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Case Report

Postpartum aHUS Secondary to a Genetic Abnormality in Factor H Acquired Through Liver Transplantation

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We report here a young female who underwent a successful deceased donor liver transplant for hepatic vein thrombosis. Five years after transplantation she developed postpartum atypical hemolytic uremic syndrome (aHUS). She did not recover renal function. Mutation screening of complement genes in her DNA did not show any abnormality. Mutation screening of DNA available from the donor showed a nonsense *CFH* mutation leading to factor H deficiency. Genotyping of the patient showed that she was homozygous for an aHUS *CD46* at-risk haplotype. In this individual, the development of aHUS has been facilitated by the combination of a trigger (pregnancy), an acquired rare genetic variant (*CFH* mutation) and a common susceptibility factor (*CD46* haplotype).

Key words: Complement, factor H, hemolytic uremic syndrome, genetics, liver transplantation

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is now well recognized to be a disease characterized by excessive complement activation in the microvasculature. In both the familial and sporadic forms, inherited and acquired abnormalities affecting components of the alternative complement pathway are found in ∼60% of patients. These include mutations in the genes encoding both complement regulators (factor H, factor I, membrane cofactor protein and thrombomodulin) and activators (factor B and C3); and autoantibodies against factor H (1). Multiple hits are necessary for the disease to manifest including a trigger, mutations and at-risk haplotypes in complement genes (2). We report here a unique patient who has an at-risk haplotype in a complement gene, then acquires a mutation in a complement gene through liver transplantation and finally develops aHUS in response to pregnancy.

Case History

A 21-year-old female was admitted to her local hospital with a 5-month history of abdominal pain, nausea and vomiting. Initial investigations showed bilirubin 44 μmol/L, aspartate aminotransferase 6813 U/L, gamma glutamyl transferase 38 U/L, albumin 23 g/L and INR of 3.9. Radiological investigations were compatible with a diagnosis of hepatic vein thrombosis (Budd–Chiari syndrome). Autoantibody and hepatitis viral screening were negative. A thrombophilia screen was undertaken. The factor V Leiden mutation was not present. No prothrombin gene mutations were detected. There was no evidence of activated protein C resistance. Concentration of antithrombin III, protein C activity and free protein S were all low. Antithrombin III 27 IU/mL (normal range 80–130 IU/mL), protein C activity 10 IU/dL (70–140 IU/dL) and free protein S 15 IU/dL (60–150 IU/dL). This was consistent with impaired hepatic synthetic function. Screening for lupus anticoagulant and anticardiolipin antibodies was negative. She was transferred to the Liver Unit at King's College Hospital, London where 3 days later she underwent a deceased donor liver transplant. The donor was a 41-year-old female who had suffered an intracranial hemorrhage. Donor serum creatinine was 88 μmol/L, Hb 14.3 g/dL and platelets 218 × 10^9/L. Urine output in the 24 hours prior to death was 4.4 L and there were no abnormalities on routine hematology investigations. The patient was successfully transplanted and subsequently made a good recovery. Histology of the

Table 1: *CD46* and *CFH* susceptibility alleles in the recipient and the donor

	CD46 −652A>G (rs2796267)	*CD46* −366A>G (rs2796268)	*CD46* IVS9 −78G>A (rs1962149)	*CD46* IVS12 +638G>A (rs859705)	*CD46* c.4070T>C (rs7144)
Recipient	GG	GG	AA	AA	CC
Donor	AG	AG	GA	AG	TC

	CFH −331C>T (rs3753394)	*CFH* c.184G>A Val62Ile (rs800292)	*CFH* c.1204C>T His402Tyr (rs1061170)	*CFH* c.2016A>G Gln672Gln (rs3753396)	*CFH* IVS15 −543G>A (rs1410996)	*CFH* c.2808G>T Glu936Asp (rs1065489)
Recipient	CT	GG	CT	AG	GG	GT
Donor	CT	GG	CT	AA	GG	GT

explant confirmed features of venous outflow obstruction with severe confluent parenchymal loss and extensive occlusion of the hepatic veins by thrombus. The appearances were in keeping with acute Budd–Chiari syndrome. She made a satisfactory recovery without any major complications. She was subsequently maintained on tacrolimus monotherapy with trough concentrations ranging between 6 and 8 μg/L.

Five years later she became pregnant for the first time. The pregnancy was uncomplicated apart from mild hypertension and she delivered at 36 weeks' gestation. Four weeks postpartum she developed nausea, vomiting and oliguria. She was admitted and on examination was pale with spontaneous bruising. Blood pressure was 149/105 mmHg and there was edema to mid-calf. Urinalysis revealed blood 3+ and protein 3+. Investigations showed hemoglobin 6.4 g/dL platelets 73 × 10^9/L, reticulocyte count 7%, serum creatinine 871 μmol/L, urea 33.2 mmol/L and LDH 2375 units/L. Schistocytes were seen on a peripheral blood film. Screening for paroxysmal nocturnal hemoglobinuria using flow cytometry was negative.

Complement C3 was 0.84 g/L (0.70–1.70) complement C4 0.37g/L (0.13–0.43) and ADAMTS 13 activity was 13% (normal range 60–123%). The IgG anti-ADAMTS13 titer was 3% (normal <6.1%). ADAMTS13 activity measured on a current convalescent sample was >100% (normal range 60–123) and the IgG anti-ADAMTS13 titer was 5%.

A diagnosis of atypical hemolytic uremic syndrome was made. Treatment with hemodialysis and plasma exchange was commenced. There was no recovery of renal function and she has remained on treatment with hemodialysis. The associated hematological abnormalities have resolved.

Informed consent for this report has been given by the patient. Research within the report was approved by the Northern and Yorkshire Multicentre Research Ethics Committee.

Genetic studies

DNA samples were available for analysis from both the patient (recipient of the liver transplant) and the donor. Mutation screening of the genes encoding factor H (*CFH*), factor I (*CFI*), membrane cofactor protein (*CD46*), C3 (*C3*), factor B (*CFB*), thrombomodulin (*THBD*) and factor H-related protein 5 (*CFHR5*) was undertaken using direct fluorescent sequencing as described previously in both recipient and donor (3). No abnormalities were found in the recipient. In the donor a nonsense heterozygous mutation in *CFH* (c.1107 G>A; p.Trp369Stop) was found. This mutation was not detected in DNA samples from 200 normal control individuals within the Wellcome Trust Case Control Consortium (4, 5).

Screening for genomic disorders affecting *CFH, CFHR1, CFHR2, CFHR3* and *CFHR5* was undertaken using multiplex ligation-dependent probe amplification (3). There was no evidence of either deletions or hybrid genes. Both the recipient and the donor had two copies of *CFHR1*, *CFHR3* and *CFHR4*

The *CD46* SNPs (−652A>**G** (rs2796267), −366A>**G** (rs2796268), IVS9 −78G>**A** (rs1962149), IVS12 +638G>**A** (rs859705) and c.4070T>**C** (rs7144)), which define the at-risk $CD46_{GGAAC}$ haplotype and the *CFH* SNPs (−331C>**T** (rs3753394), c.184**G**>A Val62Ile (rs800292), c.1204**T**>C p.Tyr402His (rs1061170), c.2016A>**G** p.Gln672Gln (rs3753396), IVS15 −543**G**>A intron 15 (rs1410996), c.2808G>**T** p.Glu936Asp (rs1065489)), which define the at-risk CFH_{TGTGGT} (also known as *CFH*-H3) were genotyped using direct sequencing. The results for the recipient and donor are shown in Table 1. The recipient is homozygous for the at-risk $CD46_{GGAAC}$ haplotype. Both the donor and recipient are heterozygous for c.1204T>C p.Tyr402His (rs1061170).

The donor *CFH* mutation (c.1107 G>A; p.Trp369Stop) is in exon 8 (encodes SCR 6 of factor H) and c.1204T>C p.Tyr402His (rs1061170) is in exon 9 (encodes SCR 7 of factor H) (Figure 1A). We were therefore able, using a long range PCR with allele-specific primers designed to amplify these two exons, to show that the mutation was on the same allele as c.1204C (the His allele).

Protein studies

We used monoclonal antibodies specific for the Tyr402 (MBI-6) and His402 (MBI-7) variants of factor H in both

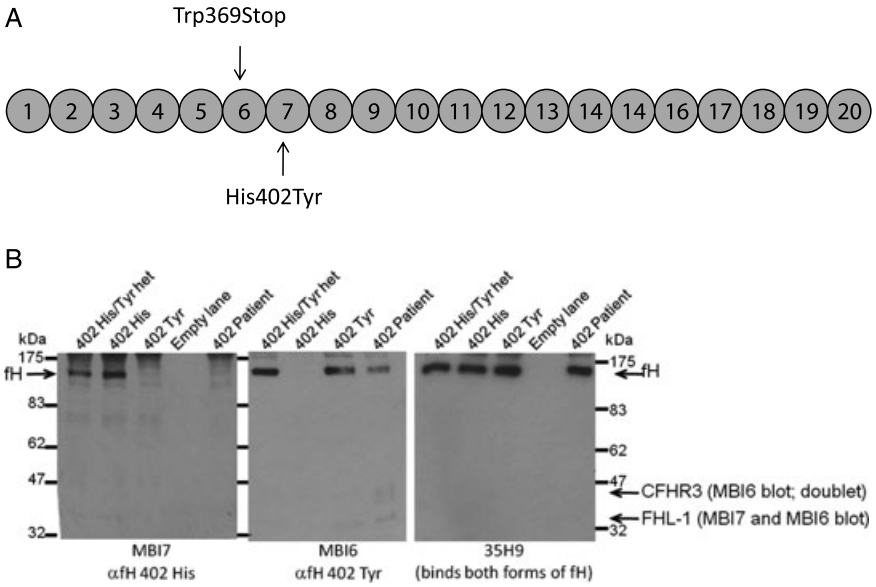

Figure 1: (A) Factor (fH) consists of 20 contiguous, homologous modules called short consensus repeat (SCR) domains each comprising ~60 amino acids. His402Tyr is in SCR7, the mutation found in the donor DNA results in Trp369stop in SCR6. The donor is heterozygous for His402Tyr. (B) Plasma from the patient and from controls (fH 402 His and Tyr homozygotes, and a 402 His/Tyr heterozygote) were loaded onto a 7.5% gel and immunoblotted with 402 His and 402 Tyr-specific antibodies. MBI7 (anti-His) picked up fH in the heterozygote control and in the fH_{402H} homozygote; no fH was detected in the fH_{402Y} homozygote or in the patient. MBI6 (anti-Tyr) picked up fH in the heterozygote control, in the fH_{402Y} homozygote and in the patient; no fH was detected in the fH_{402H} homozygote control. No truncated products were evident in the patient sample even on prolonged exposure. Low-level expression of FHL-1 (factor H-like 1) was evident as was CFHR3 in the patient MBI6 blot (SCR3 in CFHR3 shows 97% homology to SCR7 in fH and contains a Tyr at the equivalent position to fH 402, MBI6 binds fH and CFHR3). Background bands at the top of the gel are artifacts caused by antibody in human plasma.

Western blotting and a quantitative ELISA as described previously (6). We confirmed with Western blotting that the donor histidine isoform carried the factor H mutation (Figure 1B). The quantitative ELISA showed a factor H-Tyr concentration of 77 μg/mL with the Tyr-specific assay (normal range 96–343 μg/mL). The His-specific assay detected a very low concentration <5 μg/mL of factor H-His (normal range 132–365 μg/mL). This is in agreement with the Western blot and is compatible with extrahepatic synthesis of factor H-His from the recipient's *CFH* His allele. Total factor H concentration measured using the mouse antihuman factor H monoclonal antibody OX-24(6) was 73 μg/mL (normal range 124–402 μg/mL). Screening for factor H autoantibodies was negative (3). Peripheral blood mononuclear cell expression of membrane cofactor protein, measured by fluorescent antibody cell sorting, was normal.

Therefore, the recipient has acquired through liver transplantation a *CFH* mutation that is present in only the transplanted liver and results in partial factor H deficiency. She also carries (and has always carried) two copies of the $CD46_{GGAAC}$ haplotype, which increases the risk of developing aHUS in response to a trigger such as pregnancy in an individual with a *CFH* mutation. The CD46 that is encoded by this haplotype is expressed on renal endothelium.

Discussion

We report here the development of postpartum atypical HUS in a liver transplant patient. We have shown this is associated with transmission of a donor-derived nonsense factor H mutation, which has caused factor deficiency in the recipient. In addition, we have found that the recipient is homozygous for the at-risk $CD46_{GGAAC}$ haplotype.

De novo posttransplant thrombotic microangiopathy is a well-recognized complication of renal transplantation (7) occurring in approximately 0.8% of patients (8). It usually occurs within weeks of transplantation and is often associated with high blood levels of calcineurin inhibitors (CNI). In this group it has been shown that up to 30% of patients may have a previously unrecognized mutation in *CFH* or *CFI* (9). Thrombotic microangiopathy post liver transplant is also well described (10) with a frequency of between 3% and 5%. However, in the majority of cases it occurs early and is associated with CNI toxicity or ABO-incompatible living donor transplantation. The case we describe here was unusual in that HUS developed 5 years after liver transplantation with tacrolimus levels within the therapeutic range. That the episode also appeared to be triggered by pregnancy led us to seek predisposing abnormalities within the complement pathway, which are found in 86% of pregnancy-associated aHUS (11). Because factor

H and factor I are produced predominantly by the liver, we examined the possibility that in this individual the abnormality was derived from the donor liver. Mutation screening of donor DNA revealed a heterozygous nonsense mutation in exon 8 of *CFH*. Because both the recipient and the donor were heterozygous for factor H His402Tyr, we were able using monoclonal antibodies specific for the two isoforms to show that this mutation resulted in factor H deficiency.

aHUS can be both familial and sporadic. Characteristic of the familial form of aHUS is that ~50% of individuals will not manifest aHUS despite carrying a mutation in one of the aforementioned genes. Two other factors are thought to determine the development of the disease. First, in most patients there is a trigger. Infection and pregnancy are the most frequently described triggers (1,11). Second, a further genetic variant (modifier) can increase the risk of developing the disease. This can be in the form of either an additional mutation in one of the aforementioned genes and/or the presence of a common at-risk genetic variant. It is now recognized that ~20% of aHUS patients will have mutations in more than one gene. Common at-risk genetic variants (SNPs and haplotype blocks) in *CFH, CD46* and *CFHR1* have been shown to act as additional susceptibility factors for the development of the disease (12,13). Thus the presence of a rare genetic variant (mutation), a common at-risk genetic variant (SNPs and haplotype blocks) and a trigger are usually necessary for the disease to be manifest (Figure 2) (2). In the case we present here the rare genetic variant is the *CFH* mutation acquired from the donor, the common genetic variant is the homozygous $CD46_{GGAAC}$ haplotype and finally aHUS has been triggered by pregnancy.

Membrane cofactor protein (CD46) is a transmembrane complement regulator, which is widely expressed particularly on renal endothelium. The $CD46_{GGAAC}$ haplotype is associated with an increased risk of developing aHUS particularly in those patients already known to have an inherited mutation in *CFH*, *CFI* or *CD46* (14). The case that we report here is unique in that the *CFH* mutation is acquired not inherited.

Because the liver is the major site of synthesis of soluble proteins, it is not surprising that genetic defects affecting such proteins could be transmitted following liver transplantation. This has been described previously for both metabolic disorders and coagulopathies (15,16). To our knowledge this is the first report of this phenomenon affecting a complement protein.

What are the implications of these findings for the management of patients such as the one we describe here who are on dialysis and have a factor H mutation? Renal transplantation alone would be associated with an ~80% risk of losing an allograft to recurrent disease within 2 years of transplantation (1). One option might be to undertake a renal transplant alone with prophylactic plasma exchange preoperatively, postoperatively and long term. While this approach has been used successfully (17), recurrent disease can occur and patients may become intolerant of the procedure. Another option would be to again undertake a renal transplant alone but instead of plasma exchange use a complement inhibitor as prophylaxis against recurrent disease. This has been undertaken using the anti-C5 monoclonal antibody eculizumab both in the immediate postoperative period (18) and after a patient has become intolerant of plasma exchange (19). In the future it is probable that either recombinant or purified factor H will be available for such use. Finally, a simultaneous liver–kidney transplant could be considered. The success rates for this procedure in aHUS are improving but retransplantation, as would be necessary in this case, is associated with a significant decrease in graft and patient survival (20).

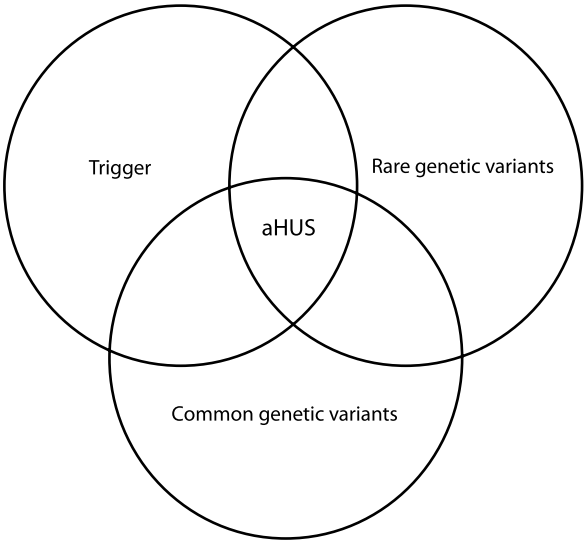

Figure 2: Manifestation of aHUS in an individual may need the presence of a trigger such as pregnancy, a rare genetic variant such as a mutation in a complement gene and a common genetic variant such as an at-risk haplotype in a complement gene (Reproduced with permission of the American Society of Hematology [ASH]).

In conclusion the unique case that we present here reinforces further the paradigm that multiple risk factors are needed for the development of aHUS (2).

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