Ⅳ.研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ueno T, Wada M, Hoshino K, Uemoto S, Taguchi T, Furukawa H, Fukuzawa M.	Impact of pediatric intestinal transplantation on intestinal failure in Japan: findings based on the Japanese intestinal transplant registry.	Pediatr Surg Int.	29巻 10号	P1065-P1070	2013
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Ⅴ.研究成果の刊行物・別刷

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ORIGINAL ARTICLE

Impact of pediatric intestinal transplantation on intestinal failure in Japan: findings based on the Japanese intestinal transplant registry

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Abstract
Introduction We assessed the impact of intestinal transplantation on Japanese pediatric patients with intestinal failure with data from the Japanese intestinal transplant registry.
Methods Standardized forms were sent to all known intestinal transplantation programs, requesting information on transplants performed between 1996 and June 30, 2012. Patients younger than 18 years were analyzed. Patient and graft survival estimates were obtained using the Kaplan–Meier method.
Results Of the 14 intestinal transplants, 4 were deceased and 10 were living donor transplants. The primary indications were: short gut syndrome ($n = 7$), intestinal functional disorder ($n = 6$), and re-transplantation ($n = 1$). The overall 1- and 5-year patient survival rates were 77 and 57 %, respectively. In transplants performed after 2006 ($n = 6$), the one-year patient survival rate was 83 %, and the 5-year survival rate was 83 %. Graft one- and 5-year survival rates were 83 and 83 %, respectively. The living-related transplant survival rate was 80 % at 1 year and 68 % at 2 years, compared to 67 and 67 % for cadaveric transplant recipients. There were no statistically significant differences in patient ($p = 0.88$) and graft ($p = 0.76$) survival rates between living donor and cadaveric transplant recipients. All current survivors discontinued PN.
Conclusion Intestinal transplantation has become an effective therapy for patients with intestinal failure who cannot tolerate PN.

Keywords Intestinal transplant · Pediatric transplant · Japanese registry

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Introduction

Intestinal failure is caused by a critical reduction of functional gut mass to below the minimal amount necessary for adequate digestion and absorption to satisfy nutrient and fluid requirements for maintenance in adults and growth in children [1]. The most common type of intestinal failure is short bowel syndrome with an estimated incidence of 3–5 cases per 100 000 births per year

[2]. Advances in neonatal intensive care, anesthesia, nutritional support, and surgical techniques have improved the survival of children, so the prevalence of common causes of short bowel syndrome, including gastroschisis, necrotizing enterocolitis, and intestinal atresia has likely increased in recent years [3]. Some survivors, however, develop irreversible intestinal failure. The prognosis for intestinal failure related to short gut syndrome and intestinal motility disorders has improved dramatically owing to the development of parenteral nutrition (PN). Some children achieve long-term survival with PN at home with a relatively good quality of life, but others develop serious side effects that can eventually lead to death. However, PN-related complications, such as loss of venous access and intestinal failure-associated liver disease (IFALD), are still major problems for patients with intestinal failure [4]. Intestinal transplantation can significantly improve their prognosis and quality of life. Early efforts to transplant the small bowel have failed due to refractory graft rejection and sepsis. Outcomes improved during the early 1990s, but survival rates were still inferior to those for other organ transplants. Over the past 5 years, individual centers have reported improved outcomes with better long-term intestinal engraftment.

The first intestinal transplant in Japan was performed in 1996. The total number of intestinal transplants in Japan has increased to 24 as of June 2011. We assessed the impact of intestinal transplantation on Japanese pediatric patients with intestinal failure based on data from the Japanese intestinal transplant registry.

Methods

Standardized forms were sent to all known intestinal transplantation programs, requesting information on intestinal transplants performed between 1996 and June 30, 2012. The data included age, sex, date of birth, date of transplant, type of donor (deceased or living), pre-transplant status (home or hospital), underlying disease, procedure, ABO blood type, immunosuppression regimen (induction and maintenance therapy), and post-transplant status (PN requirement, intravenous (IV) fluid requirement, and daily life restrictions). Patients under 18 years of age were analyzed. The data were entered into a Microsoft Excel spreadsheet and analyzed with JMP version 10.0 (SAS Institute Inc, USA). Patient and graft survival estimates were obtained using the Kaplan–Meier method. For survival analysis, failure was defined as occurring on the date of graft removal or death. A p value <0.05 was considered statistically significant. This study was approved by the institutional review board.

Results

Four programs provided data on 14 grafts in 13 patients who were received transplants between 1 April 1996, and 30 June 2012 in Japan. The participation rate was 100 %. All intestinal transplants performed in Japan are captured in the registry database. All patients were followed, unless the patient has passed way. Ten grafts were obtained from living donors, and four cases involved deceased donors. The annual number of intestinal transplants, according to organ donation type, is shown in Fig. 1. Prior to 2005, 25 % of patients who underwent transplantation were called in from home, as compared with 66 % in the last 5 years (Fig. 2).

There were nine male and five female recipients. The age distribution of the recipients is shown in Fig. 3. Two-thirds of the patients were over 6 years old. The youngest recipient was 8 months. The causes of intestinal failure requiring intestinal transplantation are shown in Fig. 4. Approximately half of the patients had conditions that result in short gut syndrome.

Most patients ($n = 13$) received isolated intestinal transplants. There was only one case of simultaneous liver-intestinal transplantation from two living-related donors. Twelve patients received grafts from donors with an identical ABO blood type. Two patients received grafts from ABO compatible donors. There were no transplants involving ABO incompatibility. All patients were on tacrolimus maintenance therapy. The types of induction therapy used are shown in Fig. 5. Antibody-based induction therapy and tacrolimus-based maintenance immunosuppression were used even if the medication was not commercially available in Japan.

Graft and patient overall survival as of June 2011 are shown in Kaplan–Meier plots (Fig. 6a, b, respectively). The one-year and 5-year patient survival rates were 77 and 57 %, respectively, comparable with rates from the international intestinal transplant registry. Five recipients died.

