Higher Risk of Posttransplant Liver Graft Failure in Male Recipients of Female Donor Grafts Might Not Be Due to Anastomotic Size Disparity

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Background. Posttransplant liver graft failure occurs most often in male recipients of livers from female donors. The respective role of donor sex itself and the size disparity in graft vessels/bile ducts according to donor sex are unclear. Thus, we aimed to evaluate the importance of donor sex with adjustment for anastomotic size disparity between female and male donor grafts.

Methods. A total of 309 male patients without hepatic tumors who underwent living donor liver transplantation were analyzed (109 female donors and 200 male donors). The primary outcome was posttransplant graft failure (ie, retransplantation or death). Survival analysis was performed using the Cox model. Analyzed anastomosis-related factors comprised graft weight, number and size of hepatic vessels/bile ducts, and anastomosis techniques. **Results.** Graft failure probabilities at 1, 6, 12, 24, and 60 months posttransplantation were 9.1%, 19.5%, 20.2%, 23.0%, and 27.0%, respectively, with female donors and 2.0%, 5.5%, 8.1%, 10.1%, and 13.5% with male donors (hazards ratio [HR], 2.29; 95% confidence interval [CI], 1.35-3.88; $P = 0.002$). Multivariable analysis confirmed the significance of donor sex (HR, 2.30; 95% CI, 1.14-4.67; $P = 0.021$) after adjustment for anastomosis-related factors. All analyzed anastomosis-related factors showed no significant association with graft failure, although size of the graft hepatic artery showed marginal significance (HR, 0.50; 95% CI, 0.25-1.01; $P = 0.053$). The significance of donor sex was lost when donor was older than 36 to 40 years (age of poor ovarian reserve and the end of female fertility). Our institutional pediatric recipient cohort validated the inferiority of female-to-male donation.

Conclusions. Donor sex appears to be an independent factor modulating graft failure risk in male liver transplant recipients.

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Posttransplant liver graft failure is more prevalent in male recipients of female donor livers compared to other donor-recipient sex combinations. Since the landmark study by Kahn et al,¹ clinical studies of liver disease patients with various etiologies and from various regions have consistently

demonstrated an increase in graft failure in female donor-to-male recipient liver transplantation²⁻⁶; hepatic estrogen receptors are considered to play an important role in this sex difference.⁷⁻¹⁰ Recent research in experimental and clinical living donor liver transplantation validated the association between female-to-male donation and transplant failure.¹¹⁻¹³ However, a few studies have shown that the association between female-to-male donation and graft failure was lost after adjustment for potential confounders.¹⁴⁻¹⁶ This conflict has not been limited to adult liver transplantation: 2 studies of pediatric recipients reported mixed results.^{17,18} The effect

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of sex has remained an unsolved key to fully understanding the process of graft failure after liver transplantation.

We considered the possibility that some previous studies had a type I error because mismatches of anastomosis size in vessels and bile ducts between female grafts and male recipients was not taken into account. This hypothesis was relevant because previous studies demonstrated that vascular complications, such as hepatic artery thrombosis, were the predominant cause of graft failure in male recipients of female donor grafts.^{2,6,16,18} Conversely, we hypothesized that the loss of significant association between female-to-male donation and graft failure after multivariable analysis in other studies could have been confounded by the strong correlation between donor sex and donor characteristics (type II error due to multicollinearity). This hypothesis was relevant because donor height appeared to be a dominant variable for reducing the strong significance of female-to-male donation shown in univariable analysis.^{14,15} Thus, we aimed to evaluate the true association between female-to-male donation and graft failure while considering the weaknesses of previous studies.

MATERIALS AND METHODS

Subjects and Data Collection

We screened the records of 313 male recipients without hepatic tumors who underwent a first living donor liver transplant between January 2003 and April 2016 in our hospital. We excluded female patients because previous studies demonstrated that donor sex was associated with graft failure only in male recipients.²⁻⁶ We excluded patients with hepatic tumors because our recent study demonstrated that the impact of donor sex on posttransplant death was different between hepatocellular carcinoma (HCC)-related death and HCC-unrelated death.¹⁹ There were no other exclusion criteria. Only 4 recipients were excluded because of missing data. The remaining 309 recipients were included in the study (109 with female donors and 200 with male donors). All data analyzed in the study were derived from our institution's electronic medical records and liver transplantation database (prospectively collected). The institutional review board of Samsung Medical Center approved this retrospective study (SMC 2017-05-054) and waived the requirement for written informed consent.

