Relative Role of Genetic Complement Abnormalities in Sporadic and Familial aHUS and Their Impact on Clinical Phenotype

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Background and objectives: Hemolytic uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment. Most childhood cases are caused by Shiga toxin–producing bacteria. The other form, atypical HUS (aHUS), accounts for 10% of cases and has a poor prognosis. Genetic complement abnormalities have been found in aHUS.

Design, setting, participants, and measurements: We screened 273 consecutive patients with aHUS for complement abnormalities and studied their role in predicting clinical phenotype and response to treatment. We compared mutation frequencies and localization and clinical outcome in familial (82) and sporadic (191) cases.

Results: In >70% of sporadic and familial cases, gene mutations, disease-associated factor H (*CFH*) polymorphisms, or anti-CFH autoantibodies were found. Either mutations or *CFH* polymorphisms were also found in the majority of patients with secondary aHUS, suggesting a genetic predisposition. Familial cases showed a higher prevalence of mutations in SCR20 of CFH and more severe disease than sporadic cases. Patients with *CFH* or *THBD* (thrombomodulin) mutations had the earliest onset and highest mortality. Membrane-cofactor protein (*MCP*) mutations were associated with the best prognosis. Plasma therapy induced remission in 55 to 80% of episodes in patients with *CFH*, *C3*, or *THBD* mutations or autoantibodies, whereas patients with *CFI* (factor I) mutations were poor responders. aHUS recurred frequently after kidney transplantation except for patients with *MCP* mutations.

Conclusions: Results underline the need of genetic screening for all susceptibility factors as part of clinical management of aHUS and for identification of patients who could safely benefit from kidney transplant.

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Hemolytic uremic syndrome (HUS) is a disorder of the microvasculature with hemolytic anemia, thrombocytopenia, and acute renal failure (1). Most childhood cases are caused by *E. coli* strains producing Shiga-like toxins (Stx-*E. coli*) (2,3). However, ~10% of cases are not caused by Stx-*E. coli* (4). This atypical form (aHUS) can be sporadic or familial (4,5) and has a poor prognosis, with a 10 to 15% mortality rate during the acute phase (6) and up to 50% of cases progressing to end-stage renal failure (ESRF).

Extensive research has established an association between aHUS and uncontrolled activation of the alternative pathway of the complement system (4). More than 120 mutations in *CFH*, *CFI*, and *MCP*, encoding the regulatory proteins complement factor H, factor I, and membrane-cofactor protein, respectively, have been reported in patients with aHUS (www.FH-HUS.org). Gain-of-function mutations in key proteins of the alternative pathway, complement factor B (CFB), and C3 have also been reported (7–9). More recently, mutations in *THBD* encoding thrombomodulin, a membrane-bound glycoprotein with anticoagulant properties that modulates complement activation on cell surfaces, have also been associated with aHUS (10). Finally, anti-CFH autoantibodies have been described in sporadic forms (11). Of note, 90% of patients with anti-CFH autoanti-

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bodies have complete deficiency of factor H–related proteins (CFHR) 1 and 3 secondary to deletion of the *CFHR1* and *CFHR3* genes (12,13), suggesting a pathogenetic link between *CFHR1/CFHR3* deletion and anti-CFH autoantibodies. Novel genetic abnormalities of *CFHR1*, *CFHR3,* and *CFHR1-CFHR4A* have recently been reported (14). Published genetic abnormalities (5,15–17) account for ~70% of familial forms and have been also found in sporadic aHUS, mainly in idiopathic, but also in few secondary forms (4,18).

In this study, we performed genetic screening for aHUS susceptibility factors in a large cohort of patients to (*1*) evaluate the prevalence of known genetic complement abnormalities in sporadic and familial aHUS, (*2*) compare the prevalence and distribution of mutations in sporadic and familial cases, (*3*) examine genotype–phenotype correlations with regard to response to plasma treatment, short- and long-term outcomes, and outcome of kidney transplantation, and (*4*) compare sporadic *versus* familial cases and childhood *versus* adult cases for the above clinical parameters.

Materials and Methods

Patients and Controls

Diagnosis of aHUS was done as described (15) (see Supplementary Material). Two hundred seventy-three patients who had been registered consecutively from 1996 to 2007 within the International Registry of Recurrent and Familial HUS/TTP were recruited: 58% from Italy, 15% from other European countries, 14% from North America, 2% from South America, 2% from Africa, 1% from Asia, and 8% from the Middle East. One hundred ninety-one were classified as sporadic and 82 as familial (31 families; 2 to 11 affected subjects/family). Among sporadic cases, 144 were idiopathic, and the others had secondary forms (Table 1). Available relatives of patients with mutations were screened to establish disease penetrance.

An appropriate panel of healthy controls was also screened (Supplementary Material). All participants provided informed written consent. The protocol was approved by the Ethics Committee of the Azienda Sanitaria Locale, Bergamo, Italy.