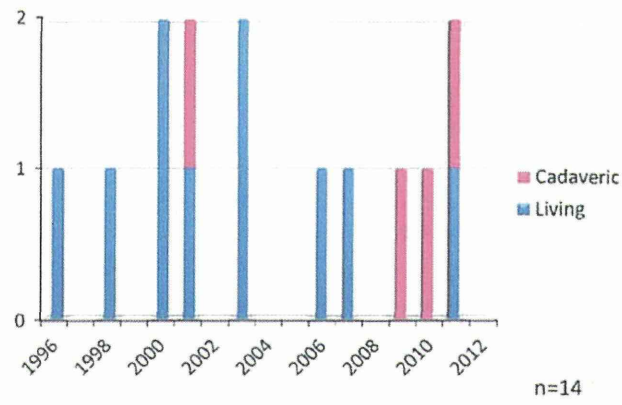

Fig. 1 Number of intestinal transplants by year

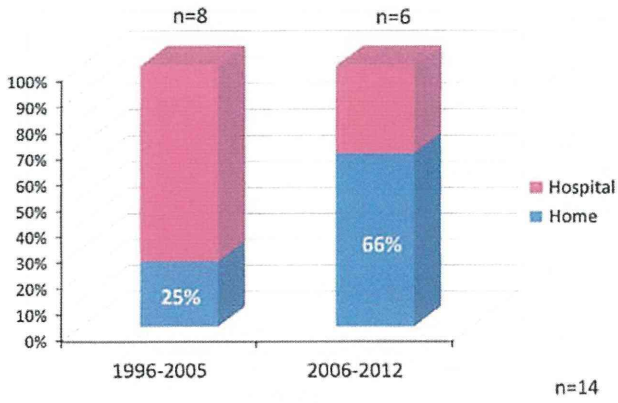

Fig. 2 Pre-transplant patient status

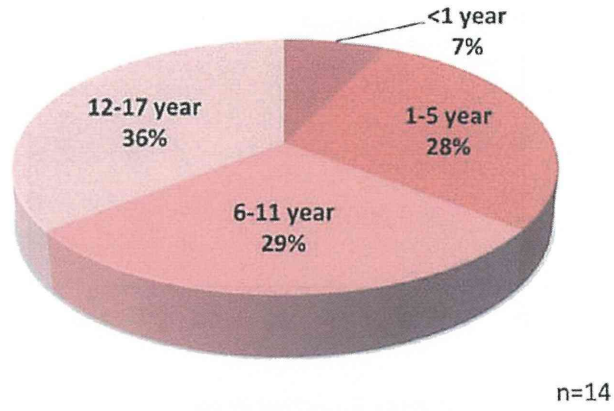

Fig. 3 Recipient age at transplant

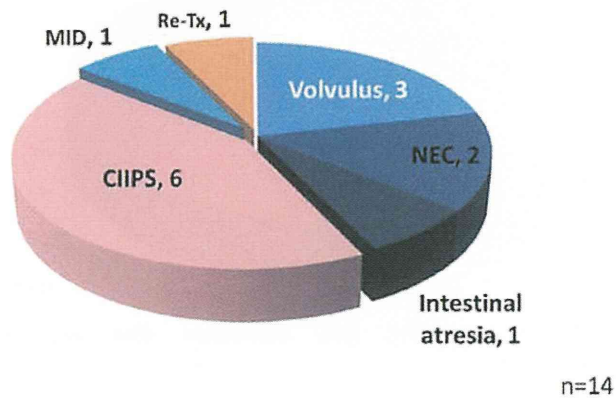

Fig. 4 Cause of intestinal failure *NEC* necrotizing enterocolitis, *CIIPS* chronic idiopathic intestinal pseudo-obstruction syndrome, *MID* microvillus inclusion disease, *Re-Tx* Re-transplant

The causes of death included sepsis ($n = 3$), post-transplant lymphoma ($n = 1$) and intra cranial hemorrhage ($n = 1$).

The 1-year overall graft survival rate was 80 % for cadaveric grafts versus 50 % for living donor grafts ($p = 0.76$), as shown in Fig. 7a. The 1-year overall patient

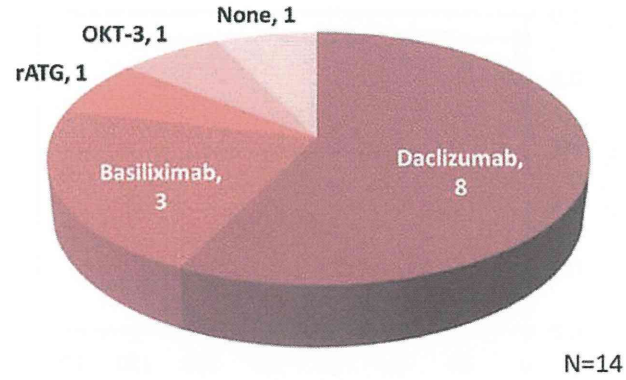

Fig. 5 Induction immunosuppression therapy *rATG* rabbit anti-thymus globulin, *OKT-3* anti-CD3 monoclonal antibody

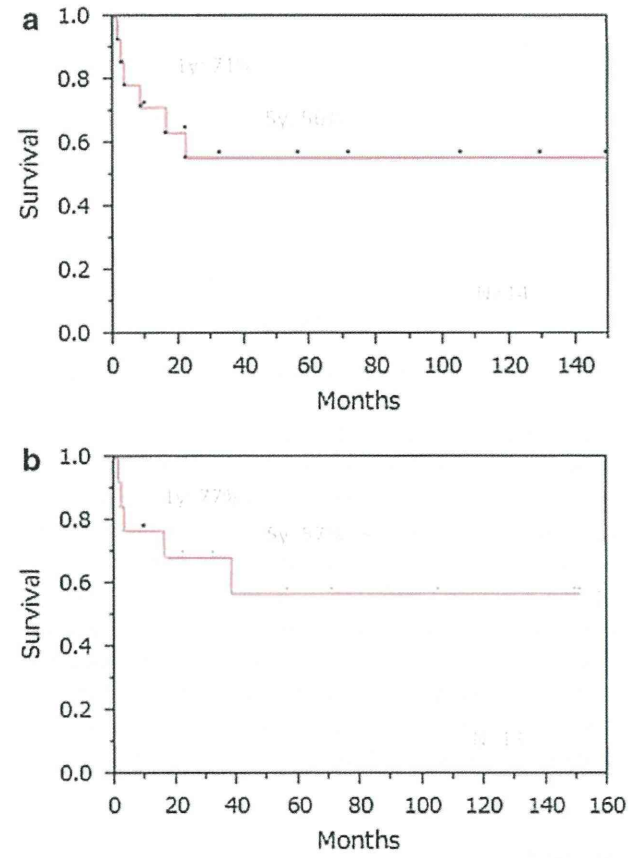

Fig. 6 Overall graft (**a**) and patient (**b**) survival

survival rate was 80 % for cadaveric grafts versus 67 % for living donor grafts ($p = 0.88$), as shown in Fig. 7b.

