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Exceptional Case

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Rare genetic variants in Shiga toxin-associated haemolytic uraemic syndrome: genetic analysis prior to transplantation is essential

Frances Dowen[1], Katrina Wood[2], Alison L. Brown[3], Jennifer Palfrey[1], David Kavanagh[4] and Vicky Brocklebank[4]

[1]Department of Renal Medicine, Sunderland Royal Hospital, Sunderland, Tyne and Wear, UK, [2]Department of Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK, [3]Renal Services Centre, Freeman Hospital, Newcastle upon Tyne, UK and [4]National Renal Complement Therapeutics Centre, Newcastle University and Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

Correspondence and offprint requests to: Vicky Brocklebank; Email: vickybrocklebank@doctors.org.uk

Abstract

We present a case of haemolytic uraemic syndrome (HUS) in a 16-year-old female with serological evidence of acute *Escherichia coli* O157:H7 infection. She progressed to established renal failure and received a deceased donor kidney transplant. Shiga toxin–associated HUS (STEC-HUS) does not recur following renal transplantation, but unexpectedly this patient did experience rapid and severe HUS recurrence. She responded to treatment with the terminal complement inhibitor eculizumab and subsequent genetic analysis revealed a rare variant in a complement gene. This highlights the importance of genetic analysis in patients with STEC-HUS prior to renal transplantation so that management can be individualized.

Key words: atypical haemolytic uraemic syndrome; complement; eculizumab; renal transplantation; STEC-HUS

Introduction

Haemolytic uraemic syndrome (HUS) is characterized by the clinical triad of acute kidney injury (AKI), thrombocytopenia and microangiopathic haemolytic anaemia (MAHA) [1]. The most common form is caused by enteric infection with Shiga toxin–producing bacteria, most frequently *Escherichia coli* serotype O157:H7 [2], known as STEC-HUS, with an estimated incidence in the UK of 7.1 per million [3]. STEC-HUS is a rare (∼5%) self-limiting illness with consequent established renal failure (ERF) [4].

The atypical form of HUS (aHUS) is usually associated with dysregulation of the complement system resulting from inherited or acquired defects. It is rare (UK incidence 0.42 per million [5]), but historically has been associated with a poor outcome (>50% ERF) [6, 7]. Additionally, due to the genetic nature of the disease, aHUS recurrence following renal transplantation and subsequent graft loss was the rule in those with mutations in liver-produced serum complement proteins [1, 8–10]. The development of the terminal complement inhibitor eculizumab has transformed the management and prognosis of aHUS and the outcome following renal transplantation [5, 11].

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In contrast, recurrence of HUS after renal transplantation does not occur in STEC-HUS [8, 9].

We report a case of HUS in a 16-year-old female with serological evidence of acute *E. coli* O157:H7 infection that resulted in ERF; she experienced rapid and severe recurrence of HUS following renal transplantation, and subsequent analysis identified a rare genetic variant in *C3*.

Case report

A 16-year-old female presented with AKI (creatinine 324 μmol/L) and a 4-day history of vomiting without diarrhoea. Her only past medical history was acne, and Roaccutane had been commenced 1 month earlier; blood tests performed at that time were all normal. Her mother had been unwell with a diarrhoeal illness 2 weeks previously.

Blood tests on admission were consistent with a thrombotic microangiopathy (TMA): haemoglobin 9.9 g/dL, platelets 41 $\times$ 10^9/L, bilirubin 38 μmol/L, lactate dehydrogenase (LDH) 1865 U/L, reticulocytes 107 $\times$ 10^9/L and red blood cell fragmentation on the blood film; Coombs test was negative and coagulation was normal. Treatment with plasma exchange was commenced (total of eight exchanges) and thrombotic thrombocytopenic purpura (TTP) was excluded (ADAMTS13 activity 76%). The National Health Protection Agency (HPA) laboratory reported that immunoglobulin M (IgM) antibodies to *E. coli* O157 lipopolysaccharide (LPS) were positive, in keeping with acute STEC-HUS [10]. Levels of C3, C4, factor H and factor I were normal. Her renal function deteriorated and she required haemodialysis, as well as ventilatory and inotropic support in the intensive care unit (ICU) consequent to a hospital-acquired pneumonia. There was no recovery in renal function and she remained on haemodialysis. A kidney biopsy was performed 3 months later, showing features of TMA; immunofluorescence showed segmental C3 deposition in the glomerular tuft. Later, 10 months after presentation, she was admitted with seizures that were attributed to hypertensive encephalopathy (computed tomography of the brain and cerebrospinal fluid examination were normal) and she was again managed in the ICU for ventilatory support and blood pressure control.

She received a donor after brain death (DBD) renal transplant with a 1:1:0 human leucocyte antigen mismatch 18 months after her initial presentation. She received basiliximab and methylprednisolone at induction with maintenance tacrolimus, mycophenolate mofetil (MMF) and prednisolone. A transplant biopsy performed 7 days post-operatively due to delayed graft function showed borderline acute T-cell–mediated rejection and no evidence of TMA; she was treated with intravenous methylprednisolone, but her renal function deteriorated concurrent with a decrease in her platelet count and an increase in LDH, with a peak creatinine of 396 μmol/L. At Day 10 she developed MAHA and was treated with plasma exchange. Tacrolimus levels were therapeutic. The following day she had multiple generalized tonic–clonic seizures, severe headache, photophobia and blurred vision, which progressed to cortical blindness over the course of 4 h in the context of a blood pressure of 190/113 mmHg. Magnetic resonance imaging (MRI) of the brain was consistent with posterior reversible encephalopathy syndrome (PRES) (Figure 1A). She was admitted to the ICU for management of hypertension and treatment with eculizumab was commenced. The neurological symptoms resolved within 48 h and over the following 2 weeks the renal function improved and haematological parameters normalized. A transplant biopsy performed 15 days post-operatively demonstrated severe acute TMA and borderline acute T-cell–mediated rejection (Figure 1Bi–ii).

Subsequent genetic analysis identified a heterozygous rare genetic variant in *C3:* c.4855A>C p.(Ser1619Arg). The functional significance has not been assessed, however, the amino acid is well conserved, *in silico* analysis suggests that the variant is possibly deleterious and structural modelling (Figure 1C) demonstrates that this is in the same *C3* domain as the variant c.4973T >C p.(Val1658Ala) demonstrated by Sartz *et al.* to be functionally significant [12].

A transplant biopsy performed 3 months post-operatively for proteinuria (urine protein:creatinine ratio 243 mg/mmol) showed no significant abnormality. A fourth transplant biopsy was performed 5 months post-operatively because the creatinine rose from 130 to 180 μmol/L. This showed acute T-cell–mediated rejection (Banff IB) (Figure 1Biii) and she was treated with intravenous methylprednisolone. Immunofluorescence, including kappa and lambda, was negative. She remains on eculizumab, prednisolone, tacrolimus and MMF, and 3 years after transplantation she is well and creatinine is 157 μmol/L.

Discussion

This patient presented with a TMA and normal ADAMTS13 activity, and although she had no history of diarrhoea, she had serological evidence (positive IgM) of recent *E. coli* O157:H7 infection consistent with a diagnosis of STEC-HUS. However, a faecal specimen was not analysed and the serodiagnosis is sensitive but not specific for verocytotoxin-producing *E. coli* (VTEC) [15]. In addition, serum antibodies to the LPS of *E. coli* 0157 have been detected in healthy people in rural communities in the UK [10]. The absence of diarrhoea does not preclude STEC-HUS [2] and ~5% of people with STEC-HUS may not have diarrhoea [16]. We recommend that all patients with HUS, even those without a history of diarrhoea, be investigated for STEC infection by culture and Shiga toxin polymerase chain reaction on a faecal specimen, as well as serological testing, to optimize sensitivity [17, 18].

STEC-HUS is said not to recur following renal transplantation [8, 9]; however, a severe TMA evolved within 1 week of transplantation, resulting in AKI and neurological manifestations. She responded promptly to treatment with the terminal complement inhibitor eculizumab. Subsequent genetic analysis revealed the p.(Ser1619Arg) heterozygous rare genetic variant in *C3* [12]. Historically, aHUS recurrence following transplantation has occurred in 60% of patients, with the highest risk observed in patients with mutations in *CFH* and *C3* [1, 19, 20]. This patient had seizures 10 months after the initial presentation and neurological symptoms during the recurrence after transplantation; it is not known whether extrarenal manifestations occur consequent to AKI, hypertension, TMA or complement dysregulation [21].

Very rare cases of STEC-HUS infection unmasking latent complement defects and triggering aHUS have been described [22–25] and Alberti *et al.* [26] also reported post-transplant recurrence leading to graft loss in two patients with STEC-HUS who were subsequently found to have complement gene mutations.

In this case, due to ongoing proteinuria, for disease resolution we looked for evidence of eculizumab deposition in the renal biopsy as described by Herlitz *et al.* in C3G [27]. That we were unable to detect immunoglobulin G (IgG) kappa deposition may reflect the differing nature of complement activation in aHUS compared with C3G.

In summary, as ERF following STEC-HUS is rare and *a priori* knowledge of an underlying complement mutation allows prophylactic eculizumab to be given, genetic screening should be performed in all individuals who develop ERF following STEC-HUS and who are being considered for transplantation, as

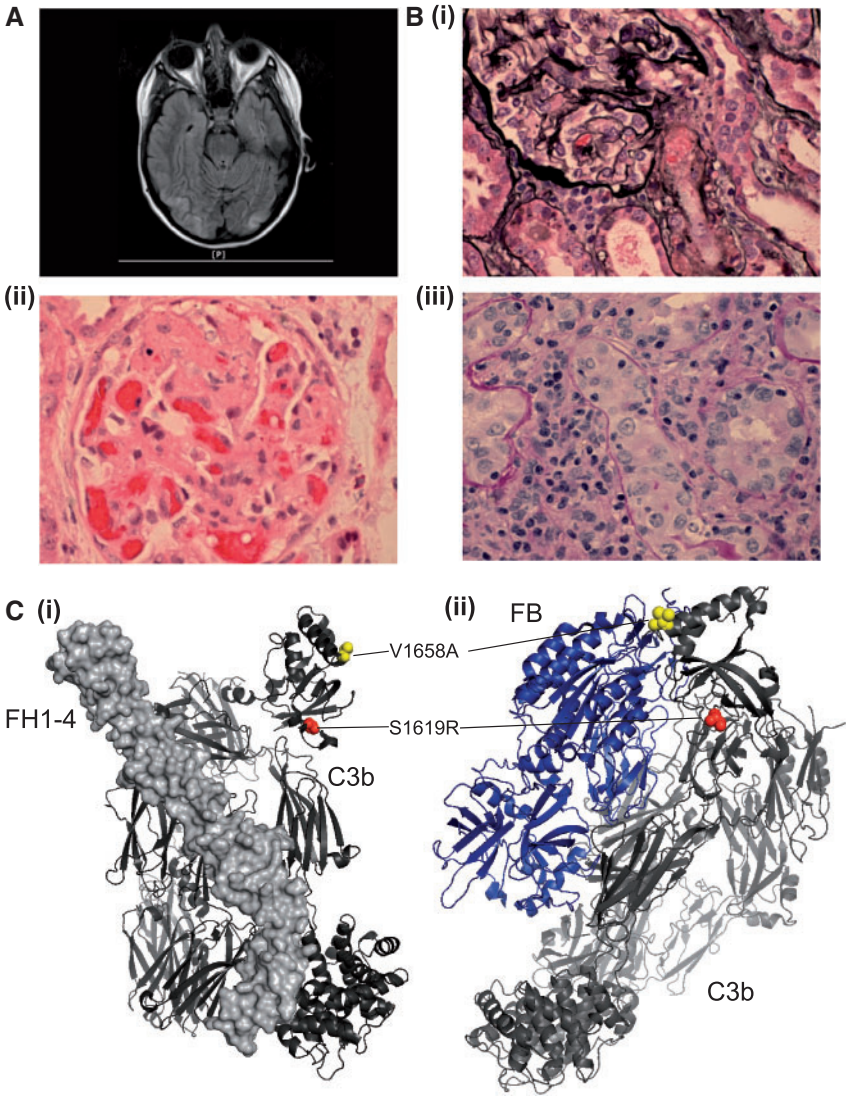

Fig. 1. Findings on brain imaging, histopathological examination and structural modelling of the *C3* variant. (**A**) MRI of the brain was performed 11 days post-transplant due to evolving neurological symptoms (multiple generalized tonic–clonic seizures, severe headache, photophobia and cortical blindness) in the context of severe hypertension. Bilateral restricted diffusion abnormalities were seen within the cerebral hemispheres posteriorly, consistent with PRES. (**B**) Histopathological examination. A transplant biopsy performed 15 days post-operatively due to clinical manifestations of TMA showed (i) thrombotic occlusion of the arteriole at the glomerular hilum (silver, ×400) and (ii) 'glomerular paralysis' with markedly congested glomerular capillaries (haematoxylin and eosin, ×400); a transplant biopsy performed 5 months post-operatively due to an increase in serum creatinine showed tubulitis indicative of T-cell–mediated rejection (iii) (periodic acid–Schiff, ×400); there were no features of TMA and there was no evidence on immunofluorescence of eculizumb IgG kappa deposition. (**C**) (i) The p.S1619R genetic variant displayed on the FH/C3b co-crystal structure. An X-ray–derived co-crystal structure of fH/C3b was used to model the mutation and displayed with Pymol (Delano Scientific). The location of the S1619R variant (red sphere) is shown within the co-crystal structure of a FH CCPs1-4 (light grey):C3b (dark grey) complex. A previously reported, functionally significant genetic variant V1658A (yellow sphere) is also shown [12]. Neither of these variants is predicted to directly oppose FH (Protein Data Base ID code 2WII) [13]. (ii) The genetic variants were also displayed on the C3b (dark grey):FB (blue) co-crystal structure (Protein Data Base ID code 2XWJ) [14]. It can be seen that the V1658A variant directly opposed FB, in keeping with functional analysis demonstrating an increase in FB binding to C3b [12]. The S1619R variant is also in the C345C domain, suggesting that this variant may also lead to increased convertase formation. *In silico* analysis suggests that this rare genetic variant is conserved [genomic evolutionary rate profiling 4 (GERP 4)] and possible damaging (Polyphen2 0.834).

recommended in a 2015 Kidney Disease: Improving Global Outcomes consensus document [21]. This personalized approach to HUS will prevent the morbidity and mortality associated with recurrent disease [28].

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Conflict of interest statement

Newcastle University has received honoraria for consultancy work (D.K.) from Alexion Pharmaceuticals, and D.K. is a director of and scientific advisor to Gyroscope Therapeutics.

References

1. Kavanagh D, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. *Semin Nephrol* 2013; 33: 508–530

2. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005, 365: 1073–1086
3. Lynn RM, O'Brien SJ, Taylor CM *et al*. Childhood hemolytic uremic syndrome, United Kingdom and Ireland. *Emerg Infect Dis* 2005; 11: 590–596
4. Siegler R, Oakes R. Hemolytic uremic syndrome; pathogenesis, treatment, and outcome. *Curr Opin Pediatr* 2005; 17: 200–204
5. Sheerin NS, Kavanagh D, Goodship TH *et al*. A national specialized service in England for atypical haemolytic uraemic syndrome–the first year's experience. *QJM* 2016; 109: 27–33
6. Noris M, Caprioli J, Bresin E *et al*. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 2010; 5: 1844–1159
7. Fremeaux-Bacchi V, Fakhouri F, Garnier A *et al*. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin J Am Soc Nephrol* 2013; 8: 554–562
8. Bassani CE, Ferraris J, Gianantonio CA *et al*. Renal transplantation in patients with classical haemolytic-uraemic syndrome. *Pediatr Nephrol* 1991; 5: 607–611
9. Ferraris JR, Ramirez JA, Ruiz S *et al*. Shiga toxin-associated hemolytic uremic syndrome: absence of recurrence after renal transplantation. *Pediatr Nephrol* 2002; 17: 809–814
10. Chart H, Cheasty T. Human infections with verocytotoxin-producing *Escherichia coli* O157–10 years of *E. coli* O157 serodiagnosis. *J Med Microbiol* 2008; 57: 1389–1393
11. Zuber J, Le Quintrec M, Krid S *et al*. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012; 12: 3337–3354
12. Sartz L, Olin AI, Kristoffersson AC *et al*. A novel C3 mutation causing increased formation of the C3 convertase in familial atypical hemolytic uremic syndrome. *J Immunol* 2012; 188: 2030–2037
13. Wu J, Wu YQ, Ricklin D *et al*. Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. *Nat Immunol* 2009; 10: 728–733
14. Forneris F, Ricklin D, Wu J *et al*. Structures of C3b in complex with factors B and D give insight into complement convertase formation. *Science* 2010; 330: 1816–1820
15. Banatvala N, Griffin PM, Greene KD *et al*. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. *J Infect Dis* 2001; 183: 1063–1070
16. Brandt JR, Fouser LS, Watkins SL *et al*. *Escherichia coli* O 157:H7-associated hemolytic-uremic syndrome after ingestion of contaminated hamburgers. *J Pediatr* 1994; 125: 519–526
17. Bryan A, Youngster I, McAdam AJ. Shiga toxin producing *Escherichia coli*. *Clin Lab Med* 2015; 35: 247–272
18. Jenkins C, Lawson AJ, Cheasty T *et al*. Assessment of a real-time PCR for the detection and characterization of verocytotoxigenic *Escherichia coli*. *J Med Microbiol* 2012; 61: 1082–1085
19. Le Quintrec M, Zuber J, Moulin B *et al*. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. *Am J Transplant* 2013; 13: 663–675
20. Noris M, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. *Am J Transplant* 2010; 10: 1517–1523
21. Goodship TH, Cook HT, Fakhouri F *et al*. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int* 2017; 91: 539–551
22. Sellier-Leclerc AL, Fremeaux-Bacchi V, Dragon-Durey MA *et al*. Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2007; 18: 2392–2400
23. Caillaud C., Zaloszyc A, Licht C *et al*. CFH gene mutation in a case of Shiga toxin-associated hemolytic uremic syndrome (STEC-HUS). *Pediatr Nephrol* 2016; 31: 157–161
24. Ahlenstiel-Grunow T, Hachmeister S, Bange FC *et al*. Systemic complement activation and complement gene analysis in enterohaemorrhagic *Escherichia coli*-associated paediatric haemolytic uraemic syndrome. *Nephrol Dial Transplant* 2016; 31: 1114–1121
25. Challis RC, Araujo GS, Wong EK *et al*. A de novo deletion in the regulators of complement activation cluster producing a hybrid complement factor H/complement factor H-related 3 gene in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2016; 27: 1617–1124
26. Alberti M, Valoti E, Piras R *et al*. Two patients with history of STEC-HUS, posttransplant recurrence and complement gene mutations. *Am J Transplant* 2013; 13: 2201–2206
27. Herlitz LC, Bomback AS, Markowitz GS *et al*. Pathology after eculizumab in dense deposit disease and C3 GN. *J Am Soc Nephrol* 2012; 23: 1229–1237
28. Wong E, Challis R, Sheerin N *et al*. Patient stratification and therapy in atypical haemolytic uraemic syndrome (aHUS). *Immunobiology* 2016; 221: 715–718

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