Liver Donation and Transplant Criteria

The sexes of the donor and recipient were not considered in selection of the donor. Acceptance criteria for liver donation were as follows: age, 65 years or younger; body mass index, less than 35 kg/m²; macrosteatosis, 30% or less²⁰; and donor residual liver volume, 30% or greater. Individuals with any type of hepatitis or fibrosis were excluded from donation.

Surgical Management

Donors underwent computed tomography angiography and magnetic resonance cholangiopancreatography to determine the anatomy of the hepatic vascular and biliary systems. Expert liver radiologists and transplant surgeons carefully discussed anatomical considerations and related surgical issues in a joint meeting prior to transplantation. All grafts consisted of segments 5 through 8 excluding the middle hepatic vein trunk. A transection plane was drawn after temporary inflow occlusion *via* the hepatic artery and portal vein

on the right side of the liver to conduct the right hepatectomy. Parenchymal resection was performed using an ultrasonic dissector (CUSA Excel; Valleylab, Boulder, CO) without the clamp-crushing method. Graft implantation was performed using the piggyback technique. The right hepatic vein of the graft was anastomosed with the recipient's right hepatic vein stump. The middle hepatic vein branches from segments 5 and 8 of the graft were anastomosed with the inferior vena cava using a vascular allograft if segments 5 and 8 were congested after perfusion with histidine-tryptophan-ketoglutarate solution or the diameters of the branches were larger than 1 cm. The inferior hepatic vein of the graft was anastomosed with the inferior vena cava if the right posterior section was congested after perfusion with histidine-tryptophan-ketoglutarate solution or the diameter of the vein was larger than 1 cm. After the portal vein anastomosis was complete, the graft was reperfused by consecutively unclamping the hepatic vein and portal vein. Subsequently, hepatic artery anastomosis was followed by biliary anastomosis. If the liver graft had multiple hepatic artery stumps, total reconstruction or partial reconstruction was selectively performed based on intrahepatic communication among the hepatic arteries.²¹ If the graft had multiple bile ducts, ductoplasty was primarily considered.²² Hepaticojunostomy was performed if there were pathologic findings in the recipients' bile duct. A bile duct stent was placed if the attending surgeons considered the risk of biliary stricture to be high. Transfusion of allogeneic blood was strictly controlled based on a restrictive and prophylactic strategy in which each blood component was transfused separately according to its respective indication.²³ Blood salvage and auto-transfusion were routinely used to reduce patient exposure to allogeneic red blood cells (RBCs).²⁴ Immunosuppression and hepatitis B virus prophylaxis were performed according to the standardized institutional protocol as described previously.¹⁹

Variables and Statistical Analysis

The primary endpoint was posttransplant graft failure (death or retransplantation). Survival analysis was performed using the Cox model with a maximum follow-up of 5 years and the results were described using hazards ratio (HR) and 95% confidence interval (CI). Anastomosis-related factors were analyzed in conjunction with donor and recipient characteristics. Factors evaluated regarding the anastomosis were graft size, number and size of hepatic vessels and bile ducts, and anastomosis technique. Donor height and weight were not included in the analysis because they were considered sex-representative (ie, risk of multicollinearity): there were no overlaps in the interquartile range for height (156-163 cm vs 169-177 cm) or weight (50.8-62.6 kg vs 63.0-75.0 kg) between female donors and male donors. Variables with a *P* value less than 0.25 after univariable analysis were entered into a multivariable model. The risk of multicollinearity was evaluated using the variance inflation factor. Model fitness was further evaluated using the Greenwood-Nam-D'Agostino calibration test.²⁵ The association between donor sex and graft failure was further tested within the subgroup of recipients 40 years or younger and within the subgroup of recipients older than 40 years because our recent study demonstrated that the impact of donor sex on posttransplant HCC recurrence differed if the donor age was over or under 40 years,¹⁹ indicating poor ovarian reserve and the end of female fertility.^{26,27} We further analyzed pediatric recipients to validate the significant

TABLE 1.**Comparison of clinical data of recipients of female donor grafts versus male donor grafts**