Genetic Analysis, Search for Autoantibodies, and CFHR1-3 Deletion

Genomic DNA was extracted from blood leukocytes (BACC2 kit; Nucleon, Amersham, UK). The coding sequence and the intronic flanking regions were directly sequenced (AB-3130-XL sequencer). Each sequence variant found in aHUS patients was searched for in healthy controls.

Screening for *CFH/CFHR1* rearrangements was performed as described (19), and the presence of a *CFH/CFHR1* hybrid gene was confirmed by long PCR with a *CFH* specific forward primer (in exon 20) and a *CFH/CFHR1* common reverse primer (in exon 23), followed by sequencing using the reverse primer. CFH autoantibodies were evaluated by ELISA (11,12,20). CFHR1-3 deletion was detected by Western blotting (12).

Biochemical Testing

C3 and C4 serum levels were evaluated by kinetic nephelometry; CFH levels were measured by radial immunodiffusion assay (The Binding Site).

Statistical Analyses

Differences in clinical and biochemical data among patients with or without mutations, patients with familial and sporadic forms, and patients with childhood and adulthood onset were analyzed by χ^2 or Fisher tests with Bonferroni's correction for multiple comparisons. The frequencies of *CFH* genotypes in aHUS patients and controls were compared by the χ^2 test. Cumulative fractions of patient-free of events (defined as the combination of ESRF or death, whichever occurred first after the onset of HUS, or the occurrence of death alone) were estimated by Kaplan-Meier analyses. *P* values for differences between groups were calculated by the log-rank test, and, when feasible, unadjusted Cox proportional hazards regression models were used to calculate hazard ratios and the corresponding 95% confidence intervals. Differences were considered statistically significant at $P < 0.05$ after Bonferroni's correction for multiple comparisons.

Results

Genetic Screening

The entire coding region of *CFH, CFI, MCP,* and *THBD* (10) were sequenced in 273 consecutive patients with aHUS. Results of genetic screening in *CFH*, *MCP,* and *CFI* in the first 156 patients have been previously published (15). For all genes, the mutation rate was higher in familial *versus* sporadic cases. Sixty-two patients, all whites, carried single *CFH* mutations (mutation rate: overall, 23%; sporadic, 16%; familial, 40%; Figure 1A; Table 1). All mutations were heterozygous, with the exception of a homozygous Y899X in a sporadic patient and a homozygous 3675–3699del in 10 patients from a consanguineous Bedouin family. All mutational events but one (causing protein interruption in short-consensus-repeat [SCR]8) in familial cases were located in SCR20 *versus* 60% of those in sporadic cases.

In four nonconsanguineous patients of African origin, six *CFH* variants were found, which were also detected in African controls ($n = 11$) but not in white controls, indicating that the *CFH* genotype is ethnicity specific.

The association of the T variant of the promoter polymorphism C-257T (rs3753394) and the D variant of the E936D polymorphism (G2808T, rs1065489) (21) with aHUS was also studied. First, only aHUS patients with white ethnicity ($n = 245$) were compared with white controls ($n = 200$). Both polymorphisms were strongly associated with aHUS (carriers frequencies C-257T, TT/CT: 0.65 aHUS, 0.44 controls, $P < 0.0001$; E936D, ED/DD: 0.51 aHUS, 0.33 controls, $P = 0.003$; allele frequencies −257T: 0.42 aHUS, 0.26 controls; 936D: 0.32 aHUS, 0.17 controls). We then also included nonwhite patients, with identical results (C-257T: $P < 0.0001$, E936D: $P = 0.003$).

CFH autoantibodies were screened in 149 patients for whom serum was available and were detected in 10 idiopathic sporadic patients: 8 without mutations (4%) and 2 with *CFH* mutations (Table 1). In all but one patient, CFH autoantibodies were associated with *CFHR1-3* deletion (12).

Ten white patients carried single *CFI* mutations (overall: 4%, sporadic: 3%, familial: 5%; Figure 1B; Table 1), all heterozygous. Eighteen white patients carried single *MCP* mutations (overall: 7%, sporadic: 7%, familial: 6%; Figure 1E; Table 1); all but two were heterozygous (15), and 90% clustered in the four extracellular SCRs. Thirteen patients (12 whites and 1 Chinese) carried single heterozygous *THBD* mutations (overall: 5%, sporadic: 3%, familial: 9%; Figure 1F; Table 1). Nine additional white

