maintains persistent infection in the livers of individuals who have

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antibody to hepatitis B core antigen (anti-HBc), but not HBsAg without causing any clinical liver dysfunction [16]. Because HBV is frequently transmitted to liver transplant recipients from anti-HBc positive and HBsAg-negative donors, there is growing recognition that most anti-HBc positive healthy individuals have latent HBV infection in their liver tissues [16–21]. Moreover, we and other researchers have shown the reactivation of latent HBV infection in some leukemia patients under newly introduced immunosuppressive therapy or after bone marrow transplantation [22–26]. These findings suggest that most healthy individuals who are positive for anti-HBc have latent infections as the episomal form of HBV. Importantly, active HBV replication was found in the liver tissue of latent HBV carriers without any detectable HBV-DNA in their serum [16].

The aim of this study was to clarify whether episomal HBV infection can occur in extrahepatic tissues. We investigated the molecular forms of HBV in the lymph nodes and PBMC of latent HBV carriers who lacked viremia. We chose these subjects to exclude the possibility of contamination by HBV genomes originating from the viral particles present in the serum. Our findings showed that the HBV genome could be present in the lymph nodes and PBMC of latent HBV carriers, although these human lymphatic tissues lack the ability to support viral replication.

## 2. Patients and methods

### 2.1. Patients

Between April 5, 1996 and August 22, 2003, 724 patients underwent living-donor liver transplantation (LDLT) at Kyoto University. Before surgery, the liver function of all donors was examined by blood chemistry and serological analyses of HBV markers including HBsAg, antibodies to HBsAg, anti-HBc, hepatitis B e antigen(HBeAg), and antibodies to HBeAg using commercial enzyme immunoassay kits as described previously [27]. Of the original 724 patients, 103 donors (14.2%) were positive for anti-HBc and negative for HBsAg. From these 103 patients, the liver tissues, lymph nodes, PBMC and serum of 21 donors were available for further analyses. These 21 anti-HBc positive individuals included 10 men and 11 women, aged 24 to 63 years (mean age, 43.4 years). None had a history of liver dysfunction, blood transfusion, drug abuse, or family history of HBV infection. From the remaining 621 donors without any HBV markers, 10 were randomly selected as the negative control group (six men and four women). None of the donors enrolled in this study was positive for HBV-DNA in their sera at the time of operation. All subjects provided written informed consent and the study was conducted in accordance with the principles of the Declaration of Helsinki.

### 2.2. Tissue samples

Liver tissue samples were obtained at the time of transplantation, frozen immediately, and stored at −80 °C until use. Blood samples were obtained before surgery, and samples from the lymph node in the hepatoduodenal ligament were taken by biopsy during the operation. DNA was extracted from the liver tissue, lymph node, and serum using procedures as described previously [28]. DNA extraction from the PBMC was performed using the Gene Trapping by Liquid Extraction kit (Takara, Tokyo, Japan) according to the manufacturer's protocol.

### 2.3. PCR amplification of HBV-DNA

HBV-DNA was amplified by nested or semi-nested polymerase chain reaction (PCR) using AmpliTaq Gold (Perkin–Elmer Cetus, Norwalk, CT) [16]. Primer sets for amplification of the S, pre-S, Core(C) /pre-C, and X regions have been described previously [28]. We defined a sample as HBV-DNA positive when amplification products were detected in two or more of four regions in the same sample in three or more independent experiments. As a positive control, DNA samples were prepared from liver tissues of patients with hepatocellular carcinoma (HCC) who were positive for HBsAg. As negative controls, PCR was performed using DNA samples extracted from liver tissues of healthy donors without any HBV markers, PCR buffer without DNA, or water only.

### 2.4. Selective detection of cccDNA and pregenomic RNA of HBV

To detect the cccDNA forms of HBV-DNA, PCR amplification was performed using DNA samples treated with mung bean nuclease and primer sets specific for the X region spanning DR1 and DR2 across the gap of the relaxed circular DNA (rcDNA). Mung bean nuclease cleaves a part of the single stranded gap and triple stranded region selectively. Thus, the sequences around the DR region in HBV rcDNA are expected to be digested by this nuclease [29]. In contrast, the digestion with mung bean nuclease prior to PCR amplification does not affect the same region of cccDNA [16]. In addition, to enhance the efficiency of cccDNA amplification, cellular DNA samples were digested with EcoRI (5 U) at 37 °C for 2 h before the PCR analysis [30]. Isolation of total RNA from lymphatic and liver tissues, RT-PCR, and southern blotting analyses were performed as described previously [16].

### 2.5. Detection of the integrated form of HBV-DNA

To discriminate the integrated viral DNA from the episomal HBV-DNA forms, the host genomic DNA (high molecular weight fraction; HMW) was separated from the low molecular weight fraction by applying the modified alkali-lysis procedure used to isolate plasmid DNA, as previously described [16]. Inverse-PCR is based on the digestion of DNA with certain restriction enzymes and circularization of cleavage products before amplification using primers synthesized in the opposite orientation to those normally employed for PCR [31–34]. As amplification of the S region of HBV-DNA was found to be the most sensitive among the four sets of primers for the HBV genome, selective digestion of the HBV-S region was performed using the restriction enzyme DdeI or RsaI followed by the amplification of this fragment using inversely designed primer sets specific for the S region. Accordingly, 4 μg of extracted DNA was digested with DdeI or RsaI in a total volume of 50 μL at 37 °C for 4 h. After confirmation of complete digestion by agarose gel electrophoresis, the enzyme was heat inactivated at 70 °C for 15 min. After the purification of the digested DNA using a PCR clean-up system (Promega, Madison, WI), samples were incubated with T4 DNA ligase (New England BioLabs, Beverly, MA) at 16 °C for 6 h. Finally, circularized DNA was used as the template for PCR amplification. The primer sets used for inverse-PCR amplification were as follows: R-HBS1.5′-GGGTCCTAGGAATCCTGAT-3′: R-HBR1.5′-GTATGT-TGCCCGTTTGTCCT-3′: R-HBS2.5′-GTCAACAAGAAAAACCC-CGC-3′: R-HBR2.5′-GCCTCATCTTCTTGTTGGTTC-3′: Equal amounts of restriction enzyme-digested but noncircularized DNA were used as a negative control.