Variables	Female donor (n = 109)	Male donor (n = 200)	P
Graft factors			
Donor age, y	36 (26-44)	27 (21-36)	<0.001
ABO blood type incompatible donor	6 (5.5)	16 (8.0)	0.415
Blood-unrelated donor	40 (36.7)	24 (12.0)	<0.001
Macrosteatosis degree $\geq 5\%$	49 (45.0)	94 (47.0)	0.730
Graft ischemia time, min	133 (107-164)	119 (103-140)	0.005
Graft weight, kg	640 (573-724)	741 (657-816)	<0.001
Graft-to-recipient weight ratio, %	0.90 (0.81-1.03)	1.06 (0.93-1.20)	<0.001
Multiple hepatic artery stumps	2 (1.8)	7 (3.8)	0.501
Selective anastomosis (vs total)	1 (0.9)	2 (1.0)	>0.99
Size of the primary hepatic artery, mm	2.0 (2.0-2.5)	2.5 (2.0-3.0)	0.001
Recipient hepatic artery type			0.081
Right hepatic artery	51 (47.2)	109 (55.3)	
Left hepatic artery	53 (49.1)	73 (37.1)	
Others	4 (3.7)	15 (7.6)	
Portal vein thrombosis	20 (18.3)	31 (15.5)	0.519
Multiple portal vein stumps	10 (9.2)	8 (4.0)	0.064
Size of the primary portal vein, mm	17.0 (15.0-20.0)	18.0 (17.0-20.0)	0.002
Size of the primary hepatic vein, mm	25.0 (23.0-30.0)	27.0 (24.0-30.0)	0.066
Anastomosis of the hepatic vein V5	84 (77.1)	137 (68.5)	0.111
Size of the hepatic vein V5, mm ^a	8.0 (6.0-10.0)	9.0 (7.0-11.0)	0.036
Anastomosis of the hepatic vein V8	58 (53.2)	124 (62.0)	0.134
Size of the hepatic vein V8, mm ^a	8.0 (6.0-9.0)	8.0 (7.0-10.0)	0.010
Anastomosis of the inferior hepatic vein	40 (36.7)	55 (27.5)	0.094
Size of the inferior hepatic vein, mm ^a	19.0 (13.8-20.0)	17.0 (13.0-20.5)	0.662
Multiple bile duct stumps	27 (24.8)	53 (26.5)	0.787
Size of the primary bile duct, mm	6.0 (5.0-7.0)	6.0 (5.0-7.0)	0.458
Hepatico-jejunostomy (vs only duct-to-duct)	6 (5.5)	10 (5.0)	0.848
Ductoplasty	20 (18.3)	47 (23.5)	0.294
Bile duct stent	7 (6.4)	22 (11.0)	0.187
Surgical factors			
Operative time > 10 h	49 (45.0)	67 (33.5)	0.047
RBC transfusion amount (units)	3 (0-5)	2 (0-4)	0.091
Recipient factors			
Age, y	48 (43-55)	50 (46-56)	0.035
Body mass index, kg/m ²	23.7 (20.7-26.3)	24.1 (21.9-26.1)	0.318
Hypertension	12 (11.0)	18 (9.0)	0.569
Diabetes	39 (35.8)	83 (41.5)	0.326
HBV prophylaxis era			0.898
Monoprophylaxis (2002-2007)	38 (34.9)	66 (33.0)	
Entecavir (2008-2011)	43 (39.4)	78 (39.0)	
Entecavir (2011-2014)	28 (25.7)	56 (28.0)	
Etiology			0.392
Viral	78 (71.6)	157 (78.5)	
Alcoholic	19 (17.4)	26 (13.0)	
Others	12 (11.0)	17 (8.5)	
MELD score	23 (15-32)	20 (15-33)	
Albumin, g/dL	2.8 (2.6-3.2)	2.9 (2.6-3.3)	0.206
Platelet count ($\times 10^9/L$)	54 (39-83)	62 (43-86)	0.108
Refractory ascites	48 (44.0)	82 (41.0)	0.605
Hepatic encephalopathy			0.772
None	69 (63.3)	120 (60.0)	
Grade I-II	29 (26.6)	61 (30.5)	
Grade III-IV	11 (10.1)	19 (9.5)	
Continuous renal replacement	6 (5.5)	9 (4.5)	0.695

Descriptive statistics are described as frequency (%) or median (25th percentile, 75th percentile).