Table 1. Genetic abnormalities in patients with aHUS

Screened Subjects		Mutations								
		CFH[a] (n = 273)	CFI (n = 273)	C3 (n = 146)	THBD (n = 273)	MCP (n = 273)	Combined (n = 273)	Other[b] (n = 48)	CFH Ab (n = 149)	Total
Sporadic patients	191	30 (16%)	6 (3%)	8 (4%)	6 (3%)	13 (7%)	3 (2%)	4 (2%)	8 (4%)	78 (41%)
Idiopathic	144	23 (16%)	4 (3%)	7 (5%)	5 (3%)	12 (8%)	3 (2%)	3 (2%)	8 (6%)	65 (45%)
Secondary:										
Malignancy and chemotherapy	1	—	—	—	—	—	—	—	—	—
Malignant hypertension	14	1 (7%)	—	—	—	1 (7%)	—	—	—	2 (14%)
Post-transplant HUS[c] and calcineurin inhibitors	11	1 (9%)	1 (9%)	—	1 (9%)	—	—	—	—	3 (27%)
Pregnancy-related HUS	10	2 (20%)	1 (10%)	—	—	—	—	—	—	3 (30%)
Systemic disease	3	1 (33%)	—	—	—	—	—	—	—	1 (33%)
Glomerulopathy	8	2 (25%)	—	1 (12%)	—	—	—	1 (12%)	—	4 (44%)
Familial patients	82	35 (43%)	4 (5%)	4 (5%)	7 (9%)	5 (6%)	6 (7%)	0	0	61 (74%)
Overall patients	273	65 (24%)	10 (4%)	12 (4%)	13 (5%)	18 (7%)	9 (3%)	4 (2%)	8 (3%)	139 (51%)

[a]Includes two patients with CFH mutations and CFH autoantibodies and patients with the *CFH/CFHR-1* hybrid gene (n = 3 of 48 patients screened).
[b]Includes mutations in *CFB* (n = 1: R183W), *C1-inh* (n = 2: A2V and K342N), and *CFP* (n = 1: D299N).
[c]Primary cause of nephropathy was unknown in 6 of 11 patients (1 of which carries a mutation in *CFH*, 1 in *CFI*, and 1 in *THBD*), 2 patients had IgA nephropathy, 1 had diabetic nephropathy, 1 had membranoproliferative glomerulonephritis (MPGN), and 1 had reflux nephropathy.
Systemic disease: sclerodermia, systemic lupus erythematosus. Glomerulopathy: membranoproliferative glomerulonephritis, nephrotic syndrome, mesangioproliferative glomerulonephritis, membranous glomerulonephritis (see Supplemental Table 2).

