Liver transplantation for aHUS: still needed in the eculizumab era?

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Abstract

Background

The risk of disease recurrence after a kidney transplant is high in patients with atypical hemolytic uremic syndrome (aHUS) and mutations in the complement factor H (FH) gene (*CFH*). Since FH is mostly produced by the liver, a kidney transplant does not correct the genetic defect. The anti-C5 antibody eculizumab prevents post-transplant aHUS recurrence, but it does not cure the disease. Combined liver–kidney transplantation has been performed in few patients with *CFH* mutations based on the rationale that liver replacement provides a source of normal FH.

Methods

We report the 9-year follow-up of a child with aHUS and a *CFH* mutation, including clinical data, extensive genetic characterization, and complement profile in the circulation and at endothelial level. The outcome of kidney and liver transplants performed separately 3 years apart are reported.

Results

~~During the post-kidney transplant period, t~~The~~he~~ patient showed This sentence refers to the period preceeding the kidney transplant incomplete response to plasma, with relapsing episodes, progression to end-stage renal disease, and endothelial-restricted complement dysregulation. Eculizumab prophylaxis post-kidney transplant did not achieve sustained remission, leaving the child at risk of disease recurrence. A liver graft given 3 years after the kidney transplant completely abrogated endothelial complement activation and allowed eculizumab withdrawal.

Conclusions

Liver transplant may definitely cure aHUS and represents an option for patients with suboptimal response to eculizumab.

Keywords

Atypical hemolytic uremic syndrome
Eculizumab
Kidney transplantation
Liver transplantation
Complement pathway
Alternative
Rare diseases

Electronic supplementary material

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is a disease which is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure associated with dysregulation of the alternative pathway of complement. Dysregulation of this ~~alternate~~alternative pathway ~~includes~~ is caused by loss-of-function mutations in genes encoding the complement regulatory proteins, such as factor H (FH), membrane cofactor protein (MCP), factor I (CFI), and thrombomodulin, or gain-of-function mutations in genes encoding the two components of the alternative pathway~~,~~ C3-convertase, complement C3 (C3) and factor B (CFB), or anti-FH autoantibodies [1]. Mutations in the complement factor H gene (*CFH*) are the most commonly reported mutations, accounting for about 30 % of cases. Before the introduction of the anti-C5 monoclonal antibody eculizumab for the treatment of aHUS, about 70 % of patients with *CFH* mutations died or reached end-stage-renal disease (ESRD) within a year of presentation [2]. In patients with *CFH* mutations, the risk of aHUS recurrence after a kidney transplant is very high, ranging from 60 to 80 % [3–5]. Since FH is mostly produced by the liver, a kidney transplant does not correct the genetic defect in these patients, who remain at risk of recurrence. The natural history of the disease has been challenged by the introduction of eculizumab [6], which also effectively prevents post-transplant aHUS recurrence [7]. However, eculizumab does not cure the disease—rather it prevents the deposition of the C5b-9 complement complex on the microvascular endothelium and protects kidney transplant patients from disease recurrence. Whether patients with aHUS with or without a renal transplant should continue the lifelong prophylactic use of eculizumab, or whether its use can be interrupted at any time, has not yet been established. Combined liver–kidney transplantation has been performed in a small number of patients with *CFH* mutations who had progressed to ESRD, based on the rationale that liver replacement provides a source of normal FH, which should then prevent disease recurrence in the kidney graft [8–13].

This approach was adopted—successfully in most cases [9]—mainly before eculizumab treatment was available. Whether combined transplant is still an option for these patients, in the era of anti-complement therapies, is the subject of lively debate [14].

Here we report the case of a child with a *CFH* mutation in whom eculizumab prophylaxis post-kidney transplant could not control hematological signs of disease activity, leaving the child at risk of disease recurrence. A liver graft given 3 years after the kidney transplant completely abrogated complement activation at the endothelial level, allowed eculizumab withdrawal, and effectively cured the disease.

Methods

Ex-vivo studies with human microvascular endothelial cells ~~of dermal origin~~ and aHUS serum

Human microvascular endothelial cells of dermal origin (HMEC-1 line, a kind gift from Drs. Edwin Ades and Francisco J. Candal of the CDC and Dr. Thomas Lawley of Emory University, Atlanta, GA) were plated on glass coverslips and used when confluent. Cells were activated with 10 μM ADP (Sigma-Aldrich, St. Louis, MO) for 10 min and then incubated for 4 h with serum (from the patient or from a pool of 10 healthy controls) diluted 1:2 with test medium (Hanks' Balanced Salt Solution + 0.5 % bovine serum albumin). At the end of incubation HMEC-1 cells were stained with the rabbit anti–human C5b-9 complement complex (Calbiochem, San Diego, CA), followed by fluorescein isothiocyanate-conjugated secondary antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA), as previously reported [15]. Fluorescent staining on the endothelial cell surface was acquired by confocal inverted laser microscopy and quantified as pixel^2 per field, as reported earlier [15]. For each sample, we considered the mean of 15 fields, and values were expressed as the percentage of deposits induced by control pool serum run in parallel.

Genetic screening and anti-FH autoantibodies

Genomic DNA was extracted from blood leukocytes (BACC2 kit; Nucleon PhytoPure system; Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The coding sequence and the intronic flanking regions were sequenced directly (AB-3130-XL sequencer) [2]. Anti-FH autoantibodies were tested using an enzyme-linked immunosorbent assay as reported previously [2]. *CFHR3-R1* deletion was studied using the multiple ligation probe amplification assay [16].

Serum and plasma complement profile

Complement C3 levels in serum were measured by nephelometry [2]. Total residual complement activity in serum (CH50) was measured by the MicroVue CH50 Eq EIA kit (Quidel Corp., San Diego, CA). Levels of the soluble terminal complement complex SC5b-9 were evaluated in plasma using MicroVue SC5b-9 Plus EIA kit (Quidel Corp.). To evaluate plasma SC5b-9 levels, blood collected in ice-cold EDTA tubes was immediately centrifuged at 4 °C to avoid ex vivo complement activation, and the plasma was quickly separated and frozen at −80 °C until the assay.

Results

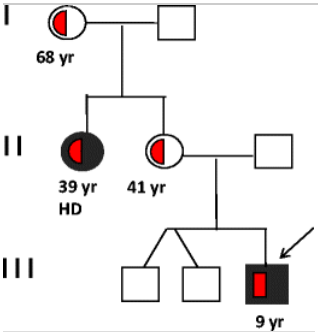
Onset and family and clinical history

Part of the pre-liver transplant course of this patient has been previously published (case #5 in [15]). Briefly, in November 2006 a 6-month-old Caucasian boy presented with hematuria and paleness following a urinary tract infection, with no history of diarrhea. He had severe anemia (hemoglobin 6.6 g/dL), thrombocytopenia (platelet count 50,000/μL), schistocytes in blood smear (20 % of red blood cells), and elevated lactate dehydrogenase (LDH; 9,000 U/L). Serum creatinine was elevated (1 mg/dL), with an estimated glomerular filtration rate of 30 mL/min/1.73 m^2, calculated using the Bedside Schwartz equation [17, 18].

C3 and C4 serum levels were normal. Both parents and two older twin brothers were in good health, while the child's maternal aunt had aHUS at the age of 26 years, progressing within a few weeks to ESRD. She was on dialysis and had refused a kidney transplantation (Fig. 1). Clinical presentation and family history led to a diagnosis of aHUS.

Fig. 1

Family pedigree. The pedigree of the proband with atypical hemolytic uremic syndrome (aHUS) carrying a c.3645 C > T mutation in the complement factor H gene (*CFH*) that caused the p.S1191L change is shown. *Squares* Male family members, *circles* female family members, *arrow* the proband. *Black circles/squares* Affected subjects, *red half-circles/square* healthy carriers of the mutation. Ages at last follow-up are shown. *yr* Years, *HD* hemodialysis treatment



The proband was treated according to contemporary guidelines [19] with plasma infusions [9 sessions of 10 ml/kg with fresh frozen plasma (FFP) substitution]. He experienced transient remission, but a relapse occurred after 2 weeks out of ~~of~~ treatment, with an upper respiratory tract infection followed by oliguric acute kidney injury. Peritoneal dialysis (PD) was performed for 7 days, and after 17 FFP infusions (10 ml/kg) and six plasma-exchange (PE) sessions, clinical remission was achieved. Following three additional relapses, in an attempt to prevent further episodes, maintenance PE was given twice a week and then once a week. However, over the following 14 months, the child experienced eight relapses, and at the age of 22 months he developed ESRD [Fig. 2; Electronic Supplementary Material (ESM) Fig. 1]. He was treated with PD for 2 years. He had several peritoneal catheter infections and was switched to hemodialysis (HD) after suffering from fungal peritonitis. He had a feeding tube from the age of 2 years, his weight was always below the 3rd percentile for age, and his height was in the 25th percentile. The child was hypertensive (125/80 mmHg, >97th percentile), which was well controlled with combined renin–angiotensin blockers, amlodipine, and calcium antagonists. The child's general condition was stable, and he showed no signs of extra-renal vascular damage.

Fig. 2

Treatments, platelet count, serum creatinine and deposition of the serum-induced C5b-9 complement complex on ADP-activated human microvascular endothelial cells ~~of dermal origin~~ (HMEC-1) in the proband during 106 months of follow-up from disease onset. *Oblique double lines* mark changes in the timescale. C5b-9 deposits were evaluated after incubation (4 h) with serum (diluted 1:2 with test medium) from the proband taken 1 month before the kidney transplant (*pre-ktx*) and before the start of eculizumab treatment (*pre-ecu*), at 15 months post-kidney transplant after eculizumab treatment ~~(~~[8 days after 300 mg eculizumab (*post-ecu 300*) and 12 days after 600 mg eculizumab (*post-ecu 600*)], at 25 months post-kidney transplant [6 days after 600 mg eculizumab (*post-ecu 600*)], at 3 weeks post-liver transplant and 7 days after 600 mg eculizumab (*post-ltx ecu 600*), and at 7.5 months after liver transplant [120 days after the last eculizumab dose (*post-ltx ecu stop*)]. Values were expressed as the percentage of deposits induced by serum from a pool of 10 healthy controls run in parallel. *Green arrows* Times of eculizumab pulse for kidney and liver transplants, respectively, *black arrows* times of kidney and liver transplants, respectively, *red arrow* time of eculizumab discontinuation. *Horizontal dashed lines*: **panel a** The lower limit of the normal range for platelet count (150,000/μL), **panel b** the upper limit of the normal range for serum creatinine (0.8 mg/dL), **panel c** the upper limit of the normal range for serum-induced C5b-9 deposits (~~><~~150 % of deposits induced by control serum pool, determined by testing serum from 10 different volunteers)

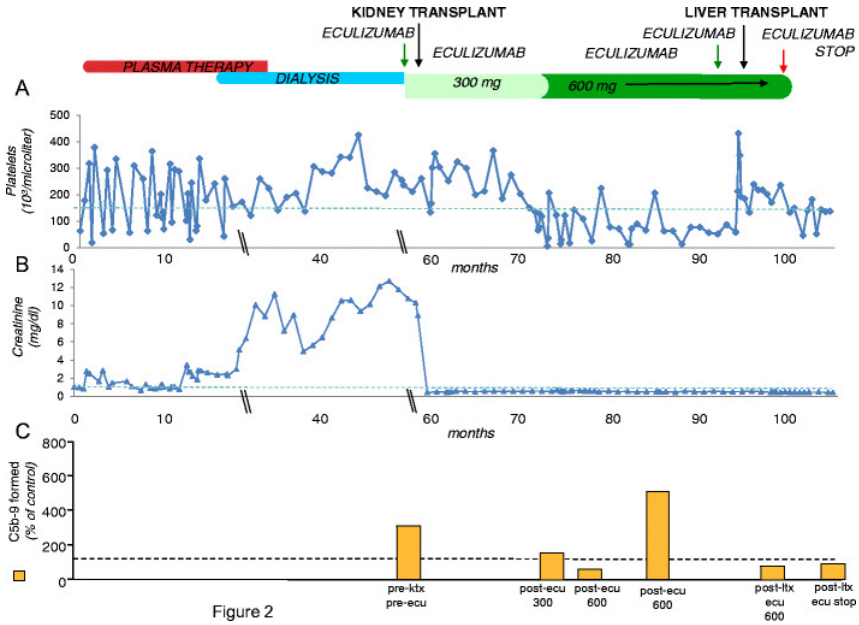


Figure 2

Genetic screening

In the proband, his healthy mother, his aunt (who was on chronic dialysis), and his healthy grandmother, we found a heterozygous c.3645C > T *CFH* mutation resulting in the amino acid change p.S1191L and in defective FH capacity to control complement activation on the cellular surface [20]. The proband was also found to be heterozygous for the *CFH* single nucleotide polymorphisms (SNPs) c.-332C > T, c.2016A > G, and c.2808G > T, which target the aHUS at-risk haplotype H3 [21, 22], and heterozygous for the last c.*897 T > C SNP of the *MCP*ggaac at-risk haplotype [23] (Table 1). In addition to the c.3645C > T *CFH* mutations, the affected maternal aunt was found to be heterozygous for the c.-332C > T *CFH* SNP and homozygous for the c.*897 T > C *MCP* SNP, while the unaffected mother was identified as carrying the c.*897 T > C *MCP* SNP in heterozygosity. No mutations were found in *MCP*, *CFI*, *C3* and *CFB*, either in the proband or in the affected aunt. Screening for deletion of the *CFHR3/R1* genes and for serum anti-FH autoantibodies in the proband was negative, and plasma ADAMTS13 activity measured in different blood samples was normal (65–97 % by the collagen binding assay; normal range 50–150 %) [24]. The proband was also screened for known gene variants previously associated with the risk of thrombosis, but no mutations or the c.20210G > A (p.R506Q, factor V Leiden) risk variant in the prothrombin gene were found [25], although he was found to be heterozygous for the c.677C > T (p.A222V) variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene [26] (Table 1).

Table 1

Results of genetic screening in the proband, the affected aunt, and the unaffected mother

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Gene	Mutation/variant (DNA)	Mutation/variant (protein)	Proband	Mother	Aunt
Complement factor H	c.3645C > T[a]	p.S1191L	Heterozygous	Heterozygous	Heterozygous
	c.-332C > T		Heterozygous	Wild-type	Heterozygous
	c.2016A > G	p.Q672Q	Heterozygous	Wild-type	Wild-type
	c.2808G > T	p.E936D	Heterozygous	Wild-type	Wild-type
Membrane cofactor protein	c.*897 T > C		Heterozygous	Heterozygous	Homozygous
F2 (prothrombin)	c.20210G > A	p.R506Q	Wild-type	Not determined	Not determined
Methylenetetrahydrofolate reductase	c.677C > T[b]	p.A222V	Heterozygous	Not determined	Not determined

[a]The c.3645C > T mutation in the complement factor H (CFH) gene results in the amino acid change p.S1191L and in defective factor H (FH) capacity to control complement activation on the cellular surface. The proband, his healthy mother, his aunt (who was on chronic dialysis), and his healthy grandmother all had this mutation

[b]Proband was found to be heterozygous for the c.677C > T (p.A222V) variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene

Endothelial-restricted complement dysregulation

At about 5 years of age, while the patient was in hematological remission on HD, screening for markers of complement activation showed a normal complement profile in the peripheral blood, with serum C3 levels of 93 mg/dL (normal range 83–180 mg/dL) and plasma SC5b-9 levels of 111 ng/mL (normal range 127–400 ng/mL; Table 2). CH50 was at the lower limit of the normal range (72 U Eq/mL, 55 % of control values; Table 2). However, the newly developed ex-vivo test for endothelial-restricted complement activation [15] showed that serum from the patient deposited high amounts of C5b-9 on ADP-activated cultured microvascular endothelial cells (298 % deposition compared to control serum; normal values <150 %; Table 2; Fig. 2).

Table 2

Platelet count, plasma SC5b-9 complement complex, serum-induced complement C5b-9 deposits, total complement activity (CH50), and eculizumab levels during the follow-up

Follow-up (months)	Sampling	Platelets (normal range 150–400,000/μL)	SC5b-9 (normal values <400 ng/mL)	C5b-9 deposits (normal values <150 % deposition compared to control)[a]	CH50 (normal range 79–187 U Eq/mL)[b]	Eculizumab levels (μg/mL)[c]
57	1 month before kidney transplant	237,000	111.1	298 %	72 (55 %)	
58	Kidney transplant					
73	15 months post-ktx; 8 days post-eculizumab 300 mg[d]	67,000	195.0	152 %	3 (2.3 %)	165
73.5	15.5 months post-ktx 12 days post-eculizumab 600 mg[d]	119,000	213.9	43 %	3 (2.3 %)	418
79	21 months post-ktx 11 days post-eculizumab 600 mg[d]	225,000	167.9	76 %	6 (4.6 %)	478
83	25 months post-ktx 1 day post-eculizumab 600 mg	12,000	105.9	656 %		
83	25 months post-ktx 6 days post-eculizumab 600 mg[d]	72,000	129.3	557 %		595
95	Liver transplant					
95	8 h post-ltx and eculizumab 900 mg		147.9	53 %	0.074 (0.06 %)	
95	1 day post-ltx and eculizumab 900 mg		161.8	52 %	Undetectable	
96	3 weeks post-ltx 7 days post-eculizumab 600 mg[d]	190,000	288.3	114 %	2.5 (1.9 %)	
98.5	3 months post-ltx 14 days post-eculizumab 600 mg[d]	219,000	481.3	58 %	1.6 (1.2 %)	
98.5	Eculizumab discontinuation					
99	22 days post-eculizumab	218,000	639.0	85 %	49.1 (37 %)	
99.5	32 days post-eculizumab	202,000	123.2	48 %	49.5 (38 %)	
100	45 days post-eculizumab	171,000	323.7	118 %		
102.5	120 days post-eculizumab	46,000	181.6	140 %		
105	197 days post-eculizumab	137,000		125 %		

ktx, Kidney transplant; ltx, liver transplant

[a]Data are expressed as % of the deposition induced by serum from a pool of 10 healthy controls run in parallel

[b]Values of CH50 as % of mean control levels are given in parenthesis

[c]Trough levels, recommended values >99 μg/mL

[d]Measurements were done immediately before the subsequent dose of eculizumab

Altogether, the data indicate that the child had normal complement regulation in the fluid phase, but that his capacity to control complement activation on cellular surfaces was defective. This finding is consistent with the presence of the p.S1191L *CFH* mutation [27].

Kidney transplantation

As soon as eculizumab became available, the child was included on the transplant list. When he was 5.3 years old (August 2011; Fig. 2) he received a cadaveric kidney and eculizumab prophylaxis to prevent post-transplant recurrences [28]. Prior to the kidney transplant, the child received PE and 600 mg eculizumab (body weight 18 kg). Eculizumab was then infused on post-transplant days 1 (300 mg) and 7 (600 mg), and then on every other week thereafter (300 mg). The induction therapy included low-dose thymoglobulin and basiliximab,

and maintenance immunosuppression with steroids, cyclosporine and mycophenolate mofetil. Kidney graft function promptly recovered, and serum creatinine had decreased to 0.4 mg/dL on day 4 post-transplant.

Shortly after the administration of the first eculizumab dose, CH50 dropped steadily to undetectable levels. During the 3-month post-transplant platelet count, serum creatinine, haptoglobin, and hemoglobin concentration were within normal ranges (Fig. 2; ESM Fig. 1).

However, at 3-months post-transplant the child developed sepsis associated with *Burkholderia cepacia* colonization of the central venous catheter (CVC), leading to a drop in platelet count to 130,000/μL and in the haptoglobin concentration to 37 mg/dL (Fig. 2; ESM Figs. 1, 2), but both promptly recovered to normal range values upon resolution of the sepsis following CVC removal and meropenem therapy. Hemoglobin and LDH concentrations were normal throughout the sepsis episode.

Fourteen months after the transplant, when the patient's weight had increased to 21 kg, his platelet count began to progressively decrease, reaching 54,000/μL, without any evidence of hemolysis. Plasma levels of SC5b-9 remained normal (176 ng/mL). One month later the platelet count was still low (67,000/μL), even though eculizumab trough levels were in the therapeutic range (165 μg/mL; minimum recommended values 50–100 μg/ml; see http://ec.europa.eu/health/documents/community-register/2015/20150330131432/anx_131432_en.pdf). The CH50 level, measured 8 days after the last 300 mg eculizumab dose and just before the subsequent dose, was 3 U Eq/ml (2.3 %), indicating almost complete inhibition of circulating C5; plasma SC5b-9 levels were normal (Table 2). Renal function was normal (serum creatinine 0.52 mg/dL), hemoglobin was at the lower limit of normal (13.5 g/dL), LDH was slightly increased (560 IU/L), and haptoglobin was slightly decreased (35 mg/mL) (Fig 2; ESM Figs. 1, 2).

Serum from blood sampled at this point caused higher than normal C5b-9 deposition on ADP-activated endothelial cells (Table 2), suggesting that 300 mg eculizumab was not sufficient to efficiently prevent C5 activation at the endothelial cell level. The eculizumab dose was therefore increased to 600 mg approximately every other week; this increase in eculizumab dose was followed by increase in the platelet count and normalization of serum-induced endothelial C5b-9 deposits (Table 2).

Anti-platelet antibodies were negative. Fearing an adverse effect from the administration of mycophenolate, the child was switched to azathioprine 2 mg/kg. One month later the platelet count dropped to 6,000/μL during a varicella-zoster virus infection, without evidence of hemolysis; it subsequently normalized following four sessions of PE (Fig. 2; ESM Figs. 1, 2). Despite the intervals between eculizumab doses (600 mg) being reduced to 7–12 days, and although trough levels were in the high therapeutic range (Table 2), in subsequent months the platelet count dropped again, this time to 10,000/μL, during several upper airway infection episodes, without evidence of hemolysis, and subsequently normalized again upon recovery from the intercurrent disease. No anti-eculizumab antibodies were detected. The search for anti-factor H autoantibodies was negative.

The complement activation assay on ADP-activated endothelial cells showed intense serum-induced C5b-9 deposition on the cell surface (Fig. 2; Table 2), indicating that transient thrombocytopenia during intercurrent infectious episodes most likely reflected complement activation despite continued eculizumab therapy. These episodes did not appear to harm the grafted kidney since no hematuria or proteinuria were detected, and graft function remained unchanged (Fig. 2). However, the repeated drops in platelet counts suggested underlying microangiopathy and a high risk of overt disease.

The frequency of severe thrombocytopenic episodes, with platelet count in steady-state conditions of 60,000–90,000/μL, but with frequent acute reductions to 5,000–10,000/μL during rhino-sinusitis episodes, the need for repeated blood sampling in a psychologically frail child, and the family's anxiety, led to poor quality of life for the child and his family. The evidence that endothelial complement dysregulation was not adequately controlled by eculizumab and the fear of treatment failure in the long-term, prompted us to explore the possibility of a liver transplant based on the rationale that as the liver is the major source of FH [29], a liver transplant should definitively correct the genetic abnormality and prevent further relapse.

Liver transplantation

Our young patient received a liver transplant when het was 8.3 years old. PE with FFP substitution and eculizumab 900 mg (22 kg body weight) preceded a 330-g cadaveric liver graft. Basiliximab and methylprednisone pulses were added to the ongoing immunosuppression schedule with prednisone, cyclosporine, and azathioprine. Eculizumab 600 mg was given at post-transplant day 1, then 600 mg weekly for 5 weeks, and biweekly for the following 2 months.

The function of the grafted liver promptly recovered post-surgery, and the kidney graft never showed signs of impaired function. The platelet count of our patient showed an also immediate recovery, increasing from the pre-transplantation mean value of 60,000/μL to 220,000/μL the day after surgery (Fig. 2), and schistocytes and hemoglobin were in the normal ranges (ESM Fig. 1). Haptoglobin normalized 1 month after transplant. Creatinine levels remained between 0.55 and 0.45 mg/dL (mean values before and after liver transplantation, respctively) (Fig. 2). Serum-induced endothelial C5b-9 deposits were in the normal range (Table 2).

Eculixumab therapy was stopped 3 months after the successful liver transplantation. Over the following 6.5 months, all markers of hemolysis, renal function and serum-induced endothelial C5b-9 deposits remained persistently in the normal range (Table 2; Fig. 2; ESM Fig. 1). During this period, the patient experienced two transient episodes of mild thrombocytopenia (Fig. 1) concomitantly with upper airway infection episodes. In both cases the thrombocytopenia fortunately resolved spontaneously without any treatment and was associated with normal serum-induced endothelial C5b-9 deposits (Fig. 1; Table 2), suggesting complement-unrelated mechanisms of platelet activation by infectious agents [30].

Discussion

The case presented here recapitulates the main features of aHUS associated with *CFH* mutations and the response of this disease to treatments, including kidney and liver transplantation. Individuals with aHUS associated with *CFH* mutations show: (1) incomplete response to plasma therapy with relapsing episodes and progression to ESRD; (2) endothelial-restricted complement dysregulation despite a normal plasma complement profile; (3) recurrence of laboratory signs of complement dysregulation and of microvascular thrombosis after kidney transplantation; (4) correction of the genetic defect after liver transplantation, as previously documented by sequencing of *CFH* mRNA [8].

The finding that within the family pedigree only two of four subjects with the p.S1191L mutation developed aHUS is consistent with previous reports of incomplete penetrance of the disease in carriers of ~~this~~*CFH* mutations [31]. One possible explanation is that for the disease to manifest, there needs to be the simultaneous presence of a mutation in combination with a common at-risk genetic variant (SNPs and haplotype blocks) and/or an environmental trigger [23]. Our patient's clinical history confirms this assumption. Indeed, both the proband and his affected maternal aunt were found to carry at-risk SNPs and haplotypes in both *CFH* and *MCP*, which likely synergized with the p.S1191L mutation to determine disease manifestation. In contrast, only the *MCP* at-risk haplotype was found in the unaffected mother. Whether the additional presence of the p.A222V variant in *MTHFR* might have contributed to the earlier onset of the disease in the proband compared to his aunt remains to be established.

The onset of aHUS and the multiple relapses in the proband were all associated with bacterial or viral infections that likely act as an environmental trigger for endothelial inflammation, complement activation [32], platelet aggregation [30], and sequestration in the microvessels. In this regard, the finding that serum taken from the proband before kidney transplant, during a period of hematological remission, induced intense C5b-9 deposition on activated endothelial cells indicated that the remission state was a metastable condition, but one nevertheless associated with underlying impaired endothelial complement regulation secondary to FH dysfunction.

The outcome of aHUS has improved greatly in recent years due to the availability of the anti-C5 monoclonal antibody eculizumab [6, 33]. This drug has also been used effectively to prevent or treat aHUS relapses after transplantation [7]. In the case described here the use of perioperative PE and eculizumab followed by chronic eculizumab administration resulted in an excellent outcome with normal kidney graft function and control of disease activity for more than 1 year post-transplant. However, eculizumab seemed to progressively lose its efficacy over time in our patient, since it did not prevent repeated decreases in platelet count (associated with laboratory signs of microangiopathic hemolysis), which were exacerbated during common mild upper respiratory tract infections, despite graft function remaining stable. The late suboptimal response to eculizumab could not be attributed to the development of anti-eculizumab antibodies or to *C5* gene variations that alter the eculizumab recognition site [34] since the response to eculizumab was very good for 1 year after the kidney transplantation. Another unlikely explanation was underdosage, since drug levels were within the range recommended by the official product information sheet (http://ec.europa.eu/health/documents/community-register/2015/20150330131432/anx_131432_en.pdf), albeit this recommended range does not rely on published data and the half-life of the drug is variable [35]. However, even doubling the dose failed to stabilize the platelet count.

In addition, ~~plasma~~ serum CH50 levels were always very low, and plasma SC5b-9 levels were persistently normal, indicating efficient control of C5 activity in the fluid phase. However, there are published reports of plasma SC5b-9 levels not correlating with aHUS disease activity [15] and of plasma SC5b-9 levels not appreciably changing after eculizumab administration [15]. At variance with these results, we found that signs of relapses paralleled increases in serum-induced ex vivo endothelial C5b-9 deposits to higher than normal levels. These results confirm our previously published observations [15] showing that ex vivo evaluation of endothelial-specific complement activation is a useful tool for monitoring eculizumab's therapeutic efficacy in aHUS, while circulating complement parameters are not.

This case parallels a very recent report describing a childhood case of aHUS associated with another *CFH* mutation in which the patient experienced frequent relapses in the native kidneys while being treated with eculizumab [36]. This finding indicates that not every patient with aHUS achieves proper control of endothelial complement activation with eculizumab.

aHUS is a heterogeneous disease, and the reason(s) why a few cases if aHUS respond suboptimally to eculizumab is a matter of conjecture, but the mechanism very likely involves genetic modifiers. MTHFR is an enzyme involved in the remethylation of homocysteine to methionine [37]. The p.A222V MTHFR polymorphic variant found in our proband is present in 20–30 % of the general population, results in a 50 % reduction in MTHFR enzyme activity [38, 39], and has been associated with the development of coronary artery disease [40] and hyperhomocysteinemia, which ultimately favors the formation of prothrombotic *N*-homocysteine-linked plasma proteins, as well as endothelial oxidative stress and dysfunction [41]. We speculate that in our patient, the *MTHFR* hypomorphic variant synergized with the p.V1191L *CFH* mutation to exacerbate platelet activation and endothelial-restricted complement activation.

Given the complex genetic and clinical features of the aHUS which our patient manifested, we reasoned that further repeated relapses would eventually cause kidney graft failure and systemic complications during follow-up and that further increases of the eculizumab dose carried the potential risk of long-term drug side-effects and the development of drug-resistant disease. A liver transplant was considered the best option, with the underlying rationale that correcting the *CFH* genetic defect [8, 9, 42], would prevent future relapses. A recent report of a successful combined liver–kidney transplantation performed under eculizumab prophylaxis in a case of aHUS with a *CFH* mutation [14, 43] provided further support for this decision.

To avoid the risk of intragraft complement activation, which can occasionally be exacerbated by surgical stress and ischemia/reperfusion injury, eculizumab was given to our patient both peri-operatively and in the early post-transplant period.

The finding that serum-induced C5b-9 endothelial deposits were consistently normalized during more than 6 months of follow-up after eculizumab therapy post-transplant had been stopped, can be taken as evidence that the liver transplantation definitively cured the disease in this boy. The child's and family's quality of life, as well as his future prospects, have improved significantly.

This is an exceptional case, and the decision to perform a liver transplant was driven by the suboptimal response to eculizumab. Eculizumab is clinically effective in inducing hematological and renal remission in aHUS, however the long-term outcome and possible side-effects are not yet known. Moreover, not all patients respond to eculizumab [34, 36], and some patients may experience loss of eculizumab effectiveness over time; the case reported here is an example of the latter. Those patients who do respond to eculizumab need repeated infusions, apparently lifelong. Unfortunately, the high cost of eculizumab therapy simply makes it unaffordable for most national health systems and private insurance companies [44]. Consequently, there is an urgent need for new complement inhibitors that are inexpensive, orally active, and effective in eculizumab-resistant patients.

Liver transplantation, in contrast to eculizumab therapy, cures aHUS definitively without the need ~~for~~of specific therapies other than standard immunosuppression to prevent graft rejection. Over 80 % of those who have been offered the possibility of liver transplantation to date have had excellent long-term outcomes [14]. The short-term mortality risk associated with initial attempts has been substantially reduced with prophylactic plasmapheresis and peri-operative eculizumab. While in industrial countries the pros and cons of eculizumab therapy versus liver and kidney transplantation are still a matter of discussion, patients living in low-income and poor countries have no choice.

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Compliance with ethical standards

The parents of the patient provided written informed consent to the analyses and the procedures reported in this paper.

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Conflict of interest The authors declare that they have no conflict of interest.

Electronic supplementary material

Below is the link to the electronic supplementary material.

ESM 1

(PPTX 78 kb)

ESM 2

(DOCX 498 kb)

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