^aAnalyzed in the subgroup of recipients who received the indicated extra vessels.

FIGURE 1. A, The probability of graft survival according to donor sex ($P = 0.002$) and (B) the ratio of graft failure probability with female donors to graft failure probability with male donors. Note that most differences in graft failure risk between the 2 groups were observed within the first 6 months.

association between donor sex and graft failure independent of the anastomosis size disparity because pediatric liver transplantation involves anastomosis size mismatch in vessels and bile ducts irrespective of donor sex. Ultimately, we analyzed 75 male children (34 with female donors and 41 with male donors) who underwent a first living donor liver transplantation in our hospital. The continuous variables were summarized as median (25th percentile and 75th percentile). Categorical variables were analyzed using the χ^2 test or Fisher exact test and presented as number (%). All reported P values were 2-sided, and a P value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL).

RESULTS

The indications for transplantation in the 309 recipients were as follows: cirrhosis secondary to viral etiology ($n = 235$: hepatitis B, $n = 212$; hepatitis C, $n = 17$; hepatitis B and C, $n = 2$; hepatitis A, $n = 4$), alcoholic cirrhosis ($n = 45$), cryptogenic cirrhosis ($n = 2$), autoimmune hepatitis ($n = 8$), toxic hepatitis ($n = 6$), bile duct obstruction ($n = 8$), metabolic diseases ($n = 3$), and Budd-Chiari syndrome ($n = 2$). All extracted livers were confirmed pathologically to be tumor-free. None

of the recipients was lost to follow-up in the absence of graft failure. There were significant differences in baseline characteristics between female donors and male donors (Table 1). Age was higher in females (36 vs 27 years; $P < 0.001$) and blood-unrelated donation was more frequent in females (36.7% vs 12.0%; $P < 0.001$). Graft weight (640 g vs 741 g; $P < 0.001$), graft-to-recipient weight ratio (0.90% vs 1.06%; $P < 0.001$), and size of the hepatic artery (2.0 mm vs 2.5 mm; $P = 0.001$), portal vein (17.0 mm vs 18.0 mm; $P = 0.002$), and hepatic veins (segment 5, 8.0 mm vs 9.0 mm; $P = 0.036$; segment 8, 8.0 [6.0-9.0] mm vs 8.0 [7.0-10.0] mm; $P = 0.010$) were significantly smaller in female donors. The size of the bile duct, multiple hepatic artery stumps, multiple portal vein stumps, and anastomoses of hepatic veins draining segments 5 or 8 were not significantly different. Graft ischemia time (133 minutes vs 119 minutes; $P = 0.005$) and operative time (proportion of >10 hours, 49% vs 67%; $P = 0.047$) were longer in recipients of female donor grafts. There were no significant differences in recipient characteristics according to donor sex with the exception of older age in male donors ($P = 0.035$).

After a median follow-up of 60 months, 55 recipients (17.8%) experienced graft failure. Graft failure probabilities at 1, 3, and 6 months posttransplantation were 9.1%, 14.7%, and 19.5%, respectively, in recipients of female donor grafts and 2.0%, 4.0%, and 5.5% in recipients of male donor grafts. Graft failure probabilities at 1, 2, and 5 years posttransplantation were 20.2%, 23.0%, and 27.0%, respectively, in recipients of female donor grafts and 8.1%, 10.1%, and 13.5% in recipients of male donor grafts (Figure 1A). Graft failure risk was significantly higher in recipients of female donor grafts (HR, 2.29; 95% CI, 1.35-3.88; $P = 0.002$). As shown in Figure 1B, most differences in graft failure risk between the recipients of male donor grafts versus female donor grafts were observed within the first 6 months. Hepatic vascular complications such as hepatic artery stricture or thrombosis were significant contributors to the higher graft failure risk during the early phase in recipients of female donor grafts as shown in Table S1 (SDC, <http://links.lww.com/TP/B530>) and Figure 2.

Results of univariable analysis indicated that graft failure risk was also significantly associated with the following variables: Model for End-stage Liver Disease score ($P = 0.012$), platelet count ($P = 0.002$), degree of hepatic encephalopathy ($P < 0.001$ for grades III-IV), preoperative use of continuous renal replacement therapy ($P = 0.001$), graft ischemia time ($P = 0.005$), operative time ($P < 0.001$), and volume of

FIGURE 2. The incidence of vascular complications at 6 months posttransplantation according to donor sex. There was a significantly higher incidence of complications when grafts were from female donors versus male donors ($P = 0.005$).