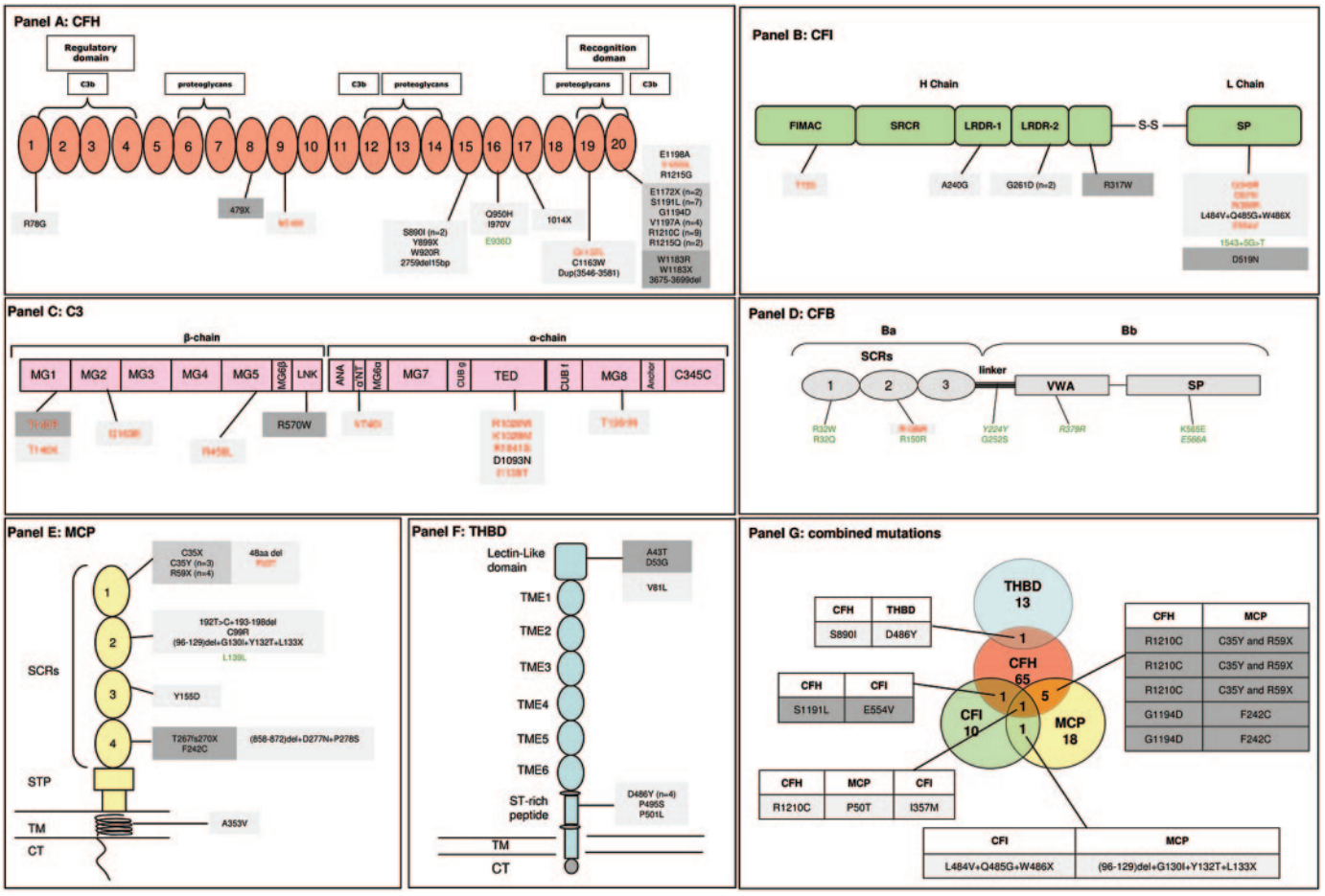


Figure 1. Summary of *CFH* (A), *CFI* (B), *C3* (C), *CFB* (D), *MCP* (E), and *THBD* (F) variants and of combined mutations (G) in aHUS patients from the International Registry of Recurrent and Familial HUS/TTP. Already published mutations are reported in black; new mutations are in red; and polymorphisms are in green (newly described polymorphisms are in italic). Mutations found only in familial aHUS patients are in dark gray squares, those found both in familial and sporadic cases are in gray squares, and mutations found only in sporadic cases are in light gray squares. A, B, E, and F include also mutational events in patients with combined mutations. (A) *CFH*: 69% of the overall independent mutations in CFH cluster in the C terminus short consensus repeat (SCR) 20. Another cluster of mutations is located in SCRs 15 to 16 (15.5%). Six mutations resulted in truncated proteins at SCR8 ($n = 1$), SCR15 ($n = 1$), SCR17 ($n = 1$), and SCR20 ($n = 3$). The aHUS-associated polymorphism in SCR16 (E936D) is marked in green. To complete *CFH* genetic analysis, we also screened exon 10, which produces factor H-like 1, a splice variant containing the first eight SCRs of CFH, including the complement regulatory domain. No mutations and/or polymorphisms were found. (B) *CFI*: 6 mutations (58%) cluster in the serine-protease domain of CFI. Of note, the intronic change 1534 + 5 G>T that was previously reported by us as a HUS-associated mutation (15) is indicated in the figure as a polymorphism because, in this report, we found this variant in a healthy control. (C) *C3*: the mutations are spread all over the gene; however, a hot spot is evidenced in the thioester-containing domain (TED domain) with five independent mutations (42%). (D) *CFB*: only one heterozygous mutation (in SCR2) has been found. Eight polymorphic variants were identified. (E) *MCP*: 17 independent mutations (94%) cluster in the four SCRs at the N terminus of MCP, and 55.5% are located within SCR1, confirming the importance of this region for complement regulation. The R59X and C35Y mutations were identified four and three times, respectively, suggesting that they may represent a mutational hot spot in *MCP*. A L139L sinonymous polymorphism has been found in *MCP* in a sporadic patient, but it was not found in healthy controls. This subject carries also a mutation in *CFH*. The amino acid syntax of MCP that takes into account the signal peptide has been adopted. (F) *THBD*: three independent mutational events cluster in the lectin-like domain and six cluster in the serine threonine rich (ST-rich) peptide. (G) Diagram showing the number of patients with single or combined mutations from the International Registry of Recurrent and Familial HUS/TTP is reported. Numbers of patients with mutations in *CFH*, *MCP*, *CFI*, and *THBD* alone are shown in the circles. The numbers of patients carrying combined mutations are shown in the overlapping areas; the amino acid changes are reported in the corresponding boxes.

patients carried mutations in more than one gene (mutation rate: overall, 3%; sporadic, 2%; familial, 7%; Figure 1G; Table 1).

All patients without mutations in the above genes were screened for *C3* ($n = 146$). Twelve heterozygous *C3* mutations were found in 12 white patients (8 sporadic and 4 familial; 1 patient carried 3 mutations; Figure 1C; Table 1); 3 of them had normal C3 serum levels (Table 2).

Of 48 patients who did not have *CFH*, *CFI*, *C3*, *MCP*, or *THBD*

Table 2. Patient characteristics

Patients	Genetic Abnormalities					CFH Ab ($n = 8$)	None ($n = 134$)
	CFH ($n = 65$)	CFI ($n = 10$)	C3 ($n = 12$)	THBD ($n = 13$)	MCP ($n = 18$)		
Disease onset	**(65)**	**(10)**	**(12)**	**(13)**	**(18)**	**(8)**	**(130)**
Children (≤18 years)	39	4	6	12	14	6	71
Adults (>18 years)	26	6	6	1	4	2	59
Male/female	30/24	4/6	7/5	7/3	12/6	4/4	61/72
Familial/sporadic	35/30[a]	4/6	4/8	7/6[a]	5/13	0/8	21/113
Recurrences	28 (55)[a]	1 (10)[b]	6 (12)	3 (10)	13 (18)[a]	3/8	36 (129)
Triggering/underlying conditions	**(41)**	**(8)**	**(11)**	**(5)**	**(15)**	**(7)**	**(104)**
Diarrhea/gastroentheritis	6	2	2	1	5	1	28
Upper respiratory tract infections	9	2	1	2	3	4	14
Malignancy and cancer chemotherapy	—	—	—	—	—	—	1
Malignant hypertension	4	—	—	—	—	—	12
De novo post-transplant HUS	1	1	—	—	—	—	8
Pregnancy related HUS	3	2	—	—	—	—	8
Systemic disease	1	—	—	—	—	—	2
Glomerulopathy	2	1	1	—	—	—	4
Extrarenal manifestations	**14 (49)**	**3 (9)**	**1 (11)**	**1 (10)**	**0 (18)**	**1 (7)**	**22 (107)**
Multivisceral involvement[c]	4	1	0	1	0	0	6
Cardiovascular disease only	5	0	0	0	0	0	2
Central nervous system only	5	2	1	0	0	1	14
Biochemical evaluation							
Reduced C3 serum levels (≤83 mg/dl)	23 (49)[a]	2 (10)	8 (11)[a]	4 (8)	4 (15)	3 (7)	22 (103)
Reduced C4 serum levels C4 (≤15 mg/dl)	2 (48)	0 (10)	2 (10)	1 (7)	1 (15)	1 (7)	6 (103)
Reduced CFH serum levels (≤350 mg/L)	6 (46)	0 (10)	0 (9)	0 (6)	0 (15)	2 (7)	2 (104)

The number of patients for whom data are available are reported between brackets. CFH group includes also patients with *CFH-CFHR1* hybrid gene (all familial cases) and two patients with CFH mutations and CFH autoantibodies. In this and all the subsequent tables, we included in the analysis also deceased affected relatives of index cases within families.

[a] $P < 0.0024$ after Bonferroni correction compared with the "none" group.

[b] $P < 0.0024$ after Bonferroni correction compared with the MCP group.

[c] Cerebral, cardiac, pulmonary, and pancreatic.

mutations, 3 (from two families) carried a *CFH/CFHR1* hybrid gene (12).

CFB and other candidate genes involved in complement pathway were also analyzed (details on selection criteria and results are in Supplementary Material, Table 1, and Figure 1D), but the mutation rate was very low.

Mutations or anti-CFH autoantibodies were identified in 139 of 273 patients (overall, 51%; sporadic, 41%; familial, 74%; Table 1). Among sporadic cases, abnormalities were found in 45% of idiopathic forms and 14 to 44% of secondary forms (Table 1). Genetic analysis in relatives of 22 sporadic patients showed that, in 21 cases, the mutation was inherited from an unaffected parent, whereas in 1, the mutation (in *CFH*) was *de novo*.

All mutations but one were found in white patients and were not found in a panel of 120 European and U.S. white controls.

In 58% of patients with sporadic aHUS and neither mutations nor antibodies, we found the CFH-257T and/or 936D variants that have been associated with aHUS (21) (Supplemental Table 1). Thus, 75% of patients with sporadic aHUS have mutations or anti-CFH antibodies or carry disease-associated *CFH* polymorphisms.

Analysis of relatives of sporadic and familial cases showed an incomplete penetrance (*CFH*, 48%; *CFI*, 50%; *C3*, 56%; *THBD*, 64%; *MCP*, 53%).

Clinical Findings

The disease became manifest mostly in childhood (≤18 years), with the exception of patients carrying *CFI* and *C3* mutations (Table 2). The earliest onset (0 to 1 years) was in patients with *CFH* or *THBD* mutations or CFH autoantibod-

