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BRIEF REPORT

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Attempted treatment of factor H deficiency by liver transplantation

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Abstract Complement factor H (FH) deficiency is one of the causes of atypical hemolytic uremic syndrome (HUS). Most patients with FH deficiency associated HUS progress to end-stage renal disease despite plasma therapy. Moreover, the disease invariably recurs in the graft kidney and causes graft failure. We confirmed FH deficiency in a 30-month-old boy with recurrent HUS of 2 years duration, and attempted an auxiliary partial orthotopic liver transplantation (APOLT) to overcome the sustained intractable dependency on plasma therapy. APOLT restored the plasma FH level, without HUS recurrence, for 7 months. However, thereafter he suffered from serious infectious complications associated with immunosuppression and finally died 11 months after APOLT. In conclusion, although APOLT showed clinical and laboratory improvement for some period in this patient, the final fatal outcome suggests that liver transplantation should be cautiously applied to patients with HUS associated with FH deficiency.

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Introduction

Hemolytic uremic syndrome (HUS) is a clinical syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. In most pediatric cases, HUS follows a prodrome of diarrhea (D+ HUS) typically caused by shiga-like toxin-producing *Escherichia coli*. However, atypical cases without preceding diarrhea (D– HUS) also occur, showing a tendency to relapse, to occur in families, and to have a poor outcome [1, 2, 3].

Inherited complement factor H (FH) deficiency is one of the recently identified causes of D– HUS [4, 5, 6, 7, 8]. FH is a serum glycoprotein that acts as a cofactor with factor I (C3b inactivator) and regulates the alternative complement pathway [9]. Plasmapheresis or plasma infusion can ameliorate the clinical symptoms of FH deficiency associated HUS, possibly by supplementing FH [10]. However, the effect of plasma therapy is transient, and the disease usually cannot be controlled by means other than continuous plasma therapy over an indefinite period. Most patients eventually progress to end-stage renal disease, regardless of plasma therapy. In addition, HUS invariably recurs in the graft kidney and causes graft failure [1, 2, 8, 11]. Given that the liver is the main source of plasma FH, liver transplantation should be considered as an alternative, and as possibly a better and more fundamental method of treatment.

We attempted an auxiliary partial orthotopic liver transplantation (APOLT) in a 30-month-old boy with recurrent D– HUS associated with FH deficiency. He had been intractably dependent on plasma transfusion for more than 2 years. We describe the clinical course and the result of APOLT.

Materials and methods

Patient

A previously healthy 3-month-old Korean boy was brought to our hospital after 2 days of vomiting, pallor, and dark urine. He showed no diarrhea or fever. The family history was negative for renal or hematological diseases. Petechiae were detected on the abdomen and the thighs. His blood pressure was 84/40 mmHg, body weight 6.5 kg, and height 63.6 cm. His hemoglobin was 5.4 g/dl, platelet count $40\times10^3/\mu$l, reticulocytes 5.6%, and direct and indirect Coombs′ tests were negative. Many fragmented erythrocytes were seen in the peripheral blood smears. Urinalysis revealed albuminuria (3+) and many red blood cells. Blood urea nitrogen was 53 mg/dl, serum creatinine 1.1 mg/dl, albumin 2.2 g/dl, and lactic dehydrogenase 1,423 U/l. Serum C3 and C4 levels were 52 mg/dl (normal 70–150 mg/dl) and 22 mg/dl (normal 10–35 mg/dl), respectively. His mother, being asymptomatic, had some fluctuations in her C3 level (64–98 mg/dl). His father's C3 level was normal (123 mg/dl). The patient's plasma FH level, measured by Western blot, was 24% of the normal level. This plasma was sampled 48 h after a fresh-frozen plasma (FFP) infusion (20 ml/kg). His mother had a FH level that was 48% of the normal level and his father had a FH at 90% of the normal level (Fig. 1).

The patient was treated with FFP infusions and was discharged in remission after 1 month. However, over the course of the next 2 years, he was readmitted 14 times due to HUS recurrence. FFP had to be used almost daily during each admission, and one to three times a week to maintain remission at the outpatient clinic between admissions. During the last two admissions, ventilator therapy on two occasions and acute peritoneal dialysis on another occasion were required to deal with the complicating dilated cardiomyopathy, pulmonary edema, and acute renal failure.

At the age of 30 months, APOLT was undertaken, with his father as the donor. This procedure was approved by the Ethics Committee of Organ Transplantation, Seoul National University Hospital.

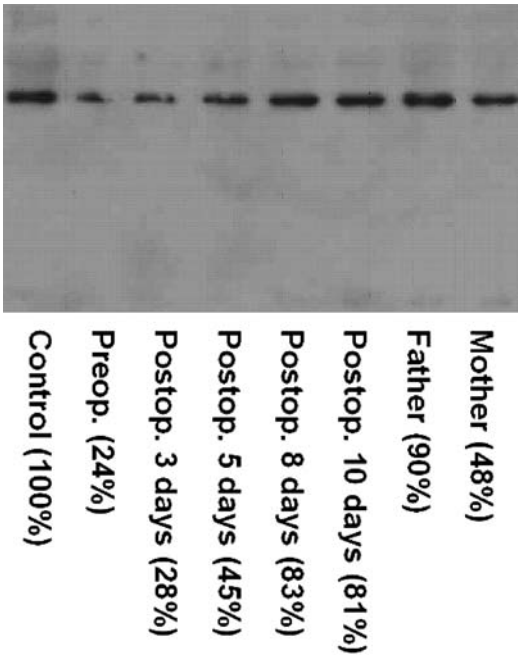

Fig. 1 The plasma factor H (FH) levels measured by Western blot. The plasma FH levels in the patient increased and were maintained above 80% of normal from the 8th postoperative day. His mother showed heterozygous FH deficiency and his father was normal

Western blot analysis of plasma FH

Plasma FH levels were measured by Western blot analysis using an ECL kit (Amersham Life Science, Buckinghamshire, UK). Goat polyclonal anti-FH antiserum (Calbiochem, San Diego, Calif., USA) was used as a primary antibody (1:2,500 dilution) and a horseradish peroxidase-labeled donkey anti-goat IgG (Santa Cruz Biotechnology, Santa Cruz, Calif., USA) as a secondary antibody (1:2,500 dilution). The pooled plasma of five healthy volunteers was used as a normal control, and the density of the 155-kilodalton FH band was measured using an Imaging Densitometer (Model G5700, BioRad, Richmond, Calif., USA) and Molecular Analyst Software, version 1.5 (BioRad).

Mutational analysis of the FH gene

Total RNA was purified from a piece of liver tissue resected during APOLT using Trizol (Gibco BRL, Grand Island, N.Y., USA), and cDNA was reverse transcribed from the RNA using an Advantage RT-for-PCR kit (Clontech, Palo Alto, Calif., USA). Ten overlapping DNA fragments covering the entire coding sequence of FH cDNA were amplified by polymerase chain reaction (PCR) and directly sequenced. In addition, genomic DNAs were extracted from the peripheral blood of the patient and of his parents. Abnormal sequences detected in the patient's FH cDNA were confirmed by direct sequencing of the genomic DNA PCR products of the patient, his parents, or of 20 normal controls.

Auxiliary partial orthotopic liver transplantation

The left lateral segment of the liver was harvested in the same way as for an ordinary living-donor liver transplantation. The weight of the graft was 260 g. After resecting the left liver in the patient, the left lateral segmental graft was orthotopically transplanted. Biliary drainage was accomplished with a Roux-en-Y hepatico-jejunostomy. Immunosuppressive therapy consisted of tacrolimus (0.5 mg/kg per day) and low-dose steroids (prednisolone 1 mg/kg per day). Microscopic examination of the resected left liver of the patient revealed grade 2/4 hemosiderin deposition.

Results

The surgical procedures were uneventful. The plasma FH level increased and was maintained above 80% of normal from the 8th postoperative day, with simultaneous normalization of peripheral blood counts and serum complement levels (Fig. 1). Plasma therapy was not required postoperatively.

The FH cDNA revealed a heterozygous missense point mutation substituting ^{926}Cys(TGT) in exon 17, the fourth Cys residue in the short consensus repeats (SCR) 15, with Phe(TTT). This mutation was inherited from his mother. In addition, ^{62}Val(GTA) in exon 2 was replaced by Ile(ATA) in one allele of the patient and the patient's father and in two alleles in the mother (Fig. 2). The genotype of this nucleotide change was determined in the 20 normal controls, and GG, GA, and AA genotypes were found in 5 (25%), 13 (65%), and 2 (10%), respectively.

After discharge on the 20th postoperative day, all laboratory findings were normal except for microscopic hematuria. Three months after APOLT, post-transplant lymphoproliferative disease, due to the Epstein-Barr virus, with high fever and cervical lymphadenopathy

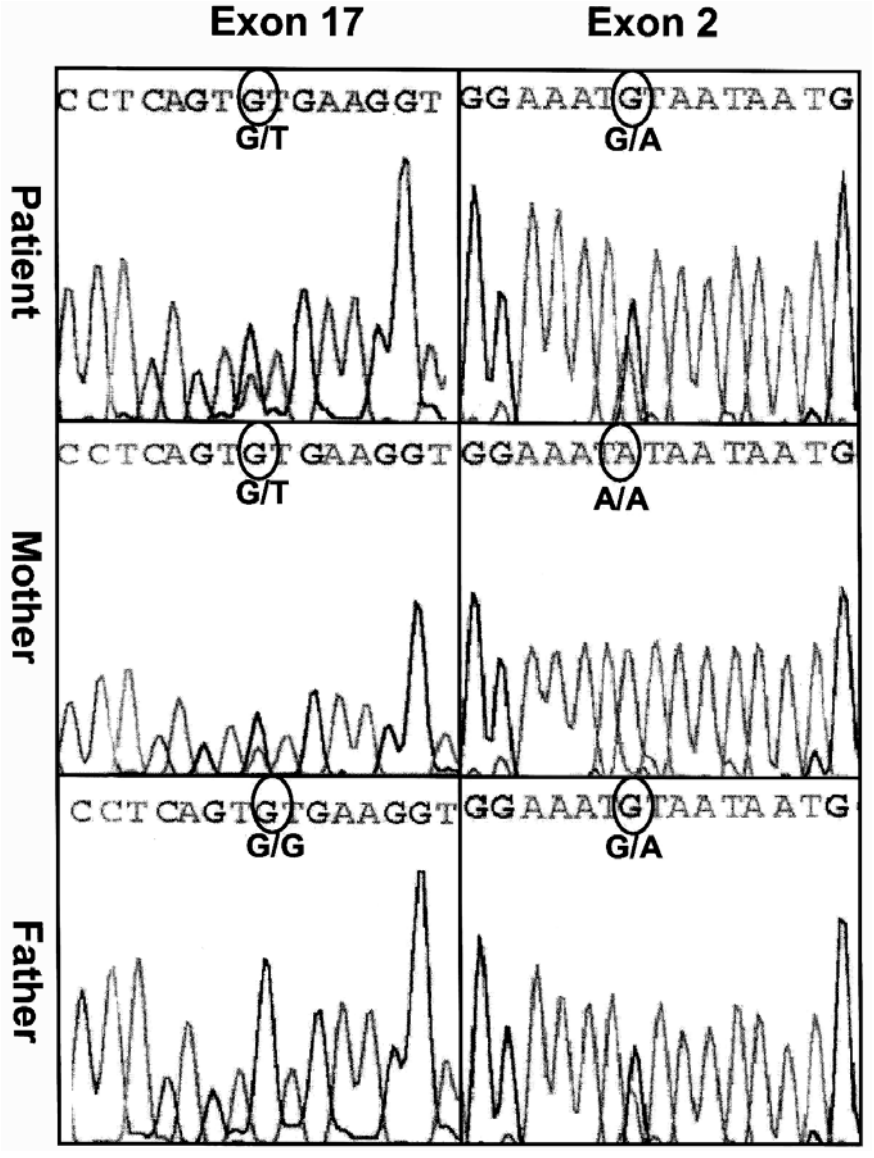

Fig. 2 Partial sequencing data of the FH gene. In exon 17, a G >T heterozygous missense point mutation was noted in the patient and in his mother, but not in the father. In exon 2, a G >A heterozygous nucleotide change was noted in the patient and in the father, and a homozygous change in the mother

developed. This was controlled by using a decreasing dose of tacrolimus and four weekly doses of rituximab, without serious problems. Seven months after APOLT, pancytopenia (hemoglobin 8.2 g/dl, white blood cells $1.52\times10^3/\mu$l, and platelets $136\times10^3/\mu$l) and azotemia (serum creatinine 3.2 mg/dl) developed. However, evidence of hemolysis was minimal (reticulocytes 3.2%, plasma hemoglobin 6.1 mg/dl). Serum C3 (72 mg/dl) and FH (80% of normal) levels were nearly normal, and the tacrolimus serum level was 5.2 ng/ml. At that time, we could not completely exclude the possibility of a recurrence of the original HUS or of tacrolimus-induced HUS. Thus, we first changed the medication from tacrolimus to cyclosporine, and subsequently we tried plasma transfusion. Neither of these measures worked, and hemodialysis was started to manage the complicating congestive heart failure, hypertension, and azotemia. Because of the persistent anemia with a low reticulocyte count (0.1%), a bone marrow biopsy was performed 1 month later. This showed a hypocellular marrow with pure red cell aplasia. Parvovirus B19 PCR was positive in the urine, serum, and bone marrow. Five doses of intravenous immunoglobulin (5 g/dose) were given for the parvovirus infection, but his anemia did not improve. Unfortunately, shortly thereafter sepsis with pneumonia caused by a methicillin-resistant *Staphylococcus aureus* led to further deterioration. Finally, in spite of the vigorous antibiotics and supportive therapy, including hemodialysis and ventilator therapy, he expired 11 months after APOLT. The direct cause of death was cardiac failure due to the aggravation of a preexisting dilated cardiomyopathy.

Discussion

The majority of patients with D– HUS associated with FH deficiency are hard to manage clinically, and progress to end-stage renal disease despite continuous plasma ther-

apy, which only has a transient effect [1, 2]. Moreover, the disease invariably recurs in the graft kidney and results in graft failure [1, 2, 8, 11]. The later clinical course is predicted to follow a fatal dependency on plasma therapy involving other organ complications, especially of the heart. This scenario forced us to consider an alternative therapy in this patient, one that could be instituted before renal function had deteriorated significantly. Thus, because the liver is the source of most plasma FH [12], we decided to perform a liver transplantation. APOLT has an advantage, as the graft liver can be safely removed and unnecessary immunosuppressive therapy avoided in the case of graft failure or lack of HUS amelioration. Whatever the exact pathogenetic role of FH in renal diseases may be, indirect evidence points to an improvement in HUS on resuming normal FH supply from a successfully grafted liver. The benefit of plasma therapy indicates that the presence of a plasma factor, and FH is the most likely factor. Recently, Remuzzi et al. [13] reported a successful simultaneous kidney and liver transplantation in a child with HUS associated with FH deficiency. The plasma FH level was restored and HUS did not recur.

D– HUS and membranoproliferative glomerulonephritis type II (MPGN II) are the most common renal diseases associated with FH deficiency [1, 2, 14]. In general, MPGN II is usually associated with absence of plasma FH (homozygous deficiency) due to the inactivation of both alleles, whereas D– HUS is associated with suboptimal FH activity (heterozygous deficiency) due to one intact and one defective allele. [1] We did not know the basal plasma FH level of our patient. The level measured 48 h after the previous FFP infusion (20 ml/kg) was 24% of the normal level. In a study [11] that measured serial serum FH levels in a patient with FH deficiency (the basal FH level was <6 mg/dl, and the normal level is >60 mg/dl), the FH levels 0.5 and 48 h after FFP administration (20 ml/kg) were 23 mg/dl and about 15 mg/dl, respectively. According to these data, our patient was presumed to have a very low basal level of FH, corresponding to homozygous deficiency, whereas, his mother had heterozygous deficiency.

By FH gene analysis, we found two heterozygous nucleotide variations in the patient, both of which were predicted to cause substitutions of wild type amino acid residues (^{926}Cys to Phe and ^{62}Val to Ile). The FH molecule is composed of 20 repetitive CCP modules, each of which contains four cysteine residues. These residues play an essential role in the protein folding of each CCP, by forming disulfide bridges. In fact, many of the reported missense point mutations involve the substitution of one of these conserved cysteine residues. [8] Hence, the ^{926}Cys to Phe mutation in this patient may be pathogenic. However, the second variation, ^{62}Val to Ile, is believed to be a polymorphism, because it was also detected in some of the normal controls. These genetic data are discordant with the plasma FH levels. The patient was biochemically homozygous and genetically heterozygous. The different plasma FH levels between the patient and his mother, both of whom had the same heterozygous mutation, could not be explained either. A possibility still remains that the ^{62}Val to Ile mutation is functional.

Although APOLT normalized the FH deficiency and prevented the recurrence of hemolytic episodes for 7 months, our patient had a fatal outcome. Moreover, the recurrence of HUS could not be completely excluded during the later downhill course, which mainly resulted from a series of infectious complications.

Therefore, one must be cautious before applying liver transplantation in patients with HUS associated with FH deficiency. APOLT may be the most suitable surgical procedure in this situation to reduce mortality associated with graft failure.

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