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Case Report

Two Patients With History of STEC-HUS, Posttransplant Recurrence and Complement Gene Mutations

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Hemolytic uremic syndrome (HUS) is a disease of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. About 90% of cases are secondary to infections by *Escherichia coli* strains producing Shiga-like toxins (STEC-HUS), while 10% are associated with mutations in genes encoding proteins of complement system (aHUS). We describe two patients with a clinical history of STEC-HUS, who developed end-stage renal disease (ESRD) soon after disease onset. They received a kidney transplant but lost the graft for HUS recurrence, a complication more commonly observed in aHUS. Before planning a second renal transplantation, the two patients underwent genetic screening for aHUS-associated mutations that revealed the presence of a heterozygous *CFI* mutation in patient #1 and a heterozygous *MCP* mutation in patient #2, and also in her mother who donated the kidney. This finding argues that the two cases originally diagnosed as STEC-HUS had indeed aHUS triggered by STEC infection on a genetic background of impaired complement regulation. Complement gene sequencing should be performed before kidney transplantation in patients who developed ESRD following STEC-HUS since they may be undiagnosed cases of aHUS, at risk of posttransplant recurrence. Furthermore, genetic analysis of donors is mandatory before living-related transplantation to exclude carriers of HUS-predisposing mutations.

Key words: Complement factor I, gene mutation, hemolytic uremic syndrome, kidney transplantation, membrane cofactor protein, Shiga-toxin

Abbreviations: aHUS, atypical HUS; AP, alternative pathway; CFB, factor B; CFH, factor H; CFI, factor I; C5b-9, terminal complement complex; ELISA, enzyme-linked immunosorbent assay; ESRD, end stage renal disease; HUS, hemolytic uremic syndrome; HUS/TTP, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura; LHD, serum lactate dehydrogenase; MCP, membrane cofactor protein; MLPA, multiplex ligation-dependent probe amplification; SP, serine protease domain; STEC, Shiga-like toxins; Stx, Shiga-like toxin; THBD, thrombomodulin.

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Introduction

Hemolytic uremic syndrome (HUS) is a rare disease characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure, with a worldwide incidence of 0.7–2 cases/year/100 000 people (1).

About 90% of patients develop typical diarrhea-associated HUS, most commonly triggered by entero hemorrhagic strains of *E. coli*, mainly of serotype O157:H7, producing Shiga-like toxins (STEC) (2,3). STEC-HUS occurs primarily in children (4). However, a large outbreak (>4000 cases) of gastroenteritis and HUS, caused by an unusual enteroaggregative STEC strain (O104:H4) and predominantly affecting adults, occurred in Germany in 2011 (5). More than 25% of infected subjects developed HUS, a proportion much higher than those recorded in outbreaks of enterohemorrhagic STEC (5).

In STEC-associated HUS, binding of Shiga-like toxin (Stx) to globotrialosyl ceramide receptors, highly expressed on glomerular endothelial cells, generates a cascade of signals resulting in expression of chemokines and adhesion molecules, complement activation, leukocyte recruitment and thrombus formation (3,4,6–8).

More than 90% of childhood cases of STEC-HUS fully recover from acute disease, although long-term renal sequelae have been reported in up to 25% of patients (9). The reasons why only a small fraction of STEC-infected

subjects develops a severe form of HUS is not clear, but a role of genetic predisposition has been hypothesized (10).

On the other hand, 10% of HUS cases are not associated to STEC infections (atypical HUS, aHUS). Mutations in genes encoding for proteins of the complement system have been shown to predispose to aHUS (1). The complement system is involved in the immediate defense against pathogens through three different pathways, the classical, the lectin and the alternative pathways, converging at the cleavage of C3. About 60% of patients with aHUS carry mutations in genes encoding regulatory proteins of the alternative pathway (AP) of complement, like factor H (*CFH*), factor I (*CFI*) and membrane cofactor protein (*MCP*), or gain-of-function mutations in the genes of the two components of the AP C3 convertase, C3 and factor B (*CFB*) (11). Genetic abnormalities of the endothelial anticoagulant and complement-inhibitory protein thrombomodulin (THBD) have also been reported in aHUS (12). In addition, anti-CFH autoantibodies have been described mostly in children that lack CFHR1 and CFHR3 (CFH-related proteins 1 and 3) due to a deletion of the corresponding genes (13–16). All the above abnormalities result in uncontrolled complement activation on endothelial cell surface (11,17–19).

The clinical outcome of aHUS is usually unfavorable. About 50% of cases progress to end stage renal disease (ESRD) and up to 25% may die during the acute phase (20).

Here we describe two patients with a clinical history of STEC-HUS, selected among 41 STEC-HUS patients of the International Registry of Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura (HUS/TTP). Both patients developed ESRD soon after disease onset and received a kidney graft, one from a deceased donor and one from a living-related donor. Disease recurrence is uncommon in patients transplanted after STEC-HUS (20), and indeed the other three STEC-HUS transplanted patients of the Registry had an excellent graft outcome. At variance both cases here presented lost the kidney graft for HUS recurrence, a complication more commonly observed in aHUS (19,20). Thus, before planning a second renal transplantation, the patients underwent genetic screening for aHUS-associated genes. We found a heterozygous *CFI* mutation in patient #1 and a heterozygous *MCP* mutation in patient #2 and also in her mother, who donated the kidney.

We argue that the two cases originally diagnosed as STEC-HUS had indeed aHUS triggered by STEC infection on a genetic background of impaired complement regulation, which predisposed to development of posttransplant aHUS recurrence.

We discuss the implications of these findings for pretransplant genetic counseling in patients who developed ESRD following STEC-HUS.

Material and Methods

The two patients were selected among those with diagnosis of STEC-HUS referred to the International Registry of HUS/TTP.

The patients and healthy controls provided informed written consent. The study was approved by the Ethics Committee of the Azienda Sanitaria Locale, Bergamo, Italy.

Genomic DNA was extracted from peripheral blood leukocytes (Nucleon BACC2 kit, Amersham, UK). The coding sequences and the intronic flanking regions of complement genes were screened by direct sequencing (AB-3730 sequencer).

CFHR3-CFHR1 deletion was detected by Multiplex Ligation-dependent Probe Amplification, MLPA (SALSA MLPA P236-A1 kit, MRC Holland).

Complement C3 and C4 levels in serum were evaluated by kinetic nephelometry; CFH and CFI levels were measured by enzyme-linked immunosorbent assay (ELISA).

Paraffin-embedded sections of kidney biopsy of patient #1 were analyzed by light microscopy after staining with hematoxylin and eosin for histopathological evaluation. Additional sections were analyzed for deposition of C3 and C5b-9 using mAb anti-C3 (Quidel, San Diego, CA) and mAb WU 13–15 anti-C9 neoantigen (a gift from Prof. R. Wurzner, Insbruck, Austria) as primary antibodies and the streptavidin–biotin–peroxidase complex system.

Patient #1

The clinical course of the patient, at present 47 years old, was partially described in a previous report (21). She was first hospitalized at the age of 26 years because of watery diarrhea, profuse vomiting and syncope. Laboratory exams showed platelet count 27,000/μL, hemoglobin 8.1 gr/dL, hematocrit 26%, LDH 2.200 U/L and serum creatinine 2 mg/dL. The diagnosis of HUS was made. Search for *E. coli* infection showed positivity for anti-Stx and anti-*E. coli* O157:H7 lipopolysaccharide antibodies in serum. A renal biopsy demonstrated classic histological features of thrombotic microangiopathy (Figure 1A–D). A diffuse and intense reactivity for C3 and C9, the latter reflecting formation of the terminal complement complex (C5b-9), was evident in glomeruli with overt ischemic alterations (Figure 1E–F).

Biochemical analyses, performed during the acute phase, showed low C3 levels (65.6 mg/dL; normal range: 90–180 mg/dL) and normal C4 levels (14.6 mg/dL; normal range: 10–40 mg/dL), suggesting activation of the AP.

Despite intensive plasma exchange the disease progressed, renal function deteriorated and hemodialysis was started. Seizures and hypertensive encephalopathy developed and the patient underwent bilateral nephrectomy that was followed by complete neurological and hematological remission. At discharge, the patient was on chronic hemodialysis but her blood pressure was well-controlled (21).

At 33 years of age the patient received a cadaveric renal allograft under immunosuppressive therapy with cyclosporine, mycophenolate mofetil and methylprednisolone; however, one year after transplantation she developed clinical and laboratory signs of HUS recurrence (platelet count 37 000/μL, hemoglobin 9 gr/dL, hematocrit 29%), with severe renal and neurological involvement (serum creatinine 4 mg/dL, seizures). No

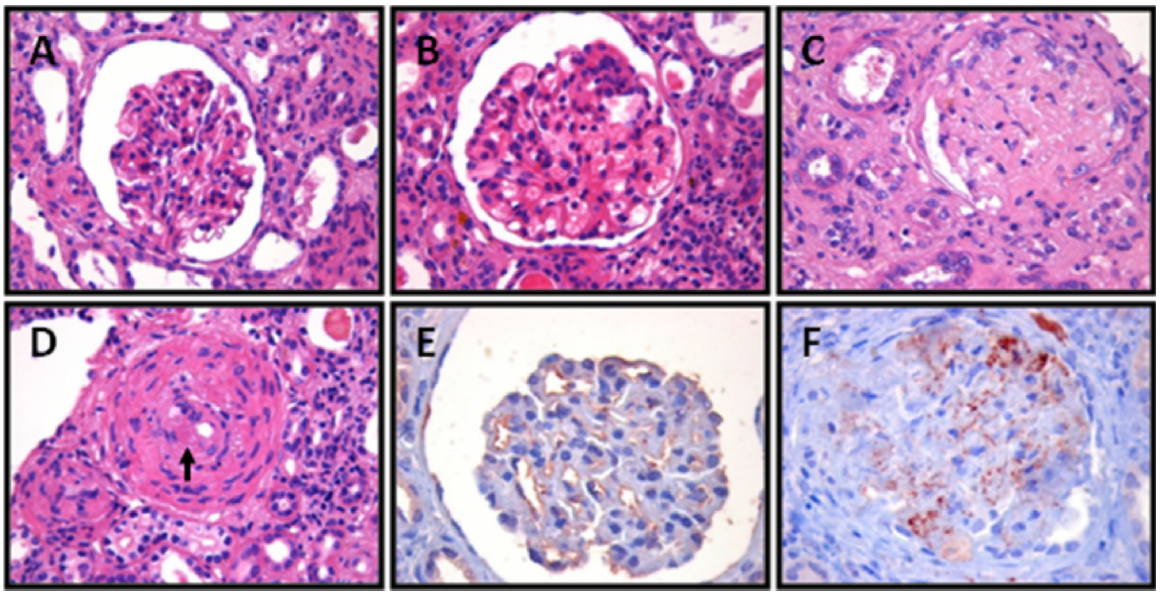

Figure 1: Renal biopsy of the native kidney from patient #1. Images show typical features of active microangiopathic process characterized by glomerular alterations of variable severity from slight ischemic changes (A) with capillary engulfment and mesangial proliferation (B) to infarction and mesangiolysis (C) (original magnification 400×). Small arteries and arterioles showed prominent intimal (black arrows) and subintimal proliferation leading to lumen occlusion (D) (original magnification 400×). Panels E and F: Immunohistochemistry showing C3 deposition on the endothelial layer of the glomerular tuft (E). C9 immunostaining demonstrates diffuse terminal complement activation (C5b-9) in glomeruli with overt ischemic alterations (F). Original magnification 400×, Streptavidin-biotin-peroxidase complex method.

improvement was achieved with plasma exchange and infusion, the patient developed ESRD and the graft was explanted. About 10 years later, before planning a second kidney transplantation, the patient was referred to our center to investigate her possible genetic predisposition to HUS and hence the risk of disease recurrence. No relevant clinical history was reported in her family.

C3 serum levels were persistently low (73 mg/dL) despite the patient was in remission without hematological signs of the disease, while C4 levels were normal (36 mg/dL). Serum levels of factor H (415 mg/L, normal range: 350–750 mg/L) and factor I (60.9 mg/L normal range: 38–65 mg/L) were normal. Genetic analyses of *CFH*, *CFI* and *MCP* were performed on leukocyte DNA and we found a new c.1234G > A heterozygous missense mutation in *CFI*, causing the aminoacidic substitution p.Val412Met (Figure 2A). This residue is localized in the serine protease domain SP (Figure 2B) that is responsible for C3b inactivation by CFI (22). The aminoacidic change Val412Met is predicted as damaging by PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/, score 0.997) and Sift (http://sift.jcvi.org, score 0.01) softwares. The variant is not reported in NCBI online database and was not found in any of 220 healthy controls. Homozygous deletion of *CFHR3-CFHR1*, previously associated with development of anti-CFH autoantibodies and aHUS (16), was excluded by MLPA (16).

Patient #2

The clinical history of this patient, a 23-year-old woman, started at the age of 16 years when she presented with diarrhea, thrombocytopenia (platelet count 33 000/μL), microangiopathic hemolytic anemia (hemoglobin 6.1 gr/dL, hematocrit 17,3%), hypertension and very severe renal failure, quickly developing ESRD (serum creatinine 21 mg/dL). Infection by *E. coli* O157:H7 producing Stx1 and Stx2 was documented in stool cultures.

At 18 years of age living-related kidney transplantation from her mother was performed. Seven months after transplantation the patient manifested graft dysfunction. A graft biopsy revealed intraglomerular thrombi, ischemic alterations and arteriolar thrombosis leading to diagnosis of thrombotic microangiopathy. Despite intensive plasma-exchange, anemia (hemoglobin 8 gr/dL), thrombocytopenia (platelet count 80 000/μL) and severe graft dysfunction (serum creatinine 10 mg/dL) persisted and 2 months later the patient lost the graft.

Before planning kidney transplantation, DNA samples of the patient, of her parents and of the two siblings were sent to our center to investigate a possible genetic predisposition to HUS and to evaluate the further risk of disease recurrence. No relevant clinical history was reported in her family. Both the parents and the siblings were healthy (Figure 3).

C3 and C4 serum levels were normal in the patient. Serum levels of factor H (474 mg/L) were also normal. Genetic analyses of *CFH, CFI, MCP, C3, CFB* and *THBD* were performed on leukocyte DNA. The patient was found to be heterozygous for the c.286 + 2T > G (also known as IVS2 + 2T > G (23)) splice site mutation in *MCP*. This mutation has been previously described in aHUS patients (23,24) and has been found to cause an abnormal splicing between exons 2 and 3, resulting in either protein truncation (24) or in the deletion of 48 aminoacids (23). The mutation was absent in 319 healthy controls. Analysis of relatives' DNA revealed the same mutation in the mother and in the brother, but not in the father and the sister (Figure 3 and 4).

Homozygous deletion of *CFHR3-CFHR1* was excluded by MLPA (16).

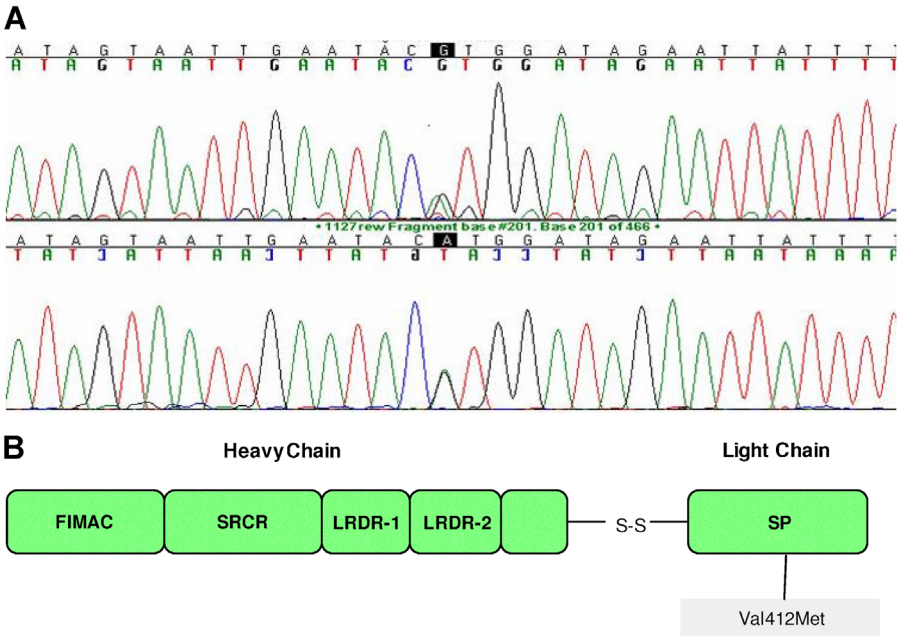

Figure 2: Sequencing electropherograms showing the c.1234G > A heterozygous mutation in *CFI* of patient #1. A: upper chromatogram, forward; lower chromatogram, reverse. B: Schematic structure of CFI protein with the localization of the mutation.

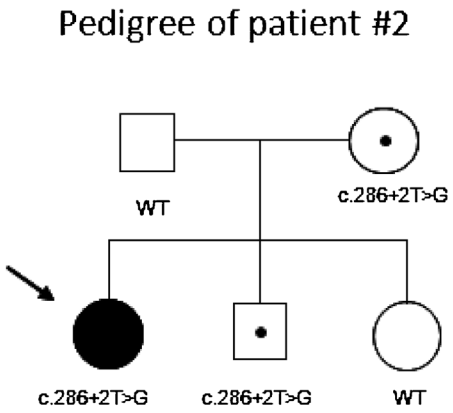

Figure 3: Family pedigree of patient #2. The black circle represents the proband carrying the heterozygous *MCP* mutation c.286 + 2T > G; healthy carriers are indicated by a black dot. WT: Wild-type.

Discussion

Here we describe two unrelated patients who debuted with STEC-HUS, developed ESRD soon after the onset of the disease and experienced HUS recurrence after kidney transplantation. This disease course is rather unusual. First, patients with STEC-HUS usually recover from acute episode, although on long term 25% of them develop some degree of renal impairment (9). Second, the disease rarely recurs in kidney transplants performed after STEC-HUS (19). STEC-released Stx is the causative agent of STEC-HUS, therefore a re-exposure to STEC would be required to trigger recurrence. In addition patients with STEC-HUS develop neutralizing anti-Stx antibodies that persist for long-time, thus protecting from recurrences (25).

Such severe clinical features instead closely resemble those reported in patients with aHUS and mutations in complement regulatory proteins. Consistently, genetic screening revealed the presence of a heterozygous *CFI* mutation associated with persistent low C3 levels in patient #1, whereas a heterozygous *MCP* splicing mutation was found in patient #2, who showed normal complement serum levels, and also in her mother, who donated the kidney.

CFI encodes a plasma serine protease that regulates the activation of complement system by inactivating C3b. Mutations in *CFI* have been reported in 4–10% of aHUS cases (1,11) but had never been previously described in association with STEC-HUS. MCP is a transmembrane protein that serves as cofactor for CFI to cleave C3b and C4b deposited on host surfaces (1). *MCP* mutations have been previously reported in 10–15% of aHUS patients (1), and also in a severe fatal case of STEC-HUS (10).

Evidence is emerging that STEC triggers complement activation, as documented by finding low C3 serum levels, and transient plasma elevations of C3 (C3b, C3c and C3d) and CFB (Ba, Bb) breakdown products and of sC5b9, during the acute phase of STEC-HUS (26). In addition, Stx was found to cause complement activation and C3 deposition

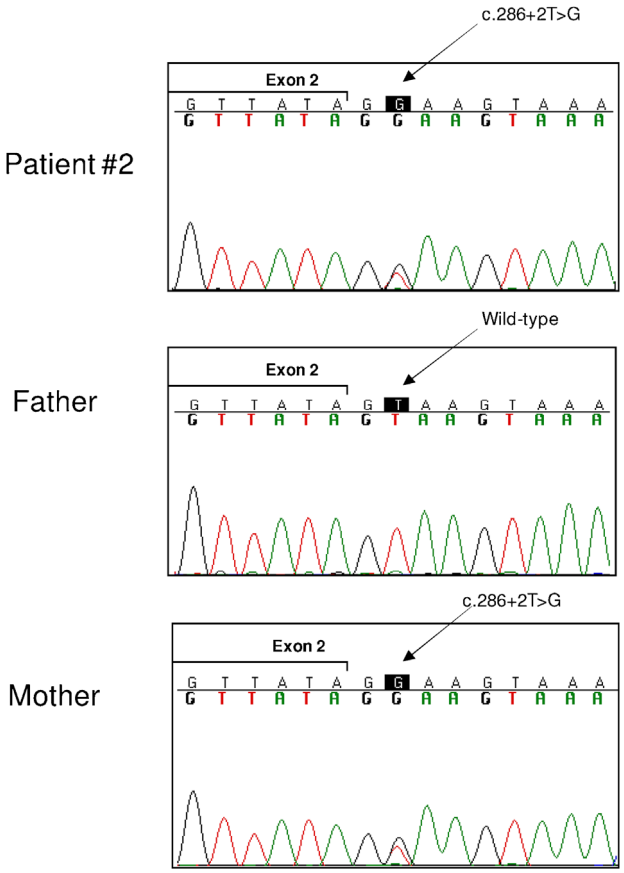

Figure 4: Sequencing electropherograms of patient #2 and her parents showing the c.286 + 2T > G heterozygous mutation in *MCP*.

on endothelial cells *in vitro* (27). Stx binding to endothelial cells upregulates mRNA expression and protein levels of cell adhesion molecules, including P-selectin (8), which has been shown to bind C3b with high affinity (27). Finally, mice deficient in CFB treated with Stx and LPS exhibited less thrombocytopenia and were protected against glomerular abnormalities and renal function impairment as compared with wild-type mice, providing in vivo evidence of the role of the AP of complement in STEC-HUS (27). The evidence that complement activation may have a pathogenic role in the microangiopathic lesions of STEC-HUS (3) provided the rationale for complement inhibitor therapy in three children with severe STEC-HUS who fully recovered with Eculizumab (28), an anti-C5 antibody that prevents the formation of the anaphylotoxin C5a and of the C5b-9 lytic complex.

The above published data and present findings of intense deposition of C3 and C5b-9 in renal biopsy of patient #1 taken at the time of STEC infection indicate that exposure to STEC may cause complement activation and trigger severe HUS in subjects with genetic alterations of complement regulatory proteins. In patient #1, the kidney transplant did not correct the *CFI* genetic defect, since the altered protein is produced by the liver and persists in the circulation after kidney transplantation (20). In such conditions, the kidney graft was not protected from complement activation triggered by ischemia and reperfusion injury posttransplantation, alloimmune response and immunosuppressive drugs, resulting in aHUS. Hence, the recurrence was due to the *CFI* mutation and had nothing to do with the patient having had STEC exposure in the past.

The above interpretation is consistent with published data that in patients with aHUS carrying genetic abnormalities in circulating regulatory proteins like CFI and CFH, the risk of recurrence after renal transplantation is very high (11).

Eculizumab has been shown to be beneficial to treat acute episodes of aHUS and also as prophylaxis of recurrences in the kidney graft (29,30) in patients with aHUS and complement gene mutations. The presence in patient #1 of a *CFI* mutation, along with the finding of intense deposition of C3 and C5b-9 in renal biopsy, would provide the rationale for a preemptive use of Eculizumab as part of a new renal transplant plan for this patient deemed at high risk for recurrent disease.

MCP is a transmembrane protein widely expressed in the kidney, thus transplantation of a kidney expressing normal MCP corrects the local genetic defect in aHUS-patient with *MCP* mutations. Consequently, these patients usually have a good graft outcome with a low incidence of posttransplant recurrence (20). Such observation in aHUS patients does not fit with the finding here that patient #2 carrying an *MCP* mutation experienced HUS recurrence in the graft. Such discrepancy can be explained by the fact that patient #2 received the graft from her mother who carries the same *MCP* mutation. Thus in this case the kidney graft produced the same dysfunctional MCP protein as the native kidney and it was not protected enough from complement hyperactivation leading to aHUS recurrence.

In conclusion, the cases here reported indicate that screening of HUS-associated complement genes should be performed in all patients on dialysis following severe forms of STEC-HUS, since they may carry complement gene mutations and be undiagnosed cases of aHUS precipitated by STEC infections.

A correct diagnosis is critical to predict the risk of recurrence after transplantation and accordingly to adopt suitable prophylactic measures. Furthermore, genetic analysis of potential donors is mandatory before living related transplantation.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Noris M, Remuzzi G. Atypical hemolytic–uremic syndrome. N Engl J Med 2009; 361: 1676–1687.
2. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 2005; 365: 1073–1086.
3. Orth D, Wurzner R. Complement in typical hemolytic uremic syndrome. Semin Thromb Hemost 2010; 36: 620–624.
4. Obrig TG, Louise CB, Lingwood CA, Boyd B, Barley-Maloney L, Daniel TO. Endothelial heterogeneity in Shiga toxin receptors and responses. J Biol Chem 1993; 268: 15484–15488.
5. Wu CJ, Hsueh PR. Ko WC. A new health threat in Europe: Shiga toxin-producing *Escherichia coli* O104: H4 infections. J Microbiol Immunol Infect 2011; 44: 390–393.
6. O'Loughlin EV, Robins-Browne RM. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. Microb Infect 2001; 3: 493–507.
7. Zoja C, Angioletti S, Donadelli R, et al. Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. Kidney Int 2002; 62: 846–856.
8. Morigi M, Galbusera M, Binda E, et al. Verotoxin-1-induced up-regulation of adhesive molecules renders microvascular endothelial cells thrombogenic at high shear stress. Blood 2001; 98: 1828–1835.
9. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: A systematic review of meta-analysis, and meta-regression. JAMA 2003; 290: 1360–1370.
10. Fang CJ, Fremeaux-Bacchi V, Liszewski MK, et al. Membrane cofactor protein mutations in atypical hemolytic uremic syndrome (aHUS), fatal Stx-HUS, C3 glomerulonephritis, and the HELLP syndrome. Blood 2008; 111: 624–632.
11. 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: 1844–1859.
12. Delvaeye M, Noris M, DeVriese A, et al. Mutations in thrombomodulin in hemolytic–uremic syndrome. N Engl J Med 2009; 361: 345–357.
13. Moore I, Strain L, Pappworth I, et al. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. Blood 2010; 115: 379–387.
14. Zipfel PF, Edey M, Heinen S, et al. Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. PLoS Genet 2007; 3: e41.
15. Dragon-Durey MA, Loirat C, Cloarec S, et al. Anti-factor H autoantibodies associated with atypical hemolytic uremic syndrome. J Am Soc Nephrol 2005; 16: 555–563.
16. Dragon-Durey MA, Blanc C, Marliot F, et al. The high frequency of complement factor H-related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. J Med Genet 2009; 46: 447–450.
17. Heinen S, Jozsi M, Hartmann A, et al. Hemolytic uremic syndrome: A factor H mutation (E1172Stop) causes defective complement control at the surface of endothelial cells. J Am Soc Nephrol 2007; 18: 506–514.
18. Walport MJ. Complement first of two parts. N Engl J Med 2001; 344: 1058–1066.
19. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. Nat Rev Nephrol 2012; 8: 622–633.
20. Noris M, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. Am J Transplant 2010; 10: 1517–1523.
21. Remuzzi G, Galbusera M, Salvadori M, Rizzoni G, Paris S, Ruggenenti P. Bilateral nephrectomy stopped disease progression in plasma-resistant hemolytic uremic syndrome with neurological signs and coma. Kidney Int 1996; 49: 282–286.
22. Nilsson SC, Sim RB, Lea SM, Fremeaux-Bacchi V, Blom AM. Complement factor I in health and disease. Mol Immunol 2011; 48: 1611–1620.
23. Fremeaux-Bacchi V, Moulton EA, Kavanagh D, et al. Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. J Am Soc Nephrol 2006; 17: 2017–2025.
24. Maga TK, Nishimura CJ, Weaver AE, Frees KL, Smith RJ. Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. Hum Mutat 2010; 31: E1445–E1460.
25. 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.
26. Robson WL, Leung AK, Fick GH, McKenna AI. Hypocomplementemia and leukocytosis in diarrhea-associated hemolytic uremic syndrome. Nephron 1992; 62: 296–299.
27. Morigi M, Galbusera M, Gastoldi S, et al. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. J Immunol 2011; 187: 172–180.
28. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. N Engl J Med 2011; 364: 2561–2563.
29. Weitz M, Amon O, Bassler D, Koenigsrainer A, Nadalin S. Prophylactic eculizumab prior to kidney transplantation for atypical hemolytic uremic syndrome. Pediatr Nephrol 2011; 26: 1325–1329.
30. Zimmerhackl LB, Hofer J, Cortina G, et al. Prophylactic eculizumab after renal transplantation in atypical hemolytic-uremic syndrome. N Engl J Med 2010; 362: 1746–1748.