## 3. Results

### 3.1. The presence of HBV-DNA in lymph nodes and PBMC of anti-HBc positive latent HBV carriers

We first examined whether HBV-DNA was present in the serum, liver, lymph node, and PBMC samples of individuals who were positive for anti-HBc but negative for HBsAg

using primers specific for the S, pre-S, pre-C/C, and X regions. The sensitivity of our nested PCR analysis used in this study has been described previously [16]. As shown in Table 1, amplification of HBV-DNA was not observed in the serum of anti-HBc positive individuals using any primer sets. In contrast, the liver tissues of 15 of the 21 (71.4%) anti-HBc positive donors were positive for HBV-DNA. These data are consistent with previous data indicating that the anti-HBc positive healthy individuals have latent HBV infection in the liver [16–21]. The lymph node samples of 11 of the 21 (52.4%) anti-HBc positive individuals were also HBV-DNA positive in the three repeated assays (Table 1). Moreover, three individuals positive for anti-HBc but negative for HBsAg also had HBV-DNA in their PBMC. These three were also positive for HBV genomes in their liver, although they lacked viral DNA in their lymph nodes from the hepatoduodenal ligament. HBV genomes were not detected in any of the liver or serum samples of donors without HBV-related serological markers. These findings suggest the presence of HBV genome in lymphatic tissues of individuals who have latent HBV infection in the liver.

### *3.2. Analyses of the replicative form of HBV in the lymphatic tissues*

To clarify whether HBV infection was maintained as an episomal form in the extrahepatic tissues, we examined the presence of HBV replicative forms, including cccDNA and intermediate RNA [35,36], in the lymph nodes of five anti-HBc positive donors who were positive for HBV genome sequences in their lymphatic tissues. For the selective detection of the cccDNA form of HBV, we performed cccDNA-specific PCR amplification accompanied by mung bean nuclease treatment (Fig. 1A) [16,29]. We first confirmed that a faint signal derived from the X region in the serum sample with a high level of HBV-DNA titer had completely disappeared after the nuclease digestion, whereas the S sequences were amplified in the same sample after the treatment with the endonuclease (Fig. 1B). This effect of endonuclease indicated the specificity of the digestion method for rcDNA in virions and agreed with previous reports [15,29]. As shown in Fig. 2A, both the liver and the lymph node tissues of all five anti-HBc positive donors were positive for HBV-DNA by conventional PCR assay using a primer set for the S region, which is conserved in both cccDNA and rcDNA molecules. Selective amplification of cccDNA detected HBV-derived cccDNA-specific bands of the expected size (658 bp) in the liver tissue of all five donors. In contrast, the amplified products originating from HBV-cccDNA were detected in none of the lymph node samples of these five donors in three replicate assays. Similar results were obtained by highly sensitive amplification of cccDNA by PCR accompanied by a single cut of cccDNA by EcoRI treatment (Fig. 2B). To further examine whether active transcription and replication were present in the lymphatic tissues, we performed RT-PCR followed by Southern blotting assay to detect the pregenomic

**Table 1**
**Serological status and the results of PCR amplification of HBV-DNA in the various tissues of anti-HBc positive donors**

| Case number | Sex | Age (year) | HBsAg/ anti-HBs | anti-HBc | HBeAg/ anti-HBe | HBV-DNA | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | LN | Liver | Serum | PBMC |
| 1 | F | 45 | −/+ | + | −/+ | + | + | − | − |
| 2 | M | 42 | −/+ | + | −/+ | + | + | − | − |
| 3 | F | 48 | −/+ | + | −/+ | − | − | − | − |
| 4 | F | 50 | −/+ | + | −/+ | − | − | − | − |
| 5 | M | 35 | −/+ | + | −/+ | − | + | − | − |
| 6 | F | 54 | −/− | + | −/− | + | + | − | − |
| 7 | M | 52 | −/+ | + | −/− | − | − | − | − |
| 8 | F | 35 | −/− | + | −/− | − | − | − | − |
| 9 | M | 40 | −/+ | + | −/+ | + | + | − | − |
| 10 | M | 37 | −/+ | + | −/+ | + | + | − | − |
| 11 | M | 63 | −/+ | + | −/− | − | − | − | − |
| 12 | F | 34 | −/− | + | −/− | + | − | − | − |
| 13 | F | 42 | −/+ | + | −/+ | + | + | − | − |
| 14 | F | 38 | −/+ | + | −/+ | − | + | − | − |
| 15 | M | 29 | −/+ | + | −/+ | − | + | − | + |
| 16 | M | 53 | −/− | + | −/+ | + | + | − | − |
| 17 | M | 37 | −/+ | + | −/+ | + | + | − | − |
| 18 | M | 48 | −/+ | + | −/− | − | + | − | + |
| 19 | M | 24 | −/+ | + | −/− | + | + | − | − |
| 20 | M | 53 | −/+ | + | −/− | − | + | − | + |
| 21 | F | 52 | −/+ | + | −/+ | + | + | − | − |

LN, lymph node; PBMC, peripheral blood mononuclear cells; HBsAg, hepatitis B surface antigen; anti-HBs, antiboby to HBsAg; anti-HBc, antibody to hepatitis B core antigen; HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg.

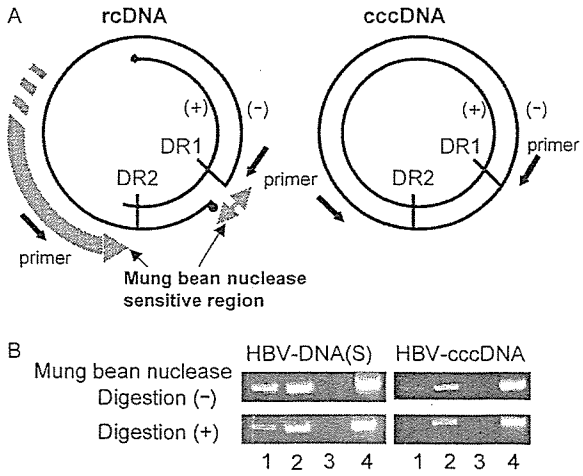


**Fig. 1. Detection of HBV cccDNA using mung bean nuclease digestion followed by PCR amplification. (A) The structure of HBV cccDNA and relaxed circular DNA (rcDNA), and mung bean nuclease sensitive region in rcDNA. Mung bean nuclease cleaves a part of the single stranded region in the minus strand. PCR primers for the detection of cccDNA were not expected to amplify the HBV rcDNA sequences across the gaps after the digestion with mung bean nuclease. (B) PCR amplification of the S region (left panel) and X region (right panel) after the selective digestion of HBV rcDNA molecules (DNA extracted from the serum of HBsAg-positive case). The digestion of mung bean nuclease was performed prior to nested-PCR with primers across the nick region of rcDNA (X region). Upper panel, mock digestion; lower panel, mung bean nuclease digestion; lane 1, serum of HBsAg-positive case; lane 2, liver tissue of HBsAg-positive case; lane 3, liver tissue of donor without any HBV serological markers; lane 4, expression plasmid of the HBV-DNA encoding the S and X region.**