TABLE 2.

Univariable Cox model to determine the association between donor/recipient characteristics and graft failure after living donor liver transplantation

Variables	HR (95% CI)	P
Donor factors		
Age, y	1.01 (0.99-1.04)	0.274
Female	2.28 (1.34-3.87)	0.002
Body mass index, kg/m ²	0.96 (0.88-1.06)	0.431
ABO blood type incompatible	0.23 (0.03-1.68)	0.148
Blood-unrelated donor	1.20 (0.64-2.23)	0.573
Recipient factors		
Age, y	0.99 (0.95-1.01)	0.270
Body mass index, kg/m ²	1.00 (0.93-1.08)	0.966
Hypertension	0.70 (0.26-1.95)	0.500
Diabetes	1.28 (0.75-2.17)	0.369
HBV prophylaxis era		0.495
Monoprophylaxis (2002-2007)		
Entecavir (2008-2011)	0.86 (0.48-1.54)	0.601
Entecavir (2011-2014)	0.60 (0.28-1.26)	0.176
Etiology		
Viral		
Alcoholic	1.33 (0.64-2.75)	0.444
Others	1.91 (0.89-4.10)	0.096
MELD score	1.03 (1.01-1.06)	0.012
Albumin, g/dL	1.19 (0.71-2.00)	0.512
Platelet count ($\times 10^9/L$)	1.01 (1.00-1.01)	0.002
Refractory ascites	0.69 (0.39-1.20)	0.186
Hepatic encephalopathy		
None		
Grade I-II	0.98 (0.51-1.88)	0.942
Grade III-IV	4.13 (2.17-7.85)	<0.001
Continuous renal replacement	3.97 (1.79-8.78)	0.001
Graft factor		
Macrosteatosis degree $\geq 5\%$	0.94 (0.56-1.61)	0.832
Graft ischemia time, min	1.01 (1.00-1.01)	0.005
Graft-to-recipient weight ratio, %	0.76 (0.25-2.32)	0.653
Multiple hepatic artery stumps	1.31 (0.32-5.37)	0.709
Selective anastomosis	2.37 (0.33-17.14)	0.394
Size of the primary hepatic artery, mm	0.54 (0.29-1.01)	0.055
Recipient hepatic artery type		0.776
Right hepatic artery		
Left hepatic artery	0.82 (0.47-1.44)	0.495
Others	0.82 (0.25-2.68)	0.741
Portal vein thrombosis	1.16 (0.59-2.30)	0.671
Multiple portal vein stumps	1.79 (0.71-4.49)	0.215
Size of the primary portal vein, mm	0.95 (0.86-1.04)	0.280
Size of the primary hepatic vein, mm	1.00 (0.95-1.06)	0.889
Anastomosis of the hepatic vein V5	0.81 (0.46-1.43)	0.467
Size of the hepatic vein V5, mm ^a	0.96 (0.87-1.06)	0.407
Anastomosis of the hepatic vein V8	0.91 (0.53-1.54)	0.712
Size of the hepatic vein V8, mm ^a	0.98 (0.87-1.10)	0.686
Anastomosis of the inferior hepatic vein	0.61 (0.32-1.15)	0.124
Size of the inferior hepatic vein, mm ^a	0.99 (0.77-1.26)	0.903
Multiple bile duct stumps	1.36 (0.77-2.42)	0.288
Size of the primary bile duct, mm	0.90 (0.77-1.04)	0.153
Hepatico-jejunostomy	2.03 (0.81-5.10)	0.131
Ductoplasty	1.42 (0.78-2.57)	0.248

Continued next page

TABLE 2. (Continued)

Variables	HR (95% CI)	P
Bile duct stent	0.93 (0.37-2.33)	0.875
Surgical factors		
Operative time > 10 h	1.00 (1.00-1.01)	<0.001
RBC transfusion (units)	1.10 (1.07-1.12)	<0.001

Descriptive statistics are described as frequency (%) or median (25th percentile, 75th percentile).
MELD, Model for End-Stage Liver Disease.