Graft survival improved over the last 5 years. The one- and five-year graft survival rates were 83 and 83 % for 2006–2011 versus 63 and 38 % for 1996–2005 ($p = 0.14$), as shown in Fig. 8a. The 1- and 5-year patient survival rates were 83 and 83 % for 2006–2011 versus 71 and 43 % for 1996–2005 ($p = 0.27$), as shown in Fig. 8b.

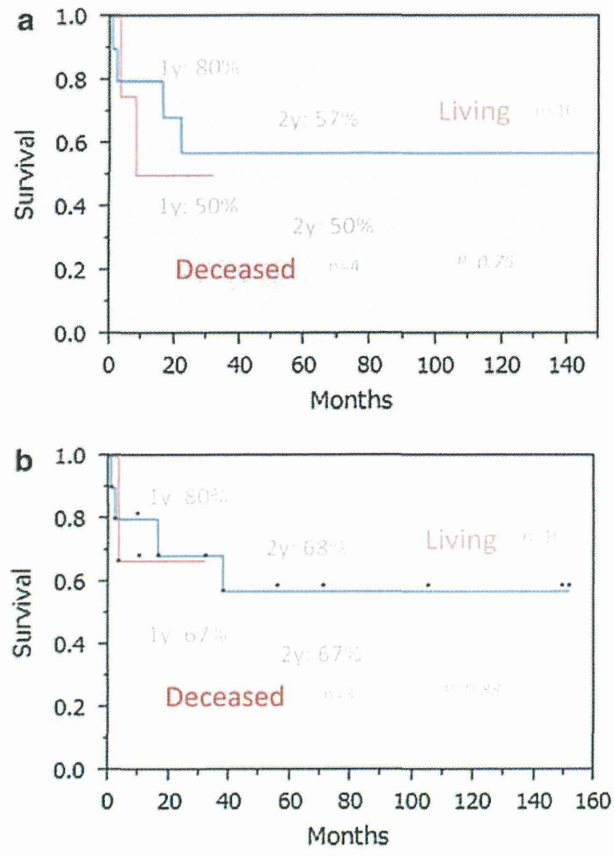

Fig. 7 Graft (a) and patient (b) survival according to graft type

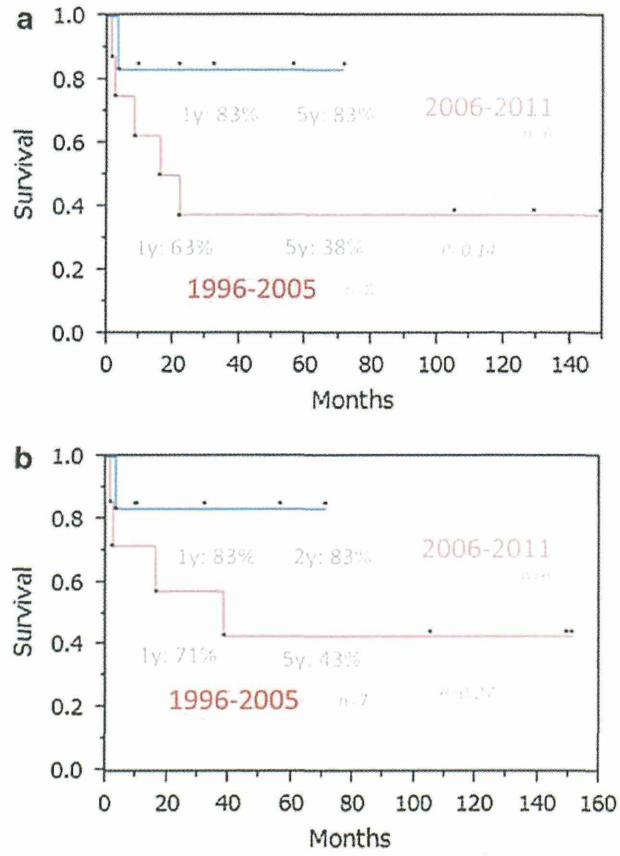

Fig. 8 Graft (a) and patient (b) survival by era

Graft function in terms of PN dependence was excellent. All patients became PN-free after intestinal transplantation, although two-thirds of patients require continuous or intermittent intravenous fluid support. Of the eight patients who were alive at the time of data collection, all patients were off parenteral nutrition, with three patients requiring intravenous fluids daily, two patients requiring intravenous fluids occasionally (Fig. 9). Most recipients stopped parenteral supplementation, eat, and have resumed normal activities. Of the seven surviving patients 1 year after transplant, six lead a full life.

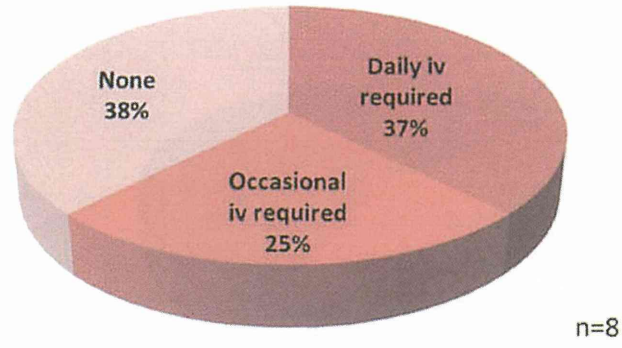

Fig. 9 Intravenous (IV) fluid requirement after intestinal transplantation

Discussion

Children with intestinal failure are at risk for numerous complications, especially PN-related complications. For example, loss of venous access and IFALD are still major problems for patients with intestinal failure because they are potentially life-threatening [4].

Catheter-related bloodstream infections were common in patients with intestinal failure [5]. Survival of children with chronic intestinal failure has increased as result of home PN. Adequate central venous accesses crucial for the successful management of home PN, but venous access can be complicated by episodes of catheter-associated infection, repeated procedures to replace catheters, and catheter-related thrombosis. Management and prevention of catheter-related thrombosis are of vital importance. [6].

IFALD can be a progressive and fatal entity in children with short gut syndrome. Parenteral fish oil-based fat emulsions are safe and may be effective in the treatment of PN-associated liver disease [7]. A lipid reduction protocol may prevent cholestasis [8]. Despite all efforts to prevent

complications, some children develop end-stage intestinal failure.

As outcomes of intestinal transplantation have improved, it has become the definitive treatment for patients with intestinal failure who cannot tolerate PN. Over the past decade, intestinal transplantation has become accepted as standard therapy for patients with life-threatening complications of PN in many countries [9, 10].

Currently, evaluation for transplant is recommended for pediatric patients with intestinal failure who are doing poorly on PN due to loss of more than 50 % of the major intravenous access sites (two out of four sites include both internal jugular veins and subclavian veins); recurrent severe catheter-related sepsis; progressive liver dysfunction; or impaired renal function due to massive gastrointestinal fluid loss.

Timely referral to an intestinal transplant program is important for children with intestinal failure because intestinal transplantation is easier and safer with adequate central venous access and normal liver function [11]. For patients who undergo intestinal transplantation, patient survival is similar to remaining on PN. The inclination is therefore to move towards earlier transplantation and avoiding the need for concomitant liver transplantation [12].

The 2011 report of the intestinal transplant registry confirmed that intestinal transplantation has become a definitive therapeutic option for patients with intestinal failure. By 2011, 2,611 intestinal transplants had been performed throughout the world with 79 participating centers worldwide. Three types of intestinal transplantation are performed: (1) isolated intestinal transplantation (1,184 cases); (2) liver and intestine transplantation (845 cases); and (3) multivisceral transplantation (619 cases). In pediatric patients, two-thirds acquired short gut syndrome as a result of congenital disease, including gastroschisis, intestinal atresia, and necrotizing enterocolitis [10].

On the other hand, only 14 intestinal transplants have been performed in patients under 18 years of age in Japan. The number is relatively small, although it is estimated that 40 pediatric patients require intestinal transplants nationwide [13]. In the Japanese experience, the 1- and 5-year overall patient survival rates are 77 and 57 %. The one-year survival rate was 83 % for the last 5 years. These are considered acceptable results for the treatment of intestinal failure. Our results in Japan are comparable with results worldwide, even though there are only one or two cases per year performed in Japan compared to over 100 intestinal transplants yearly performed in the world. In our opinion, children with intestinal failure should be treated with intestinal transplantation in Japan as well as in other countries when feasible.

There were two major reasons for the low number of intestinal transplants in Japan. One reason is the lack of available organs. For a long time, relatively few donations from deceased donors were obtainable in Japan. As with other solid organs, most intestinal transplants in Japan are performed with living-related donors. Although the situation has changed due to the new Act on Organ Transplantation, which went into effect in 2010, the number of deceased donations has not increased dramatically, especially among pediatric donors.

The financial barrier is the other, more profound reason preventing the greater use of intestinal transplantation in Japan. Since the procedure is not covered by health insurance, either the patient or the transplant center must pay the considerable costs out of pocket.

Some patients develop liver failure with short gut syndrome. These patients need simultaneous liver-intestinal transplants. A combined liver-intestine transplant has less risk of acute rejection than an isolated intestinal transplant because the liver may have protective effects on the intestine [10]. Combined liver and intestine transplants are the most frequent procedure in infants and children, accounting for half of the cases. Current organ allocation guidelines have not allowed for simultaneous combined liver–intestine organ retrieval until the law was revised in 2010; thus, simultaneous liver–intestine transplantation with a deceased donor graft had been impossible. Isolated intestinal transplantation, the preferred procedure, was offered to patients with limited IV access or recurrent line infections. Combined liver–intestine transplants are performed for treatment of irreversible liver disease caused by PN. Isolated intestinal transplantation from deceased donors following living-related liver transplantation, referred to as sequential combined liver-intestine transplantation, has been attempted.

Previously, the law on organ transplantation banned donors below 15 years of age. This is the main reason why there were relatively few pediatric transplant recipients. Intestinal transplant for infants was previously not possible because of donor-recipient size mismatch. Only a small number of pediatric transplants have been performed. Pediatric patients still await the opportunity to benefit from intestinal transplantation. Moreover, younger patients sometimes develop liver failure [3]. Multivisceral transplants are recommended for the treatment of severe gastrointestinal motility disorders [14]. However organ allocation guidelines do not allow for multivisceral organ retrieval. Further reform of allocation guidelines is needed.

This analysis found that improved induction immunosuppression is strongly associated with higher survival rates. The use of antibody induction therapy appears to be particularly important for the success of intestinal transplantation, possibly due to the large lymphoid mass of this type of graft [15]. Induction with rabbit anti-thymus globulin (rATG) minimized the amount of tacrolimus needed for

maintenance immunosuppression, facilitated the long-term control of rejection, and decreased the incidence of opportunistic infections, resulting in a high rate of patient and graft survival [16]. The combination of rATG and rituximab was an effective induction therapy according to our preliminary data. The number and severity of rejection episodes increased when the liver was not included as part of the graft. An immunosuppression regimen including rATG, rituximab, and steroids may have a protective effect against post-transplant lympho proliferative disease (PTLD) and chronic rejection [17]. Sirolimus is a safe rescue therapy in children with intestinal transplants when tacrolimus is not well tolerated. Renal function and hematologic disorders seem to improve, although other simultaneous strategies could be involved [18]. However, those medications are not commercially available with insurance coverage in Japan. Children after intestinal transplant should be managed with limited immunosuppression.

Preemptive assessments are recommended, even for patients doing well on PN, and for infants and adults with an ultra-short gut or for infants with total intestinal aganglionosis or microvillus inclusion disease, since patients with these findings have very poor survival rates on PN [15].

Early referral and listing are important for successful outcomes. Presently, because of the risks involved as well as financial reasons, transplants are rarely offered to pediatric patients in Japan. However, this treatment will undoubtedly become more common over time as the results of intestinal transplantation continue to improve.

Conclusion

Intestinal transplantation has become the definitive treatment for patients with chronic intestinal failure. Since intestinal transplantation in Japan has yielded satisfactory results, indications for the procedure should be expanded. The national health insurance should cover intestinal transplants to reduce the incidence of PN-related complications. Systems facilitating combined simultaneous liver–intestine and multi-organ transplants should be developed. We continue to work on reforming national health insurance coverage and realizing multi-organ transplantation in Japan.