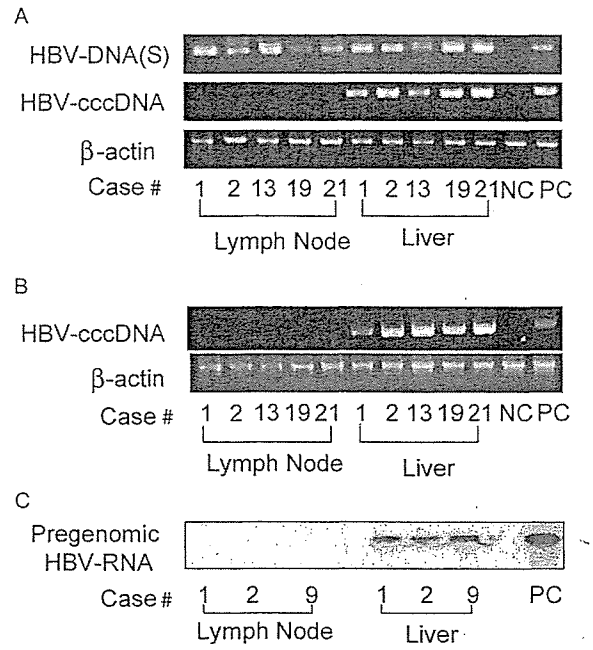


**Fig. 2. Detection of HBV cccDNA and pregenomic RNA in lymph nodes and liver tissues of anti-HBc positive healthy donors. (A) HBV-DNA was amplified using primer sets for the S region (upper panel) or the primers specific for cccDNA after the selective digestion of HBV rcDNA molecules by mung bean nuclease (middle panel). The figure shows representative results of PCR amplification using DNA samples extracted from the lymph nodes and livers of donors #1, #2, #13, #19, and #21. NC, liver tissue of donor without any HBV serological markers; PC, liver tissue of HBsAg-positive case. (B) DNA samples extracted from the lymph node and liver tissues were digested with EcoRI to linearize the cccDNA molecule, followed by PCR amplification using the primers specific for cccDNA, as described above. The figure shows representative results of PCR amplification using DNA samples extracted from the lymph nodes and livers of donors #1, #2, #13, #19, and #21. (C) Total RNA was extracted from the lymph node and liver tissues of three healthy donors positive for anti-HBc. One-step RT-PCR for amplification followed by Southern blotting analysis targeting the S region of HBV RNA was carried out. Total RNA extracted from HBsAg-positive liver tissue was used as a positive control. Lanes 1–3, lymph nodes of donors #1, #2, and #9; lanes 4–6, liver tissues of donors #1, #2, and #9; lane 7, HBsAg-positive liver.**

HBV-RNA. Total RNA extracted from both the lymph node and the liver was available in three anti-HBc positive donors for further analyses. As shown in Fig. 2C, the positive signals at the expected size representing HBV-RNA were detectable in the liver tissues of all three anti-HBc positive donors tested. However, no amplification of the HBV-RNA was observed from the total RNA samples extracted from the lymph nodes of these individuals (Fig. 2C). These findings suggested that the HBV genomes detected in the lymph nodes of anti-HBc positive individuals did not contain the replicative form of HBV.

### *3.3. The presence of the integrated form of HBV in the lymphatic tissues*

As HBV replicative forms were not detected in lymphatic tissues of anti-HBc positive individuals, we reasoned that the HBV genome might be present as an integrated form in the extrahepatic tissues. To address this question, we separated the DNA from the lymph node and liver tissues of anti-HBc positive individuals into two fractions according to their molecular sizes, as described previously [16]. Human β-actin and p53 gene sequences were amplified from the HMW fractions of all subjects, revealing that this fraction contained host chromosomal DNA (Fig. 3A for β-actin, data not shown for p53). In the liver tissues, two of six cases were positive for HBV-DNA in the HMW host chromosomal DNA fraction (Fig. 3A). Interestingly, HBV sequences were detected in three of six HMW fractions of DNA samples extracted from lymphatic tissues (lymph nodes of cases #13, #17, and #21), suggesting that the HBV genome in the lymph nodes of anti-HBc positive individuals is most likely present as an integrated form.

To further examine the presence of HBV integration in lymphatic tissues, we performed inverse-PCR-based amplification of HBV-DNA to identify the host-viral junction sequences. We selected the S region of HBV as the target sequences of inverse-PCR analyses because amplification of this region shows the highest sensitivity for detecting HBV sequences in lymphatic tissues of anti-HBc positive

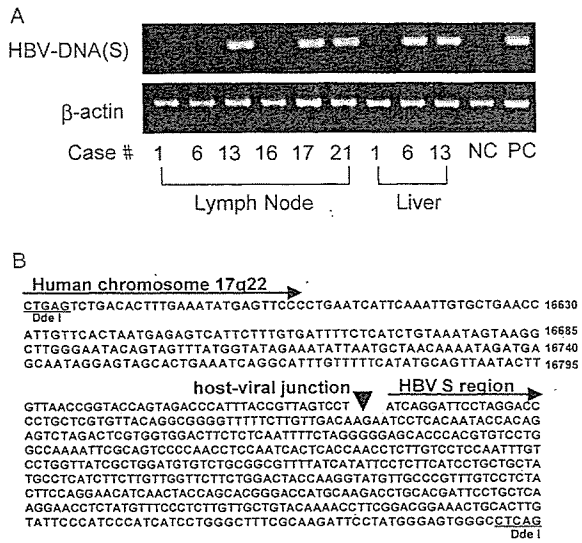


**Fig. 3. Detection of HBV integration in the lymph nodes of anti-HBc positive donors. (A) Detection of the HBV genome in a high molecular weight (HMW) fraction of extracted DNA. HMW fractions of extracted DNA from the lymph nodes and liver tissues of anti-HBc positive donors (donors #1, #6, #13, #16, #17, and #21) were separated by a modification of alkali-lysis procedure, followed by amplification of the S region of HBV-DNA using nested PCR (upper panel). To show the presence of the genomic DNA in the HMW fraction, amplification of β-actin gene was carried out using the same samples as templates (lower panel). (B) HBV-host junction sequence in the extracted DNA from the lymph node of donor #21. Host flanking DNA was located in the sequence of chromosome 17 (GenBank gi: 15281290). The HBV-DNA sequence covers from nucleotide 43 of the surface gene. The restriction enzyme DdeI restriction sites are underlined.**

individuals (Table 2). In the lymph nodes of one of three cases (case #21), one visible DNA signal was observed, which showed the different sizes from the common fragments found in all three samples, suggesting a unique HBV integrant in the inversely-amplified PCR product. The nucleotide sequence analysis in this specifically amplified fragment contained both viral and human genome sequences. As shown in Fig. 3B, the human DNA sequence located in chromosome 17q22 was identified as a host flanking sequence, which was connected with the S region of HBV-DNA. Together, these data suggest the presence of HBV integration in host chromosomal DNA of lymph nodes of anti-HBc positive latent HBV carriers.

**Table 2**
**Detection of HBV-DNA in the lymph node and liver tissues of 21 anti-HBc positive donors**

| | Number of HBV-DNA positive subjects | | | |
|---|---|---|---|---|
| | S | pre-S | pre-C/C | X |
| LN | 12/21 | 11/21 | 4/21 | 0/21 |
| Liver | 15/21 | 13/21 | 10/21 | 6/21 |

LN, lymph node; S, surface; pre-C/C, pre-core/core.

## 4. Discussion

Although several previous studies have reported the presence of HBV genomes in PBMC, it is still controversial whether HBV exists as an episomal form and replicates in extrahepatic tissues [3–15]. In this respect, it should be noted that most previous studies examined the PBMC of HBV carriers positive for HBsAg, who normally have HBV-DNA in their serum. Thus, it is possible that the detected viral genomes in PBMC were derived from viruses circulating in the serum or adsorbed to the PBMC. For example, Kock et al. reported that HBV particles can bind tightly to various types of cells so that attached viruses cannot be washed away and remain detectable in culture for many days [15]. To exclude the possibility of contamination of the circulating HBV-DNA, we examined PBMC and lymph nodes from LDLT donors positive for anti-HBc but negative for HBsAg. We have previously shown that HBV-DNA is rarely detected in the serum of these donors, despite their latent infection with the episomal form of HBV which is associated with ongoing viral replication in the liver [16,21]. Therefore, we first confirmed by PCR that HBV-DNA could not be detected in the serum in the anti-HBc positive donors to exclude the possible contamination by serum HBV-DNA in extrahepatic tissues. Our data demonstrated clearly that HBV-DNA is present in lymphatic tissues and PBMC of anti-HBc positive latent HBV carriers. Our data agree with those from a previous report showing identification of HBV-DNA sequences in PBMC of liver transplant recipients who were serum HBV-DNA negative by PCR analysis [37].

Although HBV-DNA could be detected in both the lymph nodes and PBMC of our subjects, we found no evidence of viral replication in tissues of any individual with latent HBV infection. The lack of cccDNA and pregenomic forms of RNA in the lymph nodes and PBMC strongly suggests that HBV can exist in lymphatic tissues without viral replication or proliferation. These findings are consistent with previously reported clinical outcomes in liver transplant patients, in which none of the recipients who were positive for anti-HBc but negative for HBsAg and who had HBV genomes in their liver tissues had acquired HBV reactivation after total removal of the infected liver through liver transplantation [28]. It may be emphasized that these recipients generally receive intense immunosuppressive therapy; nevertheless, none of them developed HBV reactivation after LDLT. Our results contrast with other previous studies [4–6,14]. For example, cccDNA and pregenomic forms of RNA were detected in PBMC of patients negative for HBsAg but positive for HBV-DNA in the serum [14]. As discussed above, the discrepancies between our results and those from other reports may be related to the presence or absence of circulating viral particles in the patient's serum. A considerable number of viral particles circulating in the serum was shown to contain viral RNA rather than DNA, suggesting that previous

observations of the presence of HBV infection in PBMC could be explained by adsorbed virus [15]. Since Soussan et al. showed recently that a singly spliced HBV-RNA encodes a novel HBV protein in vivo [38], there is room for further investigation to clarify whether HBV-spliced mRNA can be present in extrahepatic tissues.

Our findings also suggest the presence of integrated HBV-DNA in lymph node samples of anti-HBc positive carriers. To obtain direct evidence of HBV integration into host genomes, we used an inverse-PCR-based method using two pairs of inverse primers around the S region of HBV-DNA. It has been reported that many of the viral-host junctions cluster near the DR1 region of HBV sequences [39,40]. However, we found that amplification of the S region was the most sensitive method to detect the minimum amount of HBV genome present in lymphatic tissues of anti-HBc positive individuals. We targeted this region in the inverse-PCR analyses, and identified HBV integrants and flanking host sequences located in chromosome 17q22 in the lymph node of one case with anti-HBc. Our data agree with those in recent reports that observed viral integration in PBMC samples from patients with HBV-related acute and chronic liver disease [41,42]. However, detection of integrated viral DNA by inverse-PCR does not imply the clonal expansion of HBV in lymph nodes, and the question of whether the viral integration reflects the prior infection of proliferating bone marrow cells or the stimulation of PBMC expansion is still unanswered [3,42].

In conclusion, our data suggest that lymph nodes and PBMC do not support active replication of HBV in latent HBV carriers who are positive for anti-HBc but negative for HBsAg, in whom the ongoing viral replication occurs in the liver. Instead, HBV may persist as integrated forms in their extrahepatic tissues. Further analysis is needed to determine the molecular status of HBV infection in extrahepatic tissues of highly viremic HBV carriers.

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# Duct-to-duct biliary reconstruction in pediatric living donor liver transplantation

Okajima H, Inomata Y, Asonuma K, Ueno M, Ishiko T, Takeichi T, Kodera A, Yoshimoto K, Ohya Y. Duct-to-duct biliary reconstruction in pediatric living donor liver transplantation.
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Abstract: The results of duct-to-duct biliary reconstruction in six pediatric patients who received a living donor liver transplant aged from 2 months to 11 yr old are reported. The graft was either entire or a part of the left lateral segments. The orifice of the bile duct of the graft was anastomosed to the recipients' hepatic duct in an end-to-end fashion by interrupted suture using 6–0 absorbable material. A transanastomotic external stent tube (4 Fr) was passed through the stump of the recipients' cystic duct. Mean time for reconstruction was 24 min. All the recipients survived the operation and reinitiated oral intake on postoperative day 3. There were no early biliary complications. One 5-yr-old boy suffered from an anastomotic stenosis 9 months after transplantation. He underwent re-anastomosis by Roux-en Y (R-Y) procedure and recovered uneventfully. Duct-to-duct anastomosis in pediatric living donor liver transplantation has benefits while the complication rate is comparable to R-Y reconstruction.

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Key words: living donor liver transplantation – pediatric transplantation – duct-to-duct anastomosis – biliary reconstruction

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In pediatric liver transplantation, both cadavericand live-donated cases, R-Y hepatico-jejunostomy is often performed for biliary reconstruction because of the prevalence of BA. With the recent expansion of LDLT to adults, DD anastomosis for biliary reconstruction in LDLT is becoming more common (1–4). In pediatric LDLT even in the patients without BA, the main procedure is still the R-Y. However, there are benefits to perform a DD anastomosis, such as not having to manipulate the gastrointestinal tract, a short operative time and easy assess to the anastomosis using retrograde catheterization with an endoscope. We report our results of DD biliary reconstruction in six pediatric patients, including a young infant.

## Patients and methods

Six children (four boys and two girls, aged from 2 months to 11 yr old) underwent LDLT with DD biliary reconstruction (Table 1). The 2-month-old patient, a girl, received a part of the left lateral segment from her mother who had an incompatible blood type. The others received complete left lateral segments from one of their parents with identical blood type (Table 2).

Abbreviations: BA, biliary atresia; DD, duct-to-duct; HTK, Histidine-tryptophan-ketoglutarate; LDLT, living donor liver transplantation; PTCD, percutaneous transhepatic cholangio-drainage; R-Y, Roux-en Y.

### Operative procedure

The biliary tree of the donor was evaluated by preoperative three-dimensional CT cholangiography and intraoperative cholangiography. HTK solution was used for perfusion and preservation of the graft. Each graft had one biliary tract orifice, which was 2–4 mm in diameter. During the hepatectomy of the recipient, the bile duct was transected as proximal as possible, usually, at the level of each hepatic duct. The gallbladder was resected, but a long segment of the cystic duct was left as the entry site of the transanastomotic stent tube. After completion of the anastomosis of hepatic vein and portal vein, the graft was re-perfused before the hepatic artery was microsurgically reconstructed. The connective tissue around the recipient's common duct was preserved as much as possible, although the whole supra-pancreatic portion was dissected. The anastomotic orifice of the recipient's side was chosen according to the size of the graft bile duct. The common bile duct was used for three elder patients, and a branch patch was used for three younger patients. The blood supply of the stump of the common bile duct was confirmed by observing bleeding when making a small cut at the tip of the stump of the bile duct. Before

Table 1. Recipients' characteristics

| Case | Age | Sex | BW (kg) | Disease | Follow-up period | Complications |
|---|---|---|---|---|---|---|
| 1 | 2 mo | F | 5 | FHF[a] | 19 mo | (-) |
| 2 | 1 yr | M | 10 | Byler's disease | 2 mo | (-) |
| 3 | 3 yr | M | 9 | Byler's disease | 36 mo | (-) |
| 4 | 5 yr | M | 14 | Hepatoblastoma | 24 mo | stenosis |
| 5 | 7 yr | M | 26 | Wilson's disease | 3 mo | (-) |
| 6 | 11 yr | F | 24 | Wilson's disease | 6 mo | (-) |

[a] FHF, fulminant hepatic failure

Table 2. Details of operative data

| Case | Type of graft | GW/RBW ratio[a] | Diameter of bile duct | Time for reconstruction |
|---|---|---|---|---|
| 1 | monosegment | 5.20 | 2.5 mm | 25 min |
| 2 | left lateral | 2.10 | 3.0 mm | 35 min |
| 3 | left lateral | 2.60 | 3.0 mm | 35 min |
| 4 | left lateral | 1.07 | 3.0 mm | 20 min |
| 5 | left lateral | 1.30 | 3.0 mm | 35 min |
| 6 | left lateral | 0.76 | 3.5 mm | 18 min |

[a] GW/RBW ratio, graft weight/recipient body weight ratio

the anastomosis, a 4 Fr stent tube was guided through the remnant cystic duct and out the distal end of the recipient's bile duct (Fig. 1). End-to-end anastomosis was carried out with 6–0 absorbable sutures using the interrupted technique. The sutures were tied outside of the lumen. After completion of the posterior wall, the stent tube was inserted 2 cm into the graft. The anastomosis was then completed and the tube passed out through the abdominal wall. The stent tube was kept in place for at least 3 months.

### Postoperative result

The mean time for reconstruction was 24 min and ranged from 18 to 35 (Table 2). All six recipients recovered from the transplantation uneventfully. Oral intake was started on

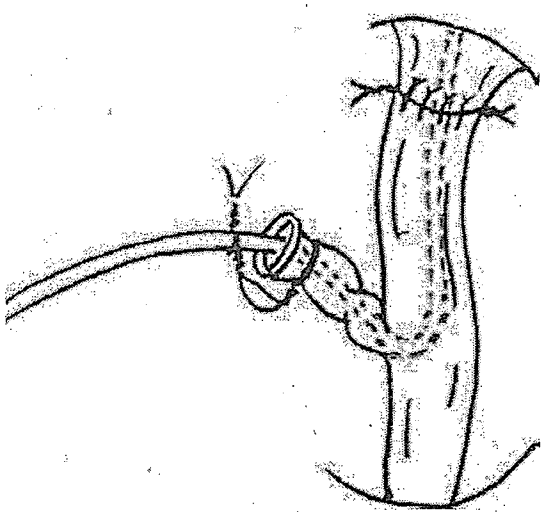

*Fig. 1.* A 4 Fr stent tube was inserted through the remnant cystic duct and into the recipient bile duct. End-to-end anastomosis was carried out with 6–0 absorbable suture using the interrupted technique.

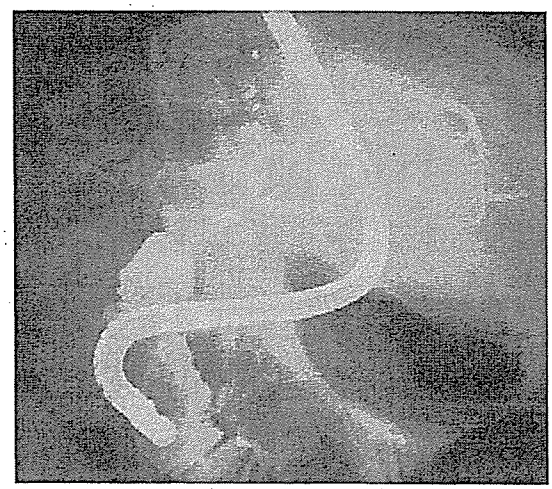

*Fig. 2.* Endoscopic retrograde cholangiography showed anastomotic stenosis on 5-yr-old boy at 9 months after transplantation.

postoperative day 3 in all cases. There were no early biliary complications. A 5-yr-old boy suffered from an anastomotic stenosis at 9 months after transplantation. In this recipient, the stent tube was mistakenly removed at 4 wk after the transplant, instead of 3 months in our protocol. Anastomotic stenosis was diagnosed by endoscopic retrograde cholangiography (Fig. 2). An attempt at balloon dilatation by endoscopic intervention failed because the guide wire could not be placed though the stenosis. Intervention through the PTCD was also performed, but failed. Therefore, re-anastomosis by R-Y was performed. He recovered uneventfully and is doing well 30 months after transplantation. None of the other patients showed signs of early or late biliary complications with a mean follow-up period of 17 months.

## Discussion

The incidence of bile duct complications in pediatric living related liver transplantation using a R-Y reconstruction is reported as high as 14% (5). Bile leakage because of anastomotic dehiscence in R-Y reconstruction is particularly serious as it may cause fatal peritonitis. Theoretically, DD anastomosis has benefits when it is compared with the R-Y procedure. These include no need for intestinal manipulation, a short operative time, a more physiologic bilioenteric continuity, less severity in case of biliary leakage and an easy assess to the anastomosis through retrograde catheterization using an endoscope. More than 75% of pediatric LDLT patients have BA and R-Y is the most common procedure in biliary reconstruction. However, in the patients with metabolic diseases or fulminant hepatic failure, the bile duct is available for reconstruction. In this series, all the children were able to start oral intake on postoperative day 3, a few days earlier than with the R-Y procedure in our institution. The mean time for D-D

reconstruction was 27 min. Because it was not necessary to make a R-Y limb, operative time was shorter. There were no early biliary complications in this series. In case of major leakage at the hepaticojejunostomy, external diversion of R-Y limb may be necessary to avoid a fatal outcome. We consider that postoperative care without such a concern is a great benefit of D-D anastomosis.

A drawback of DD reconstruction is the higher risk of postoperative stricture, which is mainly caused by tension around the anastomotic site or by poor blood supply to the recipient bile duct (1, 2). The blood at the tip of the recipient's bile duct is provided by the intramural capillary network connected to the original artery in the intra-pancreatic portion (6). Therefore, significant tension or stretch of the bile duct may cause ischemia at the tip, and lead to subsequent stricture of the anastomosis. Release of the tension by the longer dissection is important as well as the preservation of the surrounding connective tissue. Another concern with DD reconstruction is the fragility. In the present series, the diameter and the thickness of the common hepatic duct of the 2-month-old girl was compatible with those of the graft bile duct.

In our series, the 5-yr-old boy experienced anastomoric stricture. Although re-anastomosis by R-Y was necessary in this case, it could be diagnosed by retrograde cholangiography with an endoscope which could not have been possible in a R-Y. The possibility of including an endoscope for diagnosis and treatment of biliary complications, is a definite advantage in DD anastomosis.

The use of the of the stent in the biliary reconstruction after liver transplantation has long been a debatable issue, and a clear conclusion is not available. A disadvantage to a prolonged external stent, is the mild disturbance of the quality of life. When there is poor blood supply or significant tension at the anastomosis, a longer period of stent placement may sustain a patient lumen in the process of healing, although there are no scientific data to support a prolonged stent placement. The fact that only one case of a late complication in this series was the patient who lost the stent earlier than scheduled may suggest the benefit of a prolonged stent placement, although, again, no conclusive data are available.

In conclusion, DD anastomosis has benefits and the results are comparable to R-Y reconstruction in pediatric LDLT recipients.

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# Feasibility of Using the Cystic Duct for Biliary Reconstruction in Right-Lobe Living Donor Liver Transplantation

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**Duct-to-duct biliary reconstruction has been introduced in adult living donor liver transplantation (LDLT). In right-lobe grafts, however, the presence of two or three separated bile duct orifices is not rare and makes an alternative approach for reconstruction necessary. We used the cystic duct for one of the anastomoses in biliary reconstruction for 5 right-lobe living donor liver transplants with two separated ducts. Before the anastomosis, the inside lumen of the cystic duct was straightened with a metal probe. Two external drainage tubes were placed in all recipients, and posttransplant cholangiography through the tubes approximately one month after transplantation showed no leakage or stricture at any of the anastomotic sites. The drainage tubes were removed between 17 and 37 weeks after transplantation. All of the patients except one who died of chronic rejection have been doing well without any late biliary complications during follow-up periods ranging from 10 to 28 months after transplantation. In conclusion, our results indicate that biliary reconstruction using the cystic duct is feasible and safe for living donor liver transplantation and that external drainage tubes may be effective for prevention of complications. (*Liver Transpl 2005;11:1431-1434.*)**

Biliary tract complications are some of the most frequent problems after living donor liver transplantation (LDLT).[1] In an effort to prevent the serious complications that can occur in hepaticojejunostomy, duct-to-duct biliary reconstruction, especially in right-lobe LDLT, has recently become widely accepted.[2-5] The biliary tree of right-lobe grafts, however, features many anatomical variations, with absence of the right hepatic duct reported in as many as 26% of 110 resected livers.[6] In many instances, two or three bile-duct reconstructions may therefore be necessary, and modified techniques for these situations have been employed.[7] In situations where the distance between orifices permits it, two orifices have been transformed into one. In other cases, the right and left hepatic ducts have been used for separate anastomoses. Furthermore, if the two orifices are far apart, combining a hepaticojejunostomy with a duct-to-duct anastomosis has been attempted.[2] In these special cases, a modified technique using the cystic duct for the reconstruction of one of the separated orifices of the bile duct is needed. Sue et al. reported successful use of the cystic duct for biliary reconstruction in three cases.[8] However, only two of the three cases underwent a double-biliary anastomosis, and the follow-up periods were relatively short. In the study presented here, we review 5 LDLT transplants performed with a double-biliary reconstruction technique that uses the cystic duct and the right hepatic duct for two separate anastomoses as well as two external biliary stent tubes. The efficacy of biliary reconstruction using the cystic duct with an external drainage tube was assessed during both short- and long-term follow-up periods after LDLT.

## Patients and Methods

Between September 2000 and December 2004, 80 cases of living donor liver transplantation were performed at our institution. Left-lobe grafts were used in 45 cases and right lobes in 30. The remaining 5 cases underwent domino transplantation with whole-liver grafts. Duct-to-duct biliary reconstruction was performed for all of the right-lobe graft cases, 15 of whom had one hepatic duct, 15 had two, and none had triple orifices. At the back table, 8 double orifices were transformed into single ones. The other 7 cases needed two separate reconstructions, and for two of them the right and left hepatic branches of the recipient's hepatic duct could be used. The two orifices of the anterior and posterior branches of the graft bile duct were too far apart in the remaining 5 cases; however, the recipient's cystic duct was used for reconstruction of one of the orifices (Fig. 1).

The right and left hepatic ducts were dissected at the hepatic hilum during the recipient operation and cut separately to preserve the two small orifices of the bile duct. If the information obtained from the preoperative biliary computed tomography (CT) scan or intraoperative cholangiography suggested the presence of two separate orifices of the graft bile duct, the cystic duct including the neck of the gall bladder was preserved as long as possible during the recipient operation.

**Abbreviation:** LDLT, living donor liver transplantation.

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The stump of the cystic duct was tapered down to the size of the graft bile duct before reconstruction. We usually confirmed the arterial blood supply at the stump of the cystic duct by cutting the wall of the edge. Before the anastomosis, the spiral form of the inside lumen of the cystic duct was straightened by gently prodding it with a metal probe until it was dilated and entirely passable. Reconstruction was performed in an end-to-end fashion between the graft bile duct and the recipient hepatic duct or cystic duct with interrupted 6-0 PDS suture. Two 4-French polyethylene tubes, inserted via the left hepatic duct in one, via the cystic duct in one, and via the common bile duct in the other cases, were used for external drainage (Fig. 2). Cholangiography through both tubes was performed approximately one month after transplantation. Biliary complications were diagnosed clinically, biochemically, or radiologically.

## Results

Patients' demographics are shown in Table 1. There were 5 males and one female with an age range from 18 to 58 years. The underlying diseases were hepatitis C with hepatocellular carcinoma in two patients, and hepatitis B, Caroli disease, and fulminant hepatic failure in one each. The distance between the orifice of the anterior branch and the posterior branch in the right-lobe grafts of all the patients was more than 1.5 cm. The cystic duct was anastomosed to the anterior branch in three and to the posterior branch in two. The right hepatic duct was used for all other reconstructions. There were no clinical signs of leakage during the early postoperative periods, and cholangiography performed around one month after transplantation did not show either leakage or stricture of the anastomotic sites (Fig. 3). The external biliary drainage tubes were removed between 17 and 37 weeks after transplantation. Patient 1 died of chronic rejection 13 months after transplantation without any signs of biliary complications throughout the entire postoperative course. Although

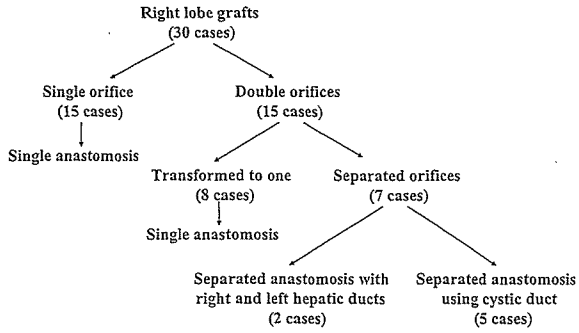


**Figure 1. Modalities of duct-to-duct reconstructions for right-lobe grafts.**

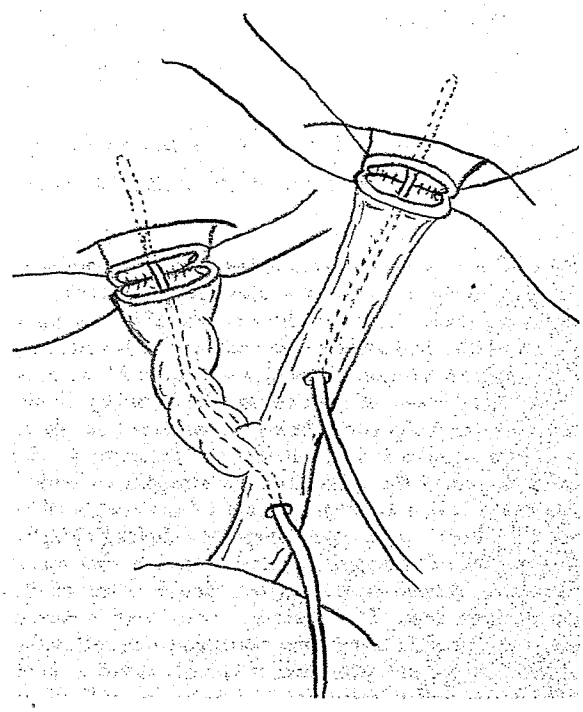

**Figure 2. Schema of separated anastomosis of two bile ducts using the cystic duct and the placement of two external drainage tubes.**

patients 2 and 3 suffered from recurrence of hepatitis C as evidenced by abnormal biochemical findings, they recovered with the aid of interferon and ribavirin therapy. Patient 5 exhibited hyperbilirubinemia due to rejection but recovered after administration of silorimus. Follow-up periods ranged from 11 to 28 months (median 14 months). All patients but one are alive and doing well without any clinical signs of biliary complications more than 10 months after transplantation.

## Discussion

During the early stages of LDLT development, the biliary system usually was reconstructed by means of a Roux-en-Y hepaticojejunostomy. Later, duct-to-duct reconstruction was introduced, especially for adult LDLT, because of its advantages over hepaticojejunostomy, such as shorter operation time and simpler biliary reconstruction. Furthermore, in the event of leakage at the anastomotic site, serious complications in duct-to-duct reconstructions are rare in contrast to hepaticojejunostomy, where division of the Roux-en-Y limb may be necessary. Therefore, duct-to-duct reconstruction for adult LDLT has been widely accepted in conjunction with the increase in right-lobe LDLT. However,

**Table 1.** Patient Demographics

| Patient | Age/Gender | Disease | Mode of Biliary Reconstruction | Follow-up Periods | Results (Biliary Complication) |
|---|---|---|---|---|---|
| 1 | 39/M | HBV | Ant-CD, Post-RHD | 13 months | Dead* (none) |
| 2 | 53/M | HCV/HCC | Ant-RHD, Post-CD | 28 months | Alive (none) |
| 3 | 58/M | HCV/HCC | Ant-CD, Post-RHD | 14 months | Alive (none) |
| 4 | 18/M | Caroli disease | Ant-RHD, Post-CD | 13 months | Alive (none) |
| 5 | 44/F | FHF | Ant-CD, Post-RHD | 10 months | Alive (none) |

Abbreviations: HBV, hepatitis B; HCV, hepatitis C; HCC, hepatocellular carcinoma; FHF, fulminant hepatic failure; Ant, anterior branch of the right hepatic duct of the graft; Post, posterior branch of the right hepatic duct of the graft; CD, cystic duct of the recipient; RHD, right hepatic duct of the recipient.
*Patient 1 died of chronic rejection.

because there are many variations in bile-duct branches, especially in the case of right-lobe grafts, some modifications are needed for multiple bile-duct reconstructions. Sometimes hepaticojejunostomy combined with duct-to duct reconstruction should be considered for reconstruction of two bile ducts that are too far apart. One of the solutions for this particular problem is to use the cystic duct for one biliary anastomosis. Although use of the cystic duct for duct-to-duct biliary reconstruction has been previously reported, there are few descriptions of this technique as an alternative to a two-bile-duct reconstruction, and none of them report long-term outcomes.[3,9]

Cholangiography performed through the external drainage tubes about one month after transplantation showed no leakage or stricture at the anastomotic site in any of our 5 recipients. During dissection of the bile duct and cystic duct for the recipient operation, we paid attention to preserving the connective tissue around the wall as much as possible to keep the blood supply and tried to ensure the patency of the cystic duct by dilating the spiral lumen before anastomosis. Although Liu et al. reported excellent results for duct-to-duct biliary reconstruction of right-lobe LDLT without biliary drainage[10] and also reported that the biliary stenting was one of the risk factors for biliary complications,[11] we still consider the use of small external drainage tubes preferable because of several advantages. First, it allows us to obtain information about bile juice production and hence about the graft function. Second, biliary drainage can be used to reduce pressure at the anasomotic site to prevent leakage. And finally, the external drainage tubes can help to keep the lumen open, which may be important particularly when dealing with a spiral cystic duct. A recent study described complete obstruction of the anastomosis of a cystic duct and a donor right anterior hepatic duct one month after transplantation because of fibrous tissue replacing the anastomotic site.[12] The

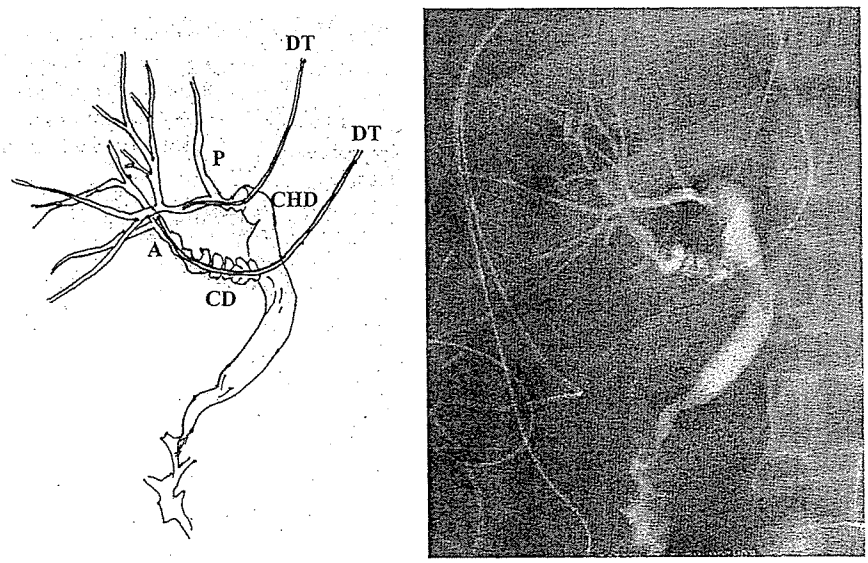


**Figure 3. Cholangiography through the external drainage tubes one month after liver transplantation in patient 1. Neither leakage nor stricture was detected. CD, cystic duct; CHD, common hepatic duct; A, anterior branch of right hepatic duct of the graft; P, posterior branch of right hepatic duct of the graft; DT, external drainage tube.**

technique described here, including the use of drainage tubes, could avoid early obstruction. In addition, cholangiography can be performed through the tubes to determine whether any leakage or stricture has occurred. However, this technique also has some disadvantages, such as a waiting period of several months before the tubes can be removed because of the possibility of bile leakage from the insertion site in the wall of the bile duct. This is especially problematic when the tubes need to be kept in place for long periods of time, such as in the case of recipients who show continuous ascites after transplantation. Moreover, immediately after we removed the drainage tubes three months post-transplantation, fever, abdominal pain, and signs of infection developed in some recipients and required administration of antibiotics.

These disadvantages are in addition to the fact that biliary strictures occur in 15 to 33% of patients who undergo LDLT with duct-to-duct biliary reconstruction, whereas such strictures do not occur as frequently in hepaticojejunostomy.[2,9,10,13] However, endoscopic access is easier in duct-to-duct reconstruction for either examination or treatment of biliary strictures. Hisatsue et al. reported that 13 of 14 patients who showed biliary stricture after duct-to-duct reconstruction were successfully treated with an internal stent.[14] It could therefore be argued that even though a cystic duct anastomosis has its problems, they are outweighed by the possibility of endoscopic access after transplantation.

In conclusion, using the cystic duct for separated biliary duct reconstruction is a feasible and safe technique as demonstrated by short-term as well as long-term results after LDLT. In addition, external drainage tubes may be effective for preventing leakage or stricture of the cystic duct anastomosis.

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# Living Domino Liver Transplantation in an Adult With Congenital Absence of Portal Vein

*Takayuki Takeichi, Hideaki Okajima, Hiroko Suda, Shintarou Hayashida, Hironori Iwasaki, Manuel Zeledon Ramirez, Mikako Ueno, Katsuhiro Asonuma, and Yukihiro Inomata*

**Congenital absence of the portal vein (CAPV) is a rare malformation of the splanchnic venous system. Although CAPV is usually detected in the pediatric age group, our patient was a 35-year-old woman. She had been diagnosed with CAPV in 1996 when she was 27 years old. In 1998, she was placed on hemodialysis due to chronic renal failure. After several episodes of encephalopathy in 2002, liver transplantation (LT) was recommended to her and her family. Since there was no suitable living donor candidate, she was put on the waiting list for a deceased donor liver transplant in Japan. In 2004, her ammonia level increased to around 300 μg/dl, and she went into a coma lasting for three days. After recovering from this event, she underwent a living domino transplantation using a whole liver donated by a familial amyloid polyneuropathy (FAP) patient. Her portal vein, which had drained directly into the inferior vena cava (IVC), was transected together with a cuff of the IVC wall and anastomosed to the graft liver portal vein in an end-to-end fashion. In conclusion, liver transplantation proved to be a safe and effective way to save this patient and improve her quality of life. (*Liver Transpl 2005;11:1285-1288.*)**

Congenital absence of the portal vein (CAPV), a rare malformation in which the portal vein does not drain into the liver but into the systemic venous blood vessels, is usually observed in cases of the pediatric age group. In 1793, John Abernethy reported the presence of a porto-caval shunt that completely bypassed the liver in an autopsy of a 10-year-old female,[1] and it was not until 1833 when another case was reported by Kiernan, again detected during autopsy.[2] This long interval indicated a very rare malformation that was noncompatible with adult life.

To date, 47 cases have been reported and 42 of them after 1979. This translates into a rate of almost two cases per year since 1980. In recent years, most of the reported cases have been living patients, mostly pediatric, although the number of adults is increasing. The majority of cases usually show no signs of encephalopathy, even in cases with documented hyperammonemia. Liver transplantation (LT) has rarely been indicated for CAPV, especially for adult cases. To our knowledge, LT has been indicated for portosystemic encephalopathy due to CAPV in only three cases, and two of them were children.[3-5] We herein present an adult patient of CAPV with hyperammonemic encephalopathy who was successfully treated with living domino LT.

## Case Report

The patient was a 35-year-old woman without any relevant history of disease during her childhood. Eight years ago, she suffered from an episode of encephalopathy due to hyperammonemia and was referred to our institution. Computed tomography (CT) scan and ultrasonographic examination showed the absence of the portal vein. Because histopathological examination of the liver biopsy showed no portal vein branches within the Glissonean sheath, diagnosis of CAPV was confirmed. Conservative treatment resulted in complete recovery from the encephalopathy, and the patient was discharged from the hospital. Two years later, however, she had to be put on hemodialysis due to chronic renal failure caused by focal segmental glomerulopathy. After the diagnosis of CAPV, her ammonia level gradually increased, and after several episodes of transient hyperammonemia with encephalopathy, LT was recommended to her and her family in 2002. Living donor LT is much more common in Japan than deceased donor LT. However, since there was no suitable living donor available among the patient's relatives, she was put on the waiting list for deceased donor LT, which is

**Abbreviations:** LDLT, living domino liver transplantation; CAPV, congenital absence of portal vein; LT, liver transplantation; FAP, familial amyloid polyneuropathy; IVC, inferior vena cava.

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