allogeneic RBC transfusion ($P < 0.001$) (Table 2). In contrast, there was no significant association with donor age/body mass index, blood-unrelated donation, recipient age, graft-to-recipient weight ratio, multiple hepatic artery/hepatic vein/bile duct stumps, anastomoses of hepatic veins draining segments 5 or 8, size of the portal vein/hepatic veins/bile duct, use of hepaticojejunostomy, ductoplasty, or bile duct stent. The size of the hepatic artery was marginally associated with graft failure ($P = 0.055$). As shown in Table 3, multivariable analysis confirmed the significantly higher graft failure risk in recipients of female donor grafts (HR, 2.30; 95% CI, 1.14-4.67; $P = 0.021$). In addition, degree of hepatic encephalopathy (grades III-IV; $P = 0.045$) and units of RBC transfusion ($P < 0.001$) also were significantly associated with mortality. The fitness of the multivariable model was confirmed by the Greenwood-Nam-D'Agostino test ($P = 0.943$), whereas the variance inflation factor of all variables was less than 2.

Figure 3 shows the results of the subgroup analysis with stratification according to donor age. Among recipients who received grafts from donors aged ≤ 40 years (Figure 3A), there was a clear trend toward higher graft failure risk in recipients of female donor grafts (HR, 2.76; 95% CI, 1.49-5.14; $P = 0.001$). In contrast, this trend was not found with

TABLE 3.

Multivariable Cox model to determine the independent contributing factors for graft failure

Variables	HR (95% CI)	P
Female donor sex	2.30 (1.14-4.67)	0.021
Transplant year 2011-2014 (vs 2002-2011)	0.30 (0.11-0.81)	0.018
Nonviral and nonalcoholic etiology (vs viral)	1.46 (0.45-4.76)	0.532
MELD score	1.01 (0.98-1.05)	0.451
Platelet count ($\times 10^9/L$)	1.004 (1.00-1.01)	0.188
Refractory ascites	0.81 (0.40-1.64)	0.556
Hepatic encephalopathy grade III-IV (vs no or grade I-II)	2.50 (1.02-6.13)	0.045
Continuous renal replacement	2.73 (0.66-11.25)	0.166
Graft ischemia time, min	1.001 (0.995-1.01)	0.692
Size of the primary hepatic artery, mm	0.50 (0.25-1.01)	0.053
Multiple portal vein stumps	0.40 (0.05-3.24)	0.389
Anastomosis of the inferior hepatic vein	0.71 (0.34-1.50)	0.375
Size of the primary bile duct size, mm	0.88 (0.73-1.07)	0.201
Hepatico-jejunostomy	1.39 (0.25-7.80)	0.706
Ductoplasty	1.85 (0.84-4.07)	0.128
Operative time > 10 h	0.78 (0.37-1.64)	0.509
RBC transfusion (units)	1.04 (1.02-1.05)	<0.001

Variables with $P < 0.25$ during univariable analysis were included in the multivariable model. A variable "ABO blood type incompatible donor" was not included because its HR is out of calculable range when included.

donors older than 40 years (Figure 3B; HR, 1.41; 95% CI, 0.50-3.97; $P = 0.513$). To validate the cutoff donor age of 40 years, we used optimal stratification by means of the Gray's test statistics: z test statistics for interaction effect between donor age and donor sex on graft failure peaked at donor age of 36 years (Figure 3C). This finding was in line with previous research demonstrating that ovarian reserve falls steadily for almost the first 4 decades of life and thereafter the attrition rate significantly accelerates at the mean age of 37.5 ± 1.2 years for the remaining premenopausal period, validating the relevance of 40 years as a cutoff donor age.^{26,27} Because 36 years was found as the optimal

cutoff value, we added the subgroup analysis with stratification according to donor age of 36 years. Among recipients who received grafts from donors 36 years or younger (Figure 3D), graft failure risk was significantly higher in recipients of female donor grafts (HR, 3.57; 95% CI, 1.81-6.99; $P < 0.001$). In contrast, graft failure risk was comparable between the 2 groups when donors are older than 40 years (Figure 3E; HR, 1.05; 95% CI, 0.45-2.48; $P = 0.909$).