Table 3. Outcome of the first episode of aHUS and at 3 years after onset

	Abnormality	CFH			CFI			C3			THBD			MCP			CFH Antibodies			None		
		Overall	Sporadic	Familial	Overall	Sporadic	Familial	Overall	Sporadic	Familial	Overall	Sporadic	Familial	Overall	Sporadic	Familial	Overall	Sporadic	Familial	Overall	Sporadic	Familial
Outcome of the first episode	**Total**[a]	**62**	**30**	**32**	**10**	**6**	**4**	**12**	**8**	**4**	**13**	**6**	**7**	**17**	**12**	**5**	**8**	**8**	**—**	**128**	**109**	**19**
	Remission	50%[b]	67%[c]	34%	40%[b]	33%	50%	42%[b]	37%	50%	54%	67%	43%	94%	92%	100%	63%	62%	—	63%	65%	47%
	Complete remission	10%[b]	13%	6%	30%[b]	33%	25%	33%[b]	25%	50%	23%[b]	33%	14%	88%	83%	100%	25%[b]	25%	—	23%[b]	22%	26%
	Partial remission	40%	53%	28%	10%	—	25%	8%	13%	—	31%	33%	29%	6%	8%	—	38%	37%	—	40%	43%	21%
	ESRF-Death	50%[b]	33%[c]	66%	60%[b]	67%	50%	58%[b]	63%	50%	46%	33%	57%	6%	8%	—	37%	37%	—	37%	35%	53%
	ESRF	31%	33%	28%	60%[b]	67%	50%	58%[b]	63%	50%	15%	33%	—	6%	8%	—	37%	37%	—	33%	34%	32%
	Death	19%[d]	—	38%	—	—	—	—	—	—	31%[d]	—	57%	—	—	—	—	—	—	4%	1%	21%
Outcome at 3 years	**Total**[a]	**64**	**30**	**34**	**10**	**6**	**4**	**12**	**8**	**4**	**13**	**6**	**7**	**17**	**12**	**5**	**8**	**8**	**—**	**119**	**101**	**18**
	Remission	23%[b,d]	40%[e]	9%	40%[b]	33%	50%	33%[f]	25%	50%	46%	67%	29%	94%	92%	100%	37%[b]	37%	—	50%[b]	53%	28%
	Complete remission	4%[b]	7%	3%	30%[b]	33%	25%	33%[b,f]	25%	50%	23%[b]	33%	14%	88%	83%	100%	12%[b]	12%	—	18%[b]	17%	22%
	Partial remission	19%	33%	6%	10%	—	25%	—	—		23%	33%	14%	6%	8%	—	25%	25%	—	32%	36%	6%
	ESRF-Death	77%[b,d]	60%[e]	91%	60%[b]	67%	50%	67%[b]	75%	50%	54%	33%	71%	6%	8%	—	63%[b]	62%	—	50%[b]	47%	72%
	ESRF	53%[b]	53%	53%	60%[b]	67%	50%	67%[b]	75%	50%	23%	33%	14%	6%	8%	—	63%[b]	62%	—	43%[b]	44%	39%
	Death	23%	7%	38%	—	—	—	—	—	—	31%	—	57%	—	—	—	—	—	—	7%	3%	33%

Complete remission is defined as normalization of both hematologic parameters (Ht > 30%; Hb > 10 g/dl; LDH < 460 U/L; plts > 150,000/μl) and renal function (s-creatinine < 1.3 mg/dl).

Partial remission is defined as normalization of hematologic parameters with renal sequelae (chronic renal failure and/or proteinuria >0.2 g/24 h). CFH group includes also patients with *CFH-CFHR1* hybrid gene (all familial cases) and two patients with CFH mutations and CFH autoantibodies.

All *P* values were computed using Bonferroni's correction for multiple tests.

[a]Number of patients for whom data are available.

[b]$P < 0.0024$ compared with patients with MCP mutations (overall).

[c]$P = 0.011$ *versus* familial, χ^2 test.

[d]$P < 0.0024$ compared with the group without mutations (overall).

[e]$P = 0.003$ *versus* familial, χ^2 test.

[f]$P < 0.0024$ compared with patients with CFH mutations (overall).

Table 4. Summary of the most relevant clinical findings

Alteration in	ESRF or Death (3 years)	Response to Plasma (outcome of episode = CR or PR/total of treated episodes)	Good Kidney Transplantation Outcome (at 1 year)
CFH	49 (77%)	57 (63%)	5 (29%)
CFI	6 (60%)	2 (25%)	2 (33%)
C3	8 (67%)	8 (57%)	4 (57%)
THBD	7 (54%)	7 (88%)	0
MCP	1 (6%)	28 (97%)	3 (100%)
CFH Ab	5 (63%)	9 (75%)	0
Non mut	60 (50%)	71 (69%)	12 (41%)
Sporadic	83 (49%)[a]	139 (69%)	19 (46%)
Familial	53 (74%)	43 (68%)	7 (30%)
Children	70 (48%)[b]	131 (78%)[c]	8 (33%)
Adults	63 (67%)	51 (53%)	18 (45%)

[a]Comparison between sporadic and familial forms: $P < 0.0001$.
[b]Comparison between children and adults: $P = 0.004$.
[c]Comparison between children and adults: $P < 0.0001$.
CR, complete remission; PR, partial remission.

ies (Supplemental Figure 1). However, in 12 to 50% of subjects, the disease occurred after the age of 25 years (up to 83 years; Supplemental Figure 2).

Triggering/underlying conditions were found in 70% of patients. Diarrhea and/or gastroenteritis and upper respiratory tract infections were frequent triggers (Table 2). Malignant hypertension either triggered or complicated the disease in 17 patients. Pregnancy-related aHUS was reported in 13 patients; 11 had *de novo* post-transplant aHUS, and 9 had other glomerulopathies (Table 2; Supplemental Table 2).

Extrarenal involvement during HUS episodes was observed in 10 to 30% of patients, with the exception of patients with *MCP* mutations (Table 2).

Low C3 levels were reported more frequently in patients with mutations in *CFH* or *C3* than in patients without mutations.

Forty to >70% of patients with *CFH*, *CFI*, *C3*, or *THBD* mutations or anti-CFH autoantibodies developed ESRF or died during the first episode or within 3 years from onset (Table 3). *CFH* mutations affecting the C-terminal SCR20 (including the *CFH-CFHR1* hybrid gene) were associated with worse short- and long-term prognosis than those affecting SCRs 1 to 19 (Supplemental Figure 3).

Complete or partial remission was the outcome of the presenting episode in patients with *MCP* mutations (Table 3). These patients had recurrences more frequently than patients with either *CFI* mutations or without mutations (Table 2). Despite this, data at 3 years confirmed a better outcome in patients with *MCP* mutations than the other groups (Table 3).

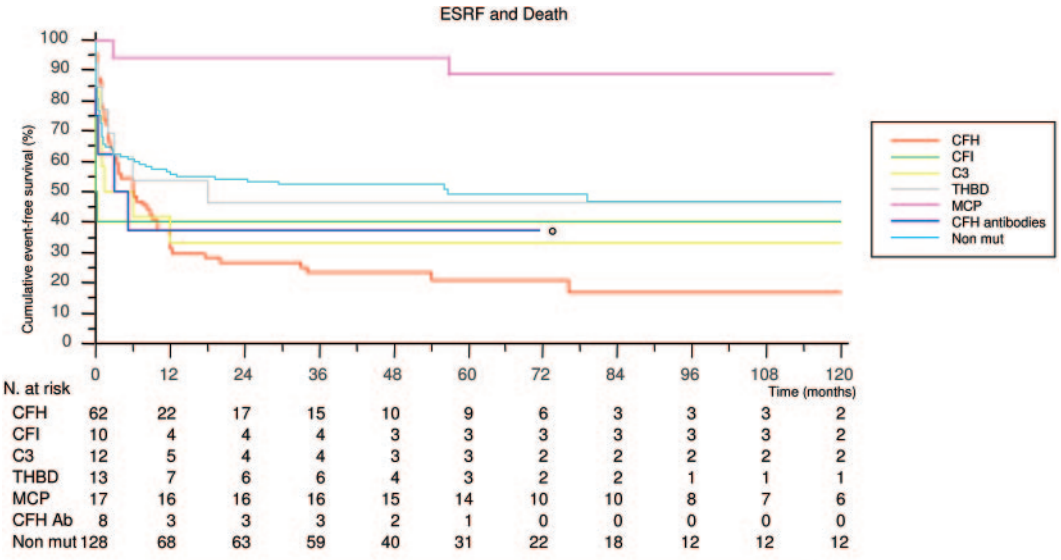
Overall, sporadic cases had a better prognosis than familial ones (Table 4). Separate analyses of groups with specific abnormalities showed a statistically significant difference between sporadic and familial cases within the *CFH*-mutated group (Table 3), possibly because of a higher prevalence of mutations in SCR20 in familial cases (Figure 1A).

Combining sporadic and familial cases, adults had a worse prognosis than children (Table 4; Supplemental Table 3).

Figure 2 shows Kaplan-Meier curves and hazard ratios for event-free survival (ESRF or death) during follow-up and confirms the best outcome for patients with *MCP* mutations. The fraction of patients still alive at any time point during follow-up is shown in Figure 3. Overall survival was worse for patients with *CFH* and *THBD* mutations than the other groups.

Plasma treatment induced complete or partial remission of 63, 25, 57, 88, and 75% of episodes in patients with *CFH*, *CFI*, *C3*, *THBD* mutations or anti-CFH autoantibodies, respectively (Tables 4 and 5). There was no difference in response to plasma infusion (complete or partial remission in 64% of episodes) *versus* plasma exchange (62%). Three patients with anti-CFH autoantibodies were given steroids together with plasma, and remission was achieved in two. Patients with *MCP* mutations underwent remission in 97% of plasma-treated episodes (Tables 4 and 5) but also in all of the 14 episodes not treated with plasma. Overall, ~70% of episodes (50% of patients) responded to plasma without differences between sporadic and familial cases. A better response to plasma treatment was observed in children than in adults (Table 4).

Transplantation outcomes in patients with *CFH* mutations were poor: 12 of 17 kidney grafts were lost for aHUS recurrence, acute rejection, or thrombosis within 1 year (Figure 4). Of the five patients with good graft outcome, three received intensive plasma prophylaxis. aHUS recurrence occurred within the first year in four out of six grafts in patients with *CFI* mutations. Simultaneous kidney and liver transplant was performed in four children with *CFH* mutations and in a child with combined *CFH/CFI* mutations. Three patients with *CFH* mutations died: two within a few days because of severe thrombotic liver complications (22,23) and one after 4 years because of hepatic encephalopathy (24). The other two patients have preserved liver and kidney function 1 year after transplantation.



Comparison	HR	95% C.I.	P value
CFH vs MCP	13.55	3.29-57.79	<0.0001
CFH vs non mut	1.73	1.19-2.51	0.0032
CFI vs MCP	7.83	1.55-39.54	0.0028
C3 vs MCP	9.62	2.02-45.75	0.0006
THBD vs MCP	6.88	1.41-33.75	0.0066
CFH Ab vs MCP	8.49	1.61-44.69	0.0028
Non mut vs MCP	6.08	1.49-24.76	0.0037

Figure 2. Cumulative Kaplan-Meier estimates of the rates of first event (ESRF or death). The fractions of patients free of ESRF or still alive at any time point according to the presence of mutations in *CFH*, *CFI*, *C3*, *THBD*, *MCP*, or CFH autoantibodies or without mutations (Non mut) are shown. The MCP group was chosen as the reference group. Hazard ratios and 95% confidence intervals calculated using the Cox proportional hazards regression model are shown. *P* values were calculated using the log-rank test. The comparisons that were statistically significant after Bonferroni correction are shown in the table. °Follow-up < 120 months.

Seven kidneys were transplanted in four patients with *C3* mutations; recurrence manifested in three grafts, of which two were lost, whereas the third recovered after four plasma exchanges. One patient with *THBD* mutation and one with CFH autoantibodies lost the kidney graft for recurrence.

Kidney transplant was performed in three patients with *MCP* mutations; all have good graft function at 13, 3, and 2 years after transplantation.

In patients without mutations or autoantibodies, 59% of the grafts were lost within 1 year.

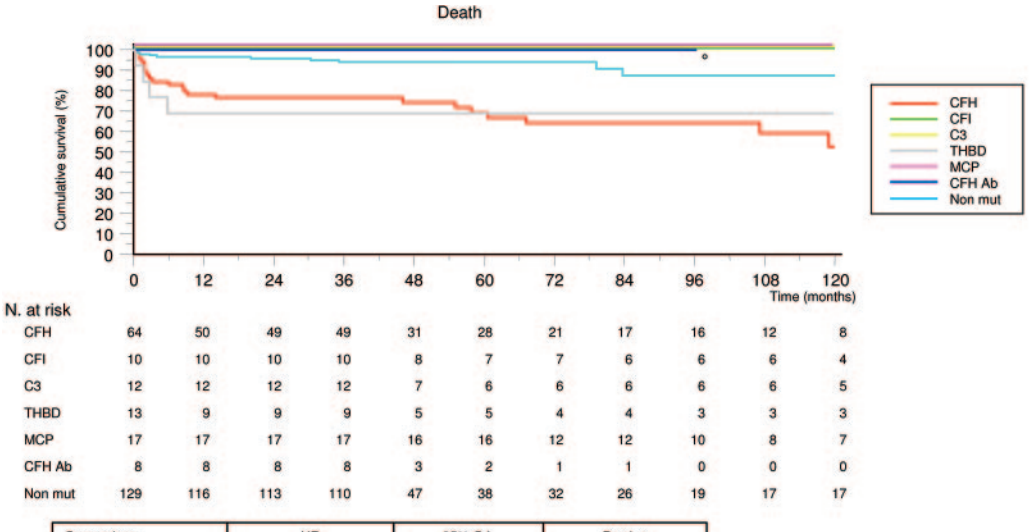
Overall, a good graft outcome was observed in 46% of sporadic and 30% of familial cases (Table 4). No difference in graft outcome was observed between children and adults (Table 4).

Discussion

This study showed that a genetic predisposition accounts for the majority of sporadic forms of aHUS and provides a detailed description of both known and new mutations and polymorphisms involved in sporadic and familial aHUS. Disease onset was generally preceded by a trigger, showing that both genetic predisposition and a precipitating event are required for the development of sporadic and familial aHUS. Finally, we provided data showing that clinical phenotype, response to treatment, and long-term outcome, including outcome after kidney transplantation, are predicted by individual gene abnormalities.

Complement gene abnormalities have been previously reported in sporadic aHUS (15,18). Here we showed that genetic abnormalities or anti-CFH autoantibodies are present in a substantial proportion of patients with sporadic idiopathic aHUS. In most patients, anti-CFH autoantibodies were associated with *CFHR1-3* deletