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Recurrence of Progressive Familial Intrahepatic Cholestasis Type 2 Phenotype After Living Donor Liver Transplantation: A Case Report

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Abstract

Background. Progressive familial intrahepatic cholestasis 2 (PFIC2) is the result of mutations in the *ABCB11*, which encodes for bile salt export pump (BSEP). An absence of BSEP in the canalicular membrane causes cholestasis and leads to the development of end-stage liver disease in the first decade of life. Liver transplantation (LT) has been considered curative for BSEP disease. However, patients with PFIC2 having undergone liver transplantation have recently been reported to develop recurrence of cholestasis together with the clinical and histological features of primary BSEP disease.

Case Report. We herein present a rare case of a patient with PFIC2 who developed post-transplant recurrence of progressive intrahepatic cholestasis due to antibodies against BSEP after living donor liver transplantation, which mimicked primary BSEP disease. The patient had mutations in the *ABCB11* gene, resulting in the complete absence of BSEP in the native liver, explaining the lack of tolerance. Immunofluorescence staining of normal human liver sections with the patient's serum and using an anti-human IgG antibody to detect serum antibodies showed reactivity to the BSEP epitope in the canalicular membrane. We suggest that the patients having undergone LT had been associated with a risk of autoantibody formation against the BSEP protein. The absence of primary tolerance for the BSEP epitopes may explain the formation of the anti-BSEP antibodies after LDLT.

Highlights

・We present a rare case of a patient with progressive familial intrahepatic cholestasis 2 who developed post-transplant recurrence of progressive intrahepatic cholestasis due to antibodies against bile salt export pump.

・This is the first case of progressive familial intrahepatic cholestasis 2, in which the patient developed post-transplant recurrence after living donor liver transplantation.

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Introduction

Progressive familial intrahepatic cholestasis (PFIC) causes the development of severe cholestasis, which progresses to cirrhosis and liver failure. PFIC is a heterogeneous group of rare genetic autosomal recessive diseases, and is categorized into three types. Progressive familial intrahepatic cholestasis type 2 (PFIC2) is typically characterized by mutations of the *ABCB11* gene, which encodes the bile salt export pump (BSEP) [1]. BSEP is expressed in the canalicular membrane of hepatocytes and is responsible for bile salt excretion into the biliary system [2]. An absence of BSEP results in the impairment of bile salt excretion into bile, with consequent serum bile salt elevation, pruritus, and severe liver disease in childhood [3, 4]. Absence of BSEP progresses to cirrhosis and liver failure in the first decade of life. Liver transplantation (LT) has been considered curative for BSEP disease [5-7]. However, in some patients with PFIC2 disease, recurrence has been observed after LT mimicking a PFIC phenotype. Several research groups have shown that an inhibitory antibody against BSEP most likely causes disease recurrence [8-12]. The present report describes a patient with PFIC2 who underwent living donor liver transplantation (LDLT) with a liver graft from his father. The recipient's clinical symptoms and laboratory findings improved after LDLT, the patient developed symptoms of cholestatic liver disease with pruritus without rejection, mimicking the PFIC2 phenotype at 6 months after LDLT, and which eventually progressed toward graft failure. We present this recurrent case of PFIC2 after LDLT that mimicked the PFIC2 phenotype and discuss the mechanism of anti-BSEP antibody formation.

Case report

The patient was born at 40 weeks' gestation by normal vaginal delivery. His birth weight was 3062 g. The prenatal history was uneventful. Jaundice appeared gradually at 1 month of age. Biliary

atresia was suspected initially, but no evidence was detected by ultrasonography. Laboratory findings demonstrated hyperbilirubinemia. Elevated transaminases and serum total bile acids (TBA) persisted, whereas gamma-glutamyl transferase (GGT) levels were normal at all times. Liver biopsy and histological examination at 6 months of age demonstrated cholestasis, fibrosis, and giant cell transformation. On the basis of these findings, he was diagnosed with PFIC-2, which has a compound heterozygous mutation of the *ABCB11* gene. Genetic testing revealed a heterozygous mutation in the *ABCB11* gene (c. T1425A, c.953_954delAA) that resulted in p. C475X and p. Lys318ArgfsX26. This finding indicated an absence of the BSEP protein. The patient developed end-stage liver disease due to PFIC2, with low GGT levels. At the age of a year and eleven months, the patient underwent LDLT with a left lateral segment obtained from his father to improve the severe pruritus and progressive hepatic failure. Histological examination of the explanted liver revealed nodular cirrhosis with hepatocanalicular cholestasis, giant cell transformation, and fibrosis. Immunohistochemistry with BSEP antibodies was abnormal with no canalicular or hepatocyte staining in the native liver (Fig 1); this indicated an absence of the BSEP protein. Transplant immunosuppression was induced with tacrolimus and corticosteroids postoperatively. The patient's post-transplant course was complicated by biopsy-proven acute cellular rejection, massive lobular necrosis, portal vein occlusion, and refractory ascites. However, his condition improved gradually by a change in the primary immunosuppressant and by reoperation. The laboratory findings and pruritus improved after the transplantation, and the patient was discharged from the hospital at 70 days after the transplant operation. This patient was medically managed with tacrolimus and corticosteroids after discharged from a hospital.

However, within a year and 7 months after transplantation, the patient again developed symptoms of cholestatic liver disease with pruritus. Laboratory findings demonstrated hyperbilirubinemia,

elevated transaminases, and plasma bile salt concentrations. Clinical course of the patient after LDLT was summarized in Table 1. Histological examination of the liver biopsy revealed cholestasis and ballooning of hepatocytes with no evidence of rejection (Fig 2). The clinical course of the low-GGT cholestasis was suggestive of antibody-mediated recurrent PFIC2. To investigate the pathological evidence of antibodies against BSEP, the patient's serum taken after the appearance of the post-transplant cholestasis was used for immunohistochemical analysis with BSEP antibodies. Anti-BSEP antibodies was analyzed. Multidrug resistance protein 2 (MRP2) on the canalicular membrane of hepatocytes corresponded with the same location in the hepatocytes where the antibodies derived from the patient's serum had localized (Fig 3a). This result showed that IgG antibodies derived from the patient's serum were targeting the canalicular membrane of hepatocytes *in vivo* and antibodies against BSEP at a 1:16000 titer. To prove the existence of BSEP-specific antibodies in the patient's serum, BSEP was introduced into a plasmid-encoding BSEP fused to the enhanced yellow fluorescent protein (EYFP). Human embryonic kidney (HEK293) cells were transiently transfected with BSEP-EFYP and expressed the BSEP-EFYP on the plasma membrane. We investigated whether IgG antibodies derived from the patient's serum targeted the HEK cells which transfected the BSEP-EFYP. As a result, IgG antibodies of the patient's serum targeted the HEK cells which transfected the BSEP-EFYP *in vitro* (Fig 3b). These findings were considered diagnostic for antibody-mediated recurrent BSEP disease. The patient was medically managed with pulse steroids and tacrolimus. At the stage after starting the immunosuppressive treatment, he had a convulsions accompanied by impaired consciousness. Magnetic resonance imaging of the brain showed the hyperintense signals in the parietal lobe on fluid attenuated inversion recovery T2 weighted images (MRI-FLAIR) (Fig 4a). As we suspected that his clinical condition was explained by posterior reversible encephalopathy syndrome, the tacrolimus was withdraw and

immunosuppressive treatment was changed to cyclosporine. The next few days after using the cyclosporine, he again had a convulsions accompanied by impaired consciousness, hemiplegia and seizures. A magnetic resonance scan of the brain showed the hyperintense signals in the occipital lobe of the cerebrum on MRI-FLAIR (Fig 4b). Subsequently, cyclosporine was also withdraw and a calcineurin inhibitor sparing regimen was started by everolimus. His clinical status and laboratory findings failed to improve. Therefore, treatment for recurrent PFIC2 was initiated with plasmapheresis (PP) for the removal of existing BSEP antibodies, followed by intravenous immunoglobulin (IVIG) (1g / kg / dose), and then rituximab (375mg / m^2), a chimeric monoclonal anti-CD20 antibody, to prevent the synthesis of anti-BSEP antibodies. This treatment resulted in decreased serum anti-BSEP antibody levels, and the patient's cholestasis improved. However, the effects of treatment lasted only temporarily. Symptoms of cholestasis and pruritus rapidly reoccurred after the end of the treatment. While awaiting a second transplantation, his condition became much worse due to septicemia and necrotizing pancreatitis. He finally eventually died of gastrointestinal bleeding at three years and four months after LDLT.

Discussion

The recurrence of a PFIC2 phenotype after LT was first reported by Keitel et al. in 2009 [8]. Several research groups subsequently reported that inhibitory antibody against BSEP most likely causes disease recurrence [9-12]. It is estimated that the prevalence of recurrent BSEP disease is 8% [12]. The risk of disease recurrence after LT depends on the cause of the patient's liver failure. Recurrence of autoimmune disease after LT, such as primary sclerosing cholangitis or primary biliary cirrhosis, is a well known problem [13]. To our knowledge, this post-transplant recurrence among the three types of PFIC has been reported so far only in patients with PFIC2 [11, 14]. Kubitz

et al. were able to detect anti-BSEP antibodies in seven more patients with post-transplant PFIC2 [15]. Recent investigations have demonstrated that graft failure after LT in patients with PFIC2 was found to be caused by antibodies against BSEP [8, 9, 11, 15]. Recurrence of PFIC2 after LT is extremely rare. We have identified 12 other recurrent cases of PFIC2 (in addition to the present case) after LT described in the literature, with the findings summarized in Table 2 [8-12].

Among the recurrent cases of PFIC2 after LT, only one case (present case) underwent LDLT.

All 13 patients had mutations in both alleles of the *ABCB11* gene (Table 2). Analysis of the mutations suggests that the BSEP protein would have been congenitally absent in all of the reported patients. Patient age at the first transplantation ranged from 10 months to 9 years (mean, 3.2 years). The indications for LT were almost always cholestasis with end-stage liver disease and pruritus that was refractory to medical treatment. The time to onset of cholestasis without rejection after LT ranged from 9 months to 17 years. For most cases, the onset of cholestasis might have been related to a reduction in immunosuppressive therapy, but we could not find the precipitating factor. Cholestasis was followed by onset of recurrent low GGT cholestasis, and histological findings of liver biopsy showed intrahepatic cholestasis, fibrosis, and giant cell transformation without rejection. Five patients underwent subsequent transplantation (Table 2). The indications for retransplantation after recurrence of PFIC2 were cholestasis with pruritus that was refractory to medical treatment, cholestasis with end-stage liver disease, or growth failure [12]. In cases with retransplantations, the time to onset of first pruritus after LT ranged from 10 months to 3 years (mean, 1.5 years). On the other hand, in the other 8 patients, the time to onset of first pruritus after LT ranged from 1.6 months to 12 years (mean, 5.9 years). The time to onset of pruritus after LT tended to be shorter in cases with retransplantations compare to the other cases. Previous studies have reported that absence of BSEP expression has been observed in many cases of PFIC2; however,

antibodies against BSEP formation after LT have not been found to be common among these patients [10, 12]. The following is a possible cause for this phenomenon. It has been suggested that severe graft damage, such as that caused by acute rejection or a severe perfusion injury after the first transplantation, may lead to the release of a large amount of BSEP protein derived from the donor graft. BSEP protein derived from the graft may trigger the expression of BSEP epitopes by antigen-presenting cells [8]. Consequently, the induction of antibodies against BSEP is conjectured to be produced by B cells in the allograft recipient. To our knowledge, 4 of the 13 reported PFIC2 patients developed severe graft damage after the first LT, whereas the other patients had no evidence of severe graft damage after LT. We suggest that the relevance of this occurrence remains controversial.

Once anti-BSEP antibodies have developed, this disease has been proven to be refractory to immunosuppressive treatment that would typically be effective in treating allograft rejection. There is no proven therapy for antibody-mediated post-transplant BSEP disease. It has been reported that a treatment consisting of rituximab, a chimeric monoclonal anti-CD20 antibody, in combination with IVIG and PP is an appropriate approach to treating the condition of antibody-mediated recurrent BSEP disease [10, 15]. However, in the present patient, the efficacy of this treatment lasted only temporarily, and the patient had relapsing features of the recurrence of a PFIC2 phenotype in a short period. Four of the 13 patients who experienced recurrence of PFIC2 after LT eventually died, whereas 9 patients were reported to yet be alive.

Post-transplant cholestatic disease is due to the binding of anti-BSEP antibodies at an extracellular, intracanalicular epitope, which blocks the transport function of BSEP [16]. It has been reported that IgG can be internalized by hepatocytes and transcytosed from blood to bile [17, 18]. Furthermore, antibodies against BSEP have a blocking function, and this has been demonstrated in rats that

received intravenous administration of serum including BSEP antibodies [9].

In the present case, BSEP at the canalicular membrane in the native liver had not been expressed, as shown in the liver biopsy (Fig 1). It is suggested that the complete absence of BSEP expression in the native liver is responsible for the failure to develop auto-tolerance toward BSEP. Consequently, BSEP derived from the graft liver is recognized as a foreign antigen after transplantation [16]. The mechanism of the graft failure in the recurrence of PFIC2 after LT is related to the formation of autoantibodies against BSEP derived from the graft liver [15, 16]. In the present case, *in vivo* findings were supported *in vitro* by a comparable membrane co-localization of IgG antibody derived from the patient in transfected HEK293 cells (Fig 3), and were found to have high-titer anti-BSEP antibodies. In line with these findings, we discovered evidence to support the existence of antibodies against BSEP, and diagnosed the post-transplant recurrence of PFIC2.

In summary, the recurrence of a PFIC2 phenotype after organ transplant from a brain-dead donor has been reported by several literatures, but this is the first case of PFIC2, in which the patient developed post-transplant recurrence due to autoantibodies against BSEP after LDLT. Although LT, including LDLT, is an effective treatment modality with good outcome and little morbidity in patients with PFIC2, antibody-mediated post-transplant BSEP disease is a major problem, and the clinical course and outcome of patients are still not satisfactory due to severe cholestasis. A review of the mechanisms discovered by several previous studies have shown that the recurrence of PFIC2 after LT is related to the formation of autoantibodies against BSEP derived from the graft liver. We recommend that patients who are undergoing LT for BSEP deficiency be monitored for the development of BSEP antibodies, and receive corresponding management of their immunosuppressive therapy. In the future, we suggest that further accumulation of cases and elucidation through immunological and genetic research are vitally important, and would contribute

toward establishing a protocol that is needed for the treatment of antibody-mediated post-transplant BSEP disease.

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Conflict of interest: The authors declare that they have no conflicts of interest.

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Table1

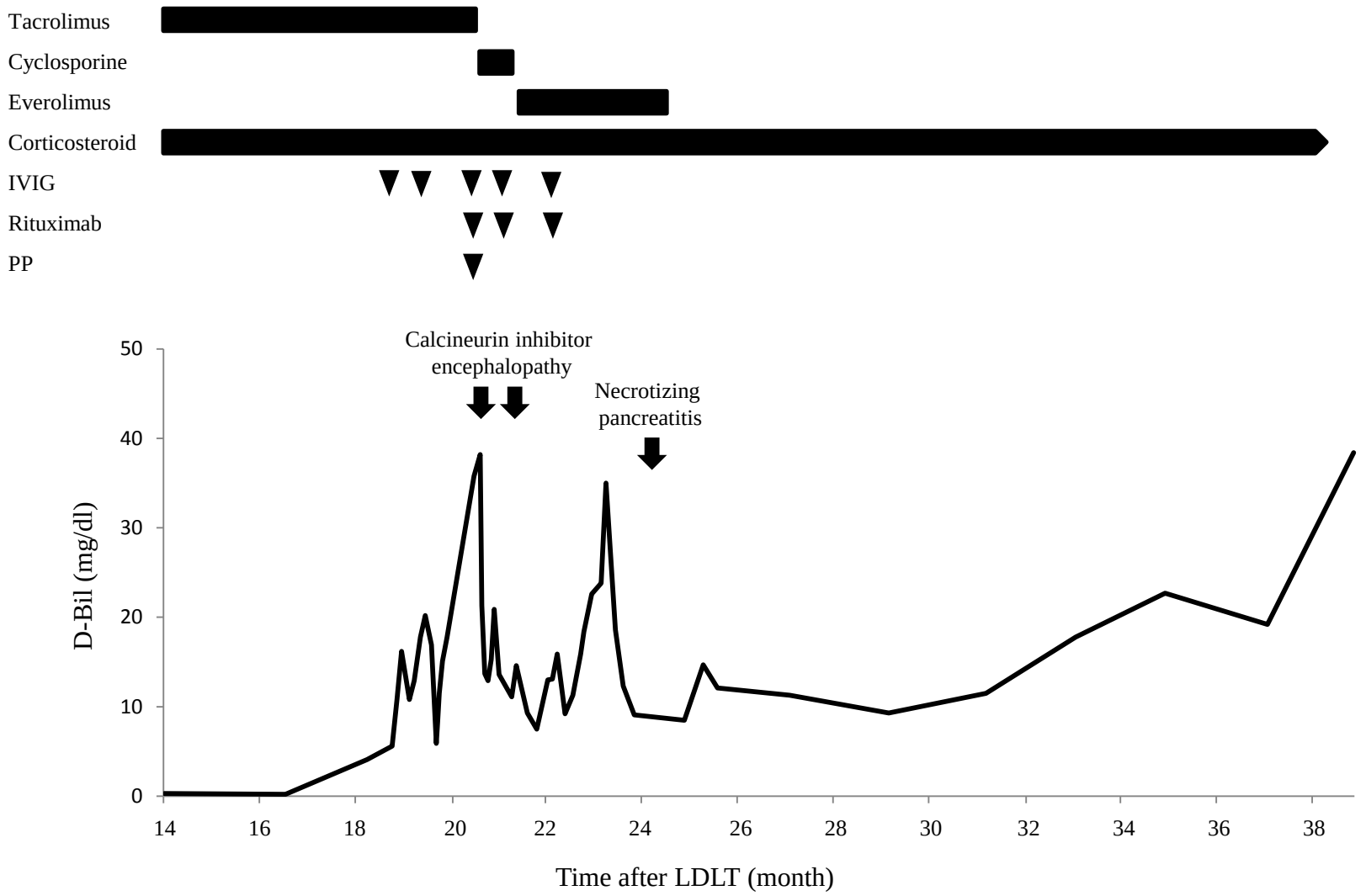

Table. 1.

Table2

Case [Reference]	Genetics findings	Age at LT (years)	Number of LT (times)	Pruritus recurrence after LT (years)	BSEP expression (native liver)	Complications after first LT	Immunosuppression after recurrence of PFIC-2	Prognosis
1 [8, 12]	Homozygous: c1331T＞C: p.Val444Ala c.2453A>T; p.Tyr818Phe c.2944G>A; p.Gly982Arg	3.5/3.5/4.9	3	1/0.4	Negative	Severe perfusion injury with massive necrosis	Basiliximab, Predonisolone, Tacrolimus, PP, Rituximab	Death
2 [9]	Homozygous: c907A>G; p.R303G	5.2	1	12/3.5/5.2	Negative	None	Tacrolimus, Corticosteroids, MMF	Disappearance of jaundice
3 [9]	Compound heterozygous: c1741C>T IVS12+1G>T; p.L581F splice site disruption	3.7	1	8.1/12/13	Negative	None	Tacrolimus, Corticosteroids, MMF	Disappearance of jaundice
4 [9]	Homozygous: IVS17+1T>A; splice site disruption	2.2	1	2.1/4	Negative	None	Tacrolimus, Azathioprine, Corticosteroids, MMF	Disappearance of jaundice
5 [11]	Homozygous: c.77-19T>A; p.Y26lfs7X	2.8	1	4.8	Negative	Acute rejection	Cyclosporine, Prednisone, Azathioprine	Disappearance of jaundice and alive at the age of 22
6 [11, 12]	Compound heterozygous: c.301delCA; p.Glu101AspX9 c.2944G>A; p.Gly982Arg	9	1	3.3/17	Negative	None	Cyclosporine, Prednisone, IVIG	Death
7 [12]	Homozygous: c.908+1G>A, splice site mutation	2.4	3	0.8	Negative	None	NA	Death
8 [12]	Compound heterozygous: c.1145-1165 deletion; pAla382_388del c.2012-8T>G; splice site mutation	3	2	3/8/8.6	Negative	None	NA	Pruritus persist and alive at age of 16
9 [12]	Compound heterozygous: c.1941delA; pGly648ValfsX6 c.2012-8T>G; splice site mutation	1.8	1	3.2/3.6	Negative	None	NA	Pruritus persist and alive at age of 7
10 [12]	Compound heterozygous: c.2787_2788insGAGAT; p.Lys930GlufsX79 c.1442T>A; p.val481Glu	0.8	2	1/1.4	Negative	None	NA	Pruritus persist and alive at age of 9
11 [10]	Homozygous: c.2787_2788insGAGAT; p.Lys930GlufsX79	0.8/3.5	2	1.7/5	Negative	Acute rejection	MMF, Cyclosporine, PP, Rituximab, IVIG	No recurrence of cholestasis
12 [10]	Homozygous: c.1639(-2) A>C; splice site disruption	5	1	12	Negative	None	Corticosteroids, PP, Rituximab, IVIG	No recurrence of cholestasis
Present case	Compound heterozygous: c.953_954delAA; p.Lys318ArgfsX26 c.T1425A; p.C475X	1.9	1	1.6	Negative	Acute rejection, Refractory ascites	Corticosteroids, PP, Rituximab, IVIG	Death

Table. 2.

Figure legends:

Fig. 1. Immunohistological findings of the native liver with BSEP antibodies showed the absence of canalicular BSEP.

Liver tissues from the control (a) and the patient (b) were stained for anti-BSEP antibody. In the control, canalicular membrane of hepatocytes expressed BSEP protein (arrow). In contrast, the patient showed no canalicular BSEP .

Fig. 2. Histological findings of the liver biopsy after recurrence of cholestasis showed ballooning of hepatocytes, but there was no evidence of rejection. (a, b) Liver tissue obtained by biopsy and stained with hematoxylin and eosin.

Fig. 3. IgG antibodies derived from the patient's serum exhibited recognition for BSEP.

(a) Immunofluorescence staining of human IgG antibody (red) and multidrug resistance protein 2 (MRP2) (green). IgG antibody derived from the patient's serum and MRP2 are co-localized at the canalicular membrane *in vivo*. (b) BSEP was fused to the enhanced yellow fluorescent protein (EYFP). Human embryonic kidney (HEK293) cells were transiently transfected with BSEP-EFYP and expressed the BSEP-EFYP on the plasma membrane. Immunofluorescence staining of human IgG antibody (red). IgG antibody derived from the patient's serum and BSEP-EYFP (green) are co-localized *in vitro*.

Fig.4. Images from the patient who developed posterior reversible encephalopathy syndrome in the brain while receiving immunosuppressive therapy.

(a) Magnetic resonance imaging of brain showed the hyperintense signals in the parietal lobe

(arrow) on fluid attenuated inversion recovery T2 weighted images (MRI-FLAIR). (b) Magnetic resonance imaging of brain showed the hyperintense signals in the occipital lobe of the cerebrum (arrow).

Table 1. Clinical course of the patient with recurrence of cholestasis after LDLT.

Table 2. Summary of PFIC2 patients with recurrence of cholestasis after LT.

Abbreviations: LT, liver transplantation; IVS, intervening sequence; BSEP, bile salt export pump; PFIC2, progressive familial intrahepatic cholestasis type 2; MMF, mycophenolate mofetil; PP, plasmapheresis; IVIG, intravenous immunoglobulin.

Figure1

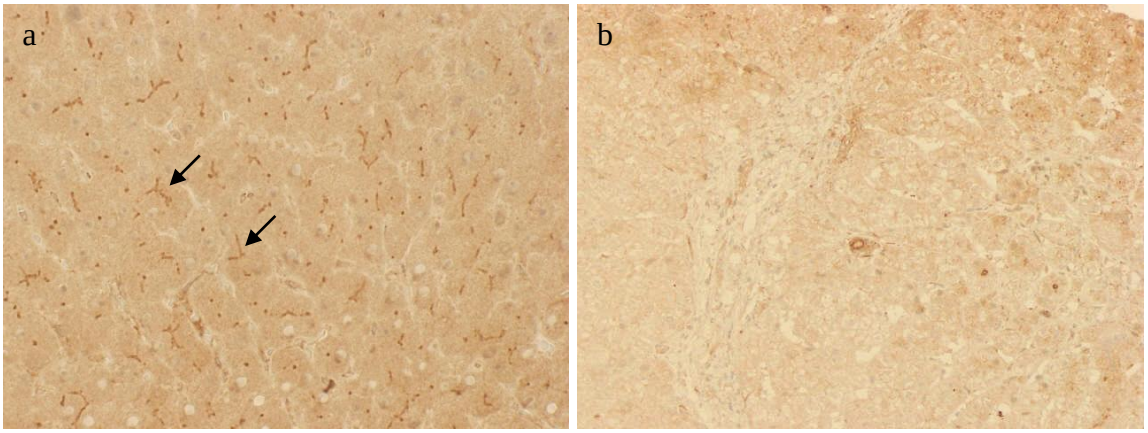

Fig. 1.

Figure2

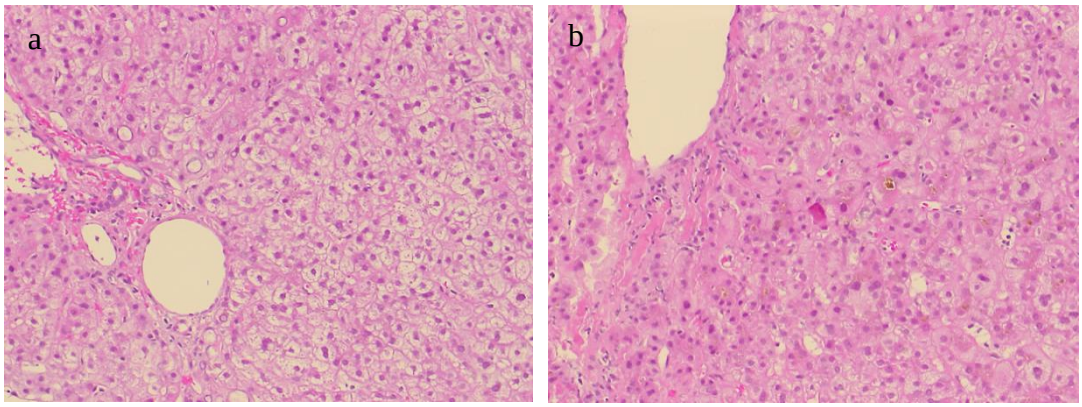

Fig. 2.

Figure3

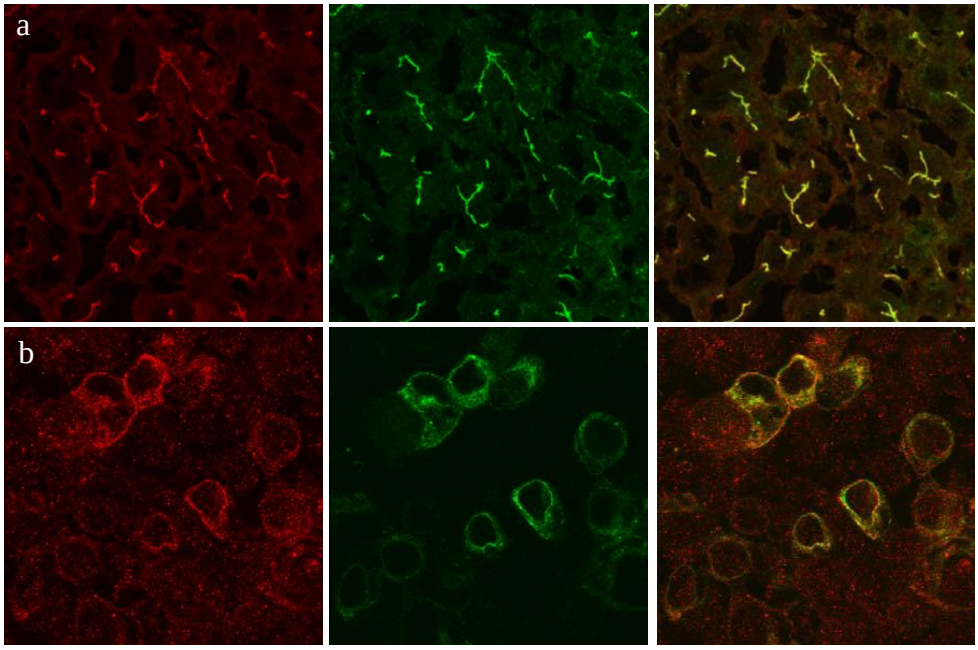

Fig. 3.

Figure4

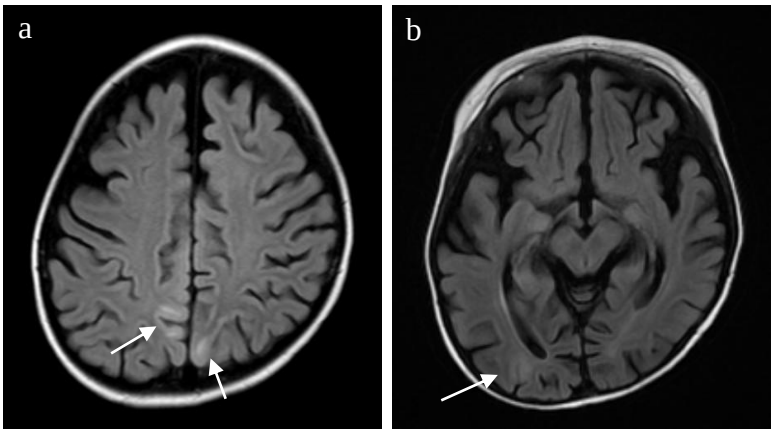

Fig. 4.