Figure 4 shows the results of analysis of pediatric recipients aged 12 months (7-48 months; range, 2-192 months). The indications for transplantation were biliary atresia ($n = 42$), cryptogenic liver failure ($n = 15$), neonatal hepatitis ($n = 4$),

FIGURE 3. Graft survival according to donor sex: (A) when donor age was ≤ 40 years (B) when donor age was > 40 years (C) interaction effect of donor sex with donor age regarding the impact on graft survival according to donor age (D) when donor age was ≤ 36 years and (E) when donor age was > 36 years. Note that grafts from female donors were significantly associated with lower graft survival compared to male donor grafts if the donor was under 40 years (or 36 years) of age; in contrast, this age-related effect was lost in donors over 40 years (or 36 years) of age.

FIGURE 4. Graft survival according to donor sex in pediatric male recipients: (A) the probability of graft survival ($P = 0.036$) and (B) the ratio of graft failure probability with female donors to graft failure probability with male donors. Female donor graft survival was significantly lower than male donor graft survival in children.

metabolic disease ($n = 6$), toxic hepatitis ($n = 3$), and others ($n = 5$). After a median follow-up of 60 months, 12 (16.0%) recipients experienced graft failures. Graft failure probabilities at 6 months, 1 year, and 2 years posttransplantation were 20.6%, 23.6%, and 23.6%, respectively, in recipients of female donor grafts and 2.4%, 4.9%, and 7.5% in recipients of male donor grafts (Figure 4A). Graft failure risk was significantly higher in recipients of female donor grafts (HR, 4.05; 95% CI, 1.09-14.96; $P = 0.036$). The difference in graft failure risk between the 2 groups was mainly observed during the early posttransplant period, as in adult recipients (Figure 4B).

DISCUSSION

In the current study of non-HCC male patients who underwent living donor liver transplantation, graft failure risk was higher with grafts from female donors; the impact of donor sex was mostly observed during the early phase (within 6 months) posttransplantation. Vascular complications (stenosis or thrombosis) were found to be a significant contributor to higher graft failure in recipients of female donor grafts during the early phase. However, the association

of donor sex with graft failure was still significant after adjustment for anastomosis-related factors (HR, 2.30) including the size of graft and its anastomosed vessels. We also found that the inferiority of female donors in graft survival significantly changed in relation to donor age around 40 years, the period of abrupt decrease in ovarian reserve and the end of female fertility in relation to a significant change in systemic female sex hormonal status,^{26,27} supporting previous studies that suggested the involvement of hepatic estrogen signaling in graft failure.^{1,3,6,11,18,19} In addition, the significance of donor sex was also found in pediatric transplantation in which the anastomosis size mismatch could be considerable and a smaller graft size would not be expected to be a problem, irrespective of donor sex. This finding again suggests the presence of anastomosis size mismatch-unrelated factors contributing to higher graft failure in male recipients of female donor grafts. This finding from a nonvirus predominant cohort also suggests the importance of donor sex in various etiologies in conjunction with previous studies of patients with various predominant etiologies^{2,4,6,11,18} including hepatitis C virus.^{6,11} Taken together, the results from the current study suggested that donor sex affects the risk of posttransplant graft failure independent of the size disparity in graft and its vessels and bile ducts and provided indirect clinical support for hepatic estrogen signaling as a contributor to the increased risk of graft failure.

Despite its retrospective design, one strength of the study was the robustness of the data. First, we only included male patients because evaluating 4 donor-recipient sex combinations (female-to-male, female-to-female, male-to-female, and male-to-male) could have caused a type II error from unnecessary multiple comparisons due to a risk of underestimation through statistical corrections, such as Bonferroni post hoc test. Moreover, an exploratory analysis of female recipients who underwent a first living donor liver transplant during the study period and met the inclusion criteria (Figure S1, SDC, <http://links.lww.com/TP/B530>) confirmed that donor sex was associated with graft failure only in male recipients.²⁻⁶ Second, we only included non-HCC patients because the different impact of a variable on HCC-related death versus HCC-unrelated death involves significant risk of confounding its impact on graft failure.^{19,28} Third, we did not include donor height in the analysis because there was almost no overlap in height distribution between female donors and male donors; thus, we were certain that including donor sex and donor height into 1 multivariable model would have distorted results. Fourth, no patients were lost to follow-up and graft failure was defined as a very objective outcome; thus, the data regarding the time-to-graft failure were highly reliable. Fifth, this study included a homogeneous living donor liver transplant population. All recipients received right hemi-liver graft without variation. Most recipients underwent elective surgery and were in stable condition without acute deterioration. Accordingly, thorough perioperative anesthetic and surgical care could be performed strictly based on the institutional standardized protocols. Finally, the sample size was sufficient to detect a real association: the power of the expected HR (2.28) was 80.5%.

Although the exact mechanisms are unknown, a few animal studies have helped elucidate mechanisms underlying the impact of female-to-male liver donation on graft failure. Estrogen mediates its biological functions by binding to specific receptors that are found in liver tissue as well as other

tissues; hepatic estrogen signaling is known to play an important role in recovering or regenerating injured liver.⁷ In mice, ovariectomy or estrogen antagonist administration increased hepatic ischemia-reperfusion injury, reduced regenerative capacity, and increased mortality after total hepatic inflow obstruction for 45 minutes in a partial liver graft model, whereas administration of 17 β -estradiol improved recovery.¹³ In rats, translocation of estrogen receptors from the cytosol to nucleus and activation of cell signaling occurred after liver injury to stimulate the healing process.^{29,31} Of note, one study further suggested the importance of estrogen receptors versus androgen receptors by demonstrating a significant increase in hepatic estrogen receptor content after hepatectomy and, in contrast, a significant reduction in hepatic androgen receptor content and serum testosterone level.³¹ A previous study in rats demonstrated that female-to-male liver transplantation significantly reduced the cytosolic estrogen receptors in grafts to the level of male liver within 10 days, whereas male-to-male liver transplantation maintain the estrogen receptor content.⁹ Abrupt defeminization of female livers by reduction of estrogen receptor activity and exposure to the male hormonal milieu can impair mechanisms of protection of grafts from female donors.

It is unclear how estrogen signaling protects the liver; however, nitric oxide (NO) pathways are thought to be involved. Endothelial NO production is promoted by estrogen signaling and NO confers vasculoprotection by reducing reactive oxygen species, promoting vasodilation, and exerting an antiatherosclerotic effect.³² A previous study in mice demonstrated that NO conferred protection against hepatic ischemia-reperfusion injury after partial hepatectomy.³³ Thus, hepatic estrogen signaling-induced upregulation of NO production might positively affect the hepatic vascular endothelium and inhibit hepatic inflow insufficiency and ischemia by preventing vasoconstriction and thrombosis, which are well-known early onset posttransplant complications. Our findings and those of previous results that male recipients of livers from female donors were at risk of graft failure due to hepatic vascular complications are in agreement with this explanation.^{2,6,16,18} A previous study in rats demonstrated that increased mortality in female-to-male partial liver transplantation was due to impaired graft perfusion, which also was in agreement with our findings.¹²

In contrast to deceased donors, living donors can be selected among multiple candidates without graft competition and the selection process is based on contributors for success or failure of transplantation. Despite a consistent trend toward higher graft failure risk in male recipients of female donor grafts, the effect of donor sex has not been addressed in the liver transplant community. The current study supported the independent association between female-to-male donation and increased graft failure and suggested that the incorporation of donor sex into the living donor selection process might help improve posttransplant clinical outcome. This conclusion is in line with our previous research that demonstrated increased posttransplant HCC recurrence in recipients who received grafts from male donors.¹⁹

This study had several limitations. First, we could not exclude the possibility of bias from unmeasured variables although we included all established contributors of posttransplant graft failure. In particular, robust matching could not be performed due to the significant size disparity-induced case loss. For instance,

after an exploratory propensity score-based 1:1 matching with the caliper width being 0.2 standard deviation of the logit-transformed propensity score, only 46 paired sets of recipients in female donor group (46 of 109) and male donor group (46 of 200) were generated. Second, mechanisms underlying the relationship between donor sex and graft failure have not been elucidated. Although the involvement of estrogen receptors can be assumed from previous studies, the mechanism of interaction between hepatic sex hormone receptors and recipient hormonal milieu and changes in posttransplant expression require additional research.

In summary, the risk of posttransplant graft failure was significantly higher in male recipients who received female donor grafts after adjustment for anastomosis-related factors such as graft weight and graft vessel/bile duct size. The significant difference between female donors and male donors regarding the association with graft failure was lost when donor was older than 36 to 40 years. Our findings indicate that donor sex is an important prognostic factor for graft failure after living donor liver transplantation.

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