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ORIGINAL ARTICLE

Apelin is a marker of the progression of liver fibrosis and portal hypertension in patients with biliary atresia

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Abstract
Purpose Apelin, the endogenous ligand of the angiotensin-like-receptor 1 (APJ), is thought to play an important role in liver disease. This study investigated the apelin expression in different stages of biliary atresia (BA) and investigated whether it is associated with the progression of disease.
Methods Liver tissues were obtained from patients at Kasai's procedure (KP), the follow-up stage after KP (Post-KP) and at liver transplantation (LT). Immunohistochemistry for apelin and its receptor APJ and real-time quantitative reverse transcriptase polymerase chain reaction for *apelin* mRNA expression were conducted.
Results The immunohistochemical study revealed that apelin was mainly localized in the perivenular areas of control liver tissue, and slightly detected in the hepatic stellate cells (HSC) and hepatocytes, whereas intense apelin immunoreactivity was detected in perivenular areas, HSC and hepatocytes of LT liver tissue. The *apelin* mRNA expression level was significantly higher in the LT group than in the KP and Post-KP group. Significant linear correlations were observed between the *apelin* mRNA level and liver fibrosis, serum total bilirubin and the grade of esophageal varices.
Conclusions The hepatic apelin–APJ system is markedly activated in the progression of BA, especially in end-stage cirrhosis. The apelin expression level accurately reflects the severity of hepatic fibrosis and esophageal varices and therefore could be used as a prognostic factor in BA patients.

Keywords Apelin · APJ · Biliary atresia · Liver fibrosis · Esophageal varices · Liver transplantation

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Introduction

Apelin, initially isolated by Tatemoto [1] and his co-workers from bovine stomach homogenates in 1998, is recognized as the endogenous ligand of angiotensin-like-receptor 1 (APJ), and the human orphan G-protein-coupled receptor, which has a close identity with the angiotensin II receptor, but does not bind angiotensin-II [2].

Apelin and its receptor are highly expressed in the central nervous system and in peripheral tissues, where it is involved in the regulation of the cardiovascular tone [3], cardiac contractility [4], glucose metabolism [5], gastrointestinal track physiology [6], and water homeostasis [7].

Recent studies have demonstrated that apelin is over-expressed in hepatic stellate cell (HSC) from both cirrhotic human and rats [8, 9] and the expression is enhanced in proliferative hepatic arterial capillaries in human cirrhotic liver [10].

Biliary atresia (BA) is characterized by complete obliteration of extrahepatic bile duct. Although bile flow can be established by a Kasai portoenterostomy (KP), progressive liver fibrosis and portal hypertension continue to develop in most patients with BA [11]. Therefore, apelin is thought to

be over-expressed in the liver of BA patients. However, no information is available regarding the apelin expression in liver tissue of BA patients. The present study was undertaken to investigate the apelin expression in different stages of BA patients, and especially focused on the correlation between apelin expression and the clinical features such as liver function, hepatic fibrosis, and esophageal varices.

Materials and methods

Patients and samples

Fifty-five BA patients including 19 males and 36 females with a mean age of 8.4 ± 8.2 years (range 37 days–29.6 years) that were treated and followed at Osaka University Hospital between June 2009 and June 2012 were included in this study. A total of 72 liver tissue samples were taken from them, including 4 wedge or needle biopsy samples taken at the time of KP, 29 needle biopsy samples from the follow-up stage after KP (Post-KP) patients, 9 liver tissues taken at time of LT, and 30 needle biopsy samples from Post liver transplantation (Post-LT) patients. Control liver samples included non-tumor containing parts of surgically removed liver tissues from three children with hepatoblastoma and two normal tissues from patients with choledochal cysts. All liver tissues in the current study were obtained after acquiring written informed consent from the parents or healthy adult donors. The protocol for this study was approved by the Ethics Committee of the Institutional Review Board of Osaka University Hospital.

Immunohistochemistry

Liver tissues were fixed in formalin and embedded in paraffin. 4 μm sections were cut, deparaffinized, and dehydrated using graded ethanol. They were incubated overnight at 4 °C with 1:800 dilution of anti-apelin rabbit antibody (Phoenix Pharmaceuticals, INC., Burlingame, CA, USA.) or 1:500 dilution of apelin receptor rabbit antibody (MBL International, Woburn, MA, USA). The sections were washed with Dako Wash Buffer (Dako, Tokyo, Japan), incubated with peroxidase labeled polymer conjugate (Envision® system) (Dako) at room temperature for 30 min and then reacted with the DAB chromogen. The sections were finally counter-stained with hematoxylin for light microscopic study. The intensity of immunostaining was scored as: 0, none; 1+, weak; 2+, moderate; 3+, intense.

Evaluation of *apelin* mRNA expression using quantitative real-time PCR

Total RNA was extracted from frozen samples using TRIzol RNA isolation reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. Template cDNA was obtained by reverse transcription of 1 μg of total RNA using a cDNA synthesis Kit (Prime Script™ RT-PCR Kit, TaKaRa, Japan). The reaction mixtures were incubated at 30 °C for 10 min, 42 °C for 30 min and 95 °C for 5 min. The cDNA was diluted five-fold for real-time PCR.

The sequences of the primers in this study were: sense primer 5′-GGCCATCACCAGCCATTCCTTG-3′ and anti-sense primer 5′-GGGCATCAGGCTCTTGTCTTCTCT-3′. The quantification of gene-expression levels for apelin was carried out by real-time quantitative PCR on an ABI ViiA™ 7 System (TaqMan, Perkin-Elmer Applied Biosystems). The SYBR Premix Ex TaqT II kit (TaKaRa) was used for real-time monitoring of amplification (45 cycles: 95 °C/15 s, 60 °C/1 min). The study used the comparative cycle threshold (Ct) method to calculate relative mRNA expression. All quantifications were normalized by the corresponding expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression (forward primer: 5′-GAAGGTGAAGGTCGGAGTCA-3′; reverse primer: 5′-GAAGATGGTGATGGGATTTC-3′).

Clinical presentation of BA patients following clinical features collected from the patients charts

Serum total bilirubin TB (mg/dl), aspirate amino-transferase AST (IU/L), alanine amino-transferase ALT (IU/L), and gamma glutamyl transpeptidase GGTP (IU/L) were measured in the Osaka University Hospital laboratory and analyzed.

Classification of esophageal and liver fibrosis

Esophagogastroduodenoscopy (EGD) and a liver biopsy were performed at the same time in Post-KP and Post-LT patients, as a routine examination to evaluate the progression of esophageal varices and liver fibrosis, respectively. The grade of an esophageal varix was classified according to Japan Society for Portal Hypertension classification, as: F0, no varicose appearance; F1, straight, small-caliber varices; F2, moderately enlarged, beady varices; F3, markedly enlarged, nodular or tumor-shaped varices [12], and the grade of liver fibrosis was classified

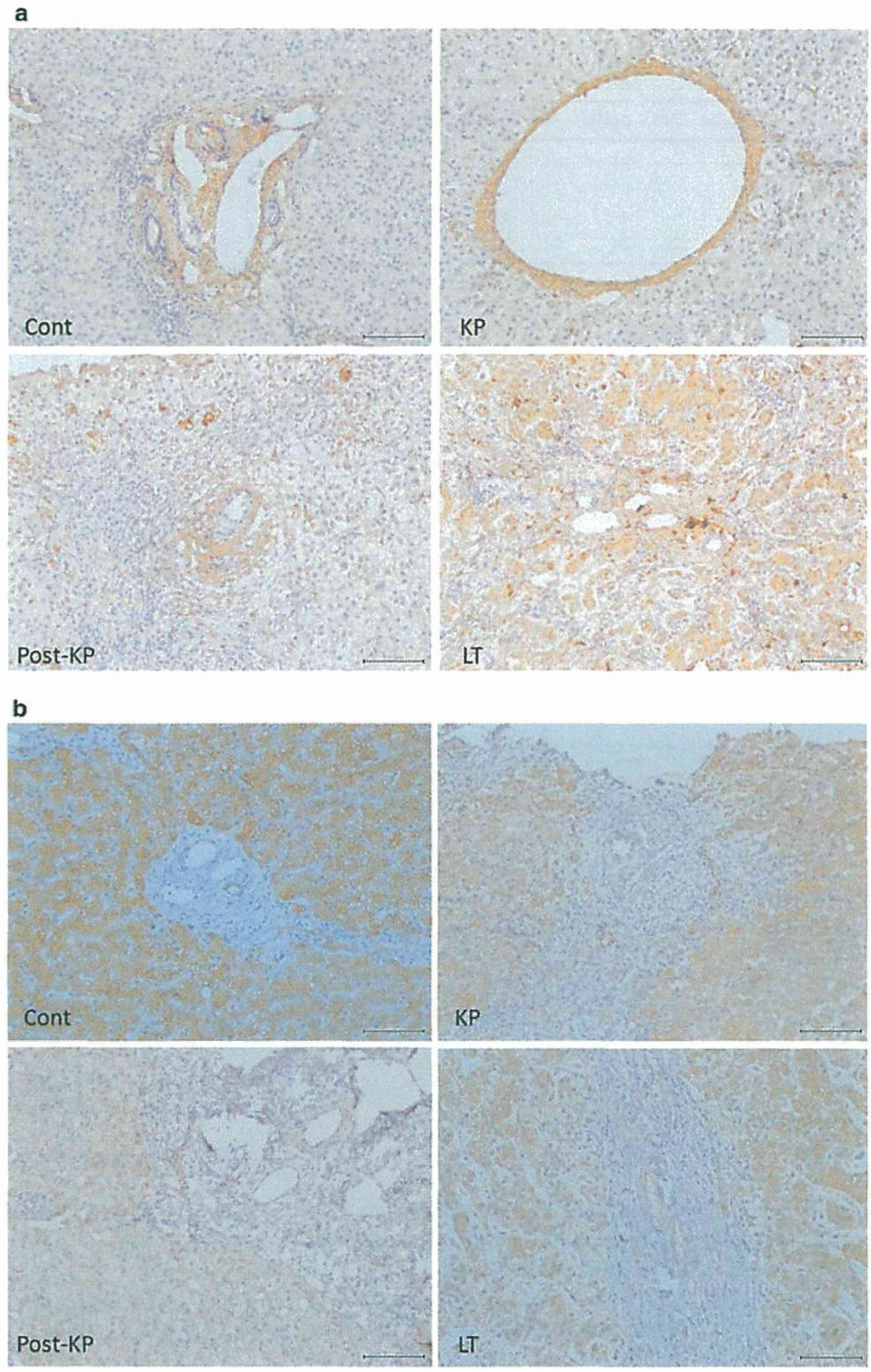

Fig. 1 The protein expression of apelin (**a**) and apelin receptor APJ (**b**) was immunolocalized in liver sections of control (*Cont*) and the stages of BA, including Kasai procedure (*KP*), *Post-KP* and liver transplantation (*LT*). **a** The control liver tissue showed that apelin was mainly localized in the perivenular areas (large portal vein and ventral vein; white arrows), and slightly detected in the HSC and hepatocytes. Apelin was observed mainly in perivenular areas and capillaries in *KP* and *Post-KP* liver tissue, and slight to moderately in HSC. Intense apelin immunoreactivity was detected mainly in perivenular areas, HSCs and hepatocytes in *LT* liver tissue. **b** APJ was almost undetected in perivenular areas but detected mainly in the hepatocytes in controls and all stages of BA

according to New Inuyama classification, as: F0, no fibrosis; F1, fibrous portal expansion; F2, bridging fibrosis; F3, bridging fibrosis with architectural distortion; and F4, liver cirrhosis [13].

Statistical analysis

Data were entered into the SPSS (Chicago IL) 16.0 software package. Mann–Whitney *U* test, Kruskal–Wallis test,

Table 1 Intensity of apelin and APJ immunostaining in different stages of BA

	CO		KP		Post-KP		LT	
	Apelin	APJ	Apelin	APJ	Apelin	APJ	Apelin	APJ
Perivenular area	2+	–	2+	+	2+	+	3+	+
HSC	+	–	+	–	2+	–	3+	–
Hepatocytes	+	2+	2+	2+	2+	2+	3+	3+

The intensity of immunostaining was scored as: –, none; 1+, weak; 2+, moderate; 3+, intense

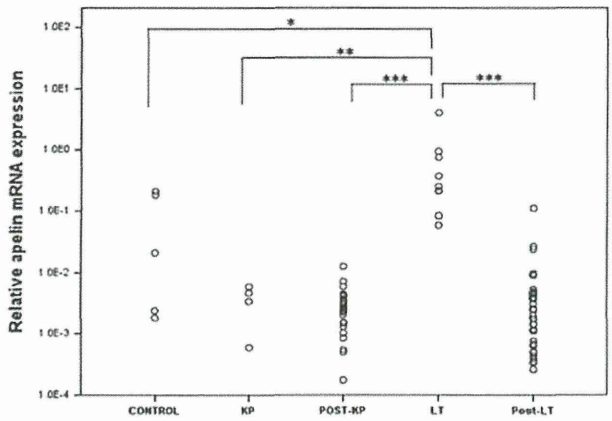

Fig. 2 Relative mRNA expression in the livers of BA patients. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Wilcoxon signed rank test, and Spearman's correlation coefficient were used. Significance levels were set at $p < 0.05$.

Results

Immunohistochemical expression of apelin and its receptor APJ (Fig. 1a, b)

Apelin was mainly localized in the perivenular areas (large portal vein and ventral vein), in control liver tissue and slightly detected in the HSC and hepatocytes. Apelin was observed mainly in perivenular areas in KP and Post-KP liver tissue, and slightly to moderately expressed in HSC and hepatocytes. Intense apelin immunoreactivity was detected in perivenular areas, HSC and hepatocytes in LT liver tissue (Fig. 1a). APJ was mainly and markedly detected in the hepatocytes, but almost undetected in the perivenular area and HSC. Slight APJ expression was also observed in perivenular areas at the KP, Post-KP and LT stage (Fig. 1b) (details summarized in Table 1).

Table 2 Details of BA patients that provided specimens in several stages

Patients	Sex	Age when the operation was performed		
		KP	Post-KP	LT
1	M	67 days	191/331 days[a]	518 days
2	F	74 days	383 days	–
3	M	88 days	–	242 days
4	F	–[b]	4.2/4.6 years[a]	–
5	F	–[b]	3.1/4.1 years[a]	–
6	F	–[b]	183 days	265 days
7	F	–[b]	408 days	977 days

[a] Patient underwent a needle liver biopsy after KP twice

[b] Patient underwent KP in other institution

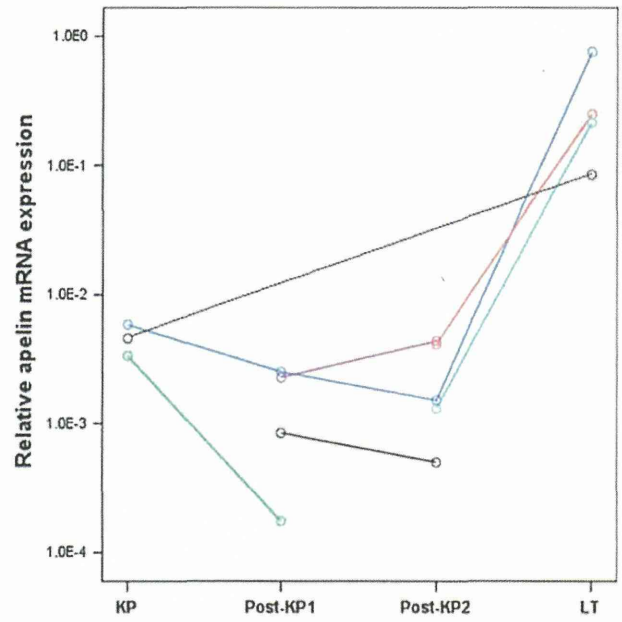

Fig. 3 Changes in the relative *apelin* mRNA expression in the same patients during the progression of BA

mRNA expression of apelin in liver tissue in BA

Apelin mRNA expression was significantly higher in the LT group than in the control, KP, Post-KP and Post-LT groups (Fig. 2: LT versus CO, $p < 0.05$; LT versus KP, $p < 0.01$; LT versus Post-KP, $p < 0.001$, and LT versus Post-LT, $p < 0.001$).

The alteration of apelin expression during the progression of BA was also analyzed in the same patients (see the details in Table 2). The result showed that *apelin* mRNA expression levels in the samples from the patients that underwent LT (patient 1, 3, 6 and 7, Fig. 3) were increased nearly ten-fold in comparison to the samples taken from them in the KP and Post-KP stages ($p = 0.068$).

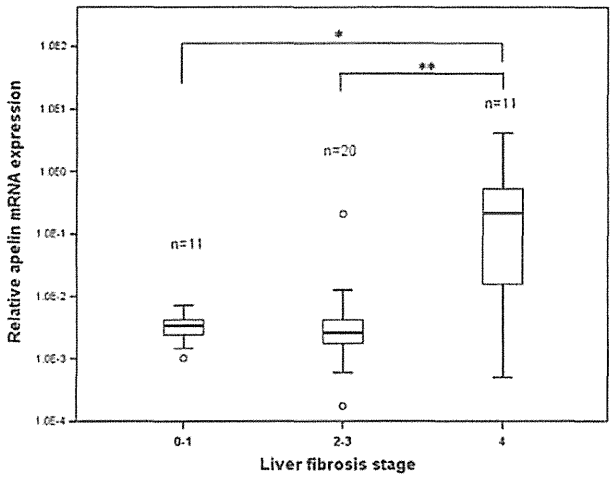

Fig. 4 Apelin increases with the progression of fibrosis in BA patients. The number of patients per group is shown above each bar. *Boxes* encompass the 25th to 75th percentile, *horizontal lines* represent the median (50th percentile), and *whiskers* extend to the smallest and highest values. (*$p < 0.05$, **$p < 0.01$)

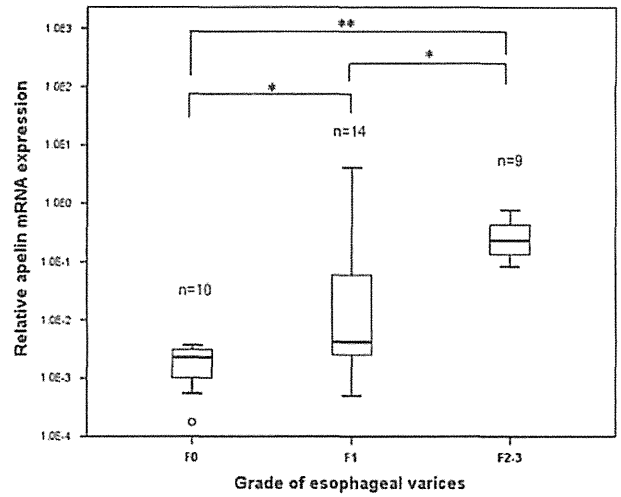

Fig. 5 *Apelin* mRNA expression with the progression of esophageal varices in BA patients. (*$p < 0.05$, **$p < 0.01$)

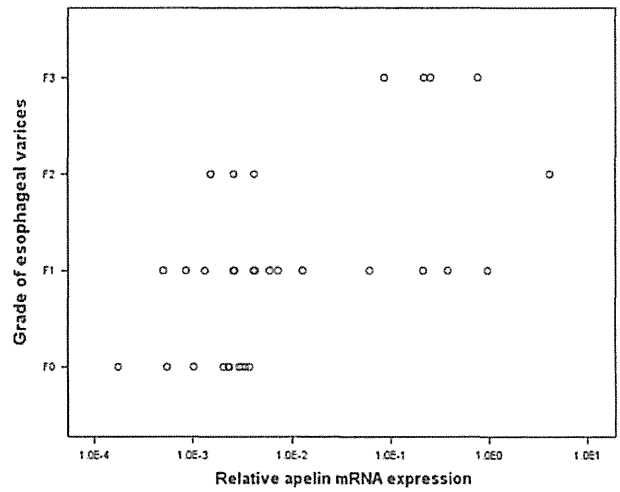

Fig. 6 Correlation between the relative *apelin* mRNA expression and the grade of esophageal varices in BA. (Spearman's $r_s = 0.522$, $p < 0.01$)

Forty-two liver samples from BA patients of KP, Post-KP and LT were subjected to real-time PCR in order to assess *apelin* mRNA expression during the progression liver fibrosis. *Apelin* mRNA expression was significantly higher at fibrosis stage 4 than at stage 0–1 and stage 2–3 (stage 4 versus stage 0–1, $p < 0.05$; stage 4 versus stage 2–3, $p < 0.01$: Fig. 4).

Endoscopy was performed in 32 patients to evaluate the grade of esophageal varices. *Apelin* mRNA expression was significantly correlated with grade of esophageal varices, a grade-dependent upregulation of *apelin* mRNA expression increased significantly during the progression of esophageal varices (grade 2–3 (F2–3) versus grade 0 (F0), $p < 0.01$; grade 1 (F1) versus grade 0 (F0) and grade 2–3 (F2–3), $p < 0.05$; Fig. 5). A significant correlation was found between the *apelin* mRNA expression in the liver tissues and the grade of esophageal varix ($r_s = 0.522$, $p < 0.01$; Fig. 6).

The relative apelin RNA expression level was significantly correlated with the serum TB level ($r_s = 0.520$ $p < 0.001$), but did not correlate with the value of AST, ALT or GGP (Table 3).

Discussion

Apelin and its receptor system have attracted widespread research interest and their pathophysiological roles are gradually emerging [14, 15]. Studies assessing the cell distribution of apelin and APJ in normal and cirrhotic human and rat liver tissues demonstrated a strong positive signal for apelin and APJ in the liver of cirrhotic animals [9] and humans [16]. Therefore, an immunohistochemical study was performed to establish whether apelin or APJ were expressed in different stages of BA liver. The results show for the first time that apelin was expressed in liver tissue of BA patients. The finding that apelin was mainly identified in HSCs and perivenular areas, especially strongly in cirrhotic liver and that APJ was mainly identified in hepatocytes is consistent with previous studies [9, 16]. However, the strong detection of apelin in hepatocytes in a cirrhotic liver has not been reported. These results indicate that apelin and the APJ system in liver tissue are also active in pediatric patients with BA, and beyond its hepatic paracrine presence in the cirrhotic liver, apelin may

Table 3 Correlation between the expression of *apelin* mRNA and laboratory data in BA patients

	Correlation coefficent	Statistical significance
TB	0.527	<0.0001
AST	0.095	n.s.
ALT	0.109	n.s.
GGP	−0.238	n.s.

Spearman correlation test

n.s. not significant

also behave as an autocrine substance in the BA liver. Hepatocytes themselves, therefore, may play an important role in the progression of liver fibrosis in BA.

Furthermore, the current study extracted total mRNA from BA liver and performed real-time PCR to assess *apelin* mRNA expression during the progression of liver fibrosis. The results showed that the *apelin* mRNA expression was significant higher in end-stage (LT) than controls, early-stage (KP, Post-KP) BA, and decreased to normal in comparison to the controls after liver transplantation. These results of mRNA expression were consistent with the findings of apelin protein expression detected by immunohistochemistry. These findings suggest that apelin may be a significant predictor of poor prognosis (need liver transplantation) in BA patients. Although the statistical analysis showed no significant alternations of *apelin* mRNA expression in the same patients during the progression of BA that may be due to the limited number of cases, because the expression in those four patients was much higher at the end stage than the early stage. In addition, the current study showed that *apelin* mRNA expression was significantly correlated with the level of serum TB; however, there was no association with liver function (AST, ALT, and GGP). This finding may indicate that apelin is associated with cholestasis, but is not affected by liver inflammation.

The development of the KP improved the prognosis for children with biliary atresia [17–20]. Despite the increasing number of patients who survive jaundice-free for an indefinite period after KP, liver fibrosis progresses in many patients [21, 22] and the risk of gastrointestinal (GI) bleeding due to HP is why BA continues to be the leading indication for pediatric liver transplantation. The current study showed a grade-dependent upregulation of *apelin* mRNA expression in liver tissue with the progression of cirrhosis and esophageal varices in BA, which demonstrated that apelin can accurately reflect the severity of cirrhosis and esophageal varices in BA and therefore could be used as a prognostic factor to estimate the timing of liver transplantation in BA patients.

These results suggest that apelin could be used as a clinical parameter in evaluating the severity of cirrhosis and esophageal varices.

Principe et al. [9] reported that rats with cirrhosis treated with the apelin receptor antagonist showed diminished hepatic fibrosis and vessel density, improved cardiovascular performance, and renal function and lost ascites. These findings suggest that the apelin–APJ system could be a candidate for a therapeutic target of anti-fibrosis and anti-portal-hypertension treatment. Further investigation is needed to establish the clinical application of the apelin–APJ system in patients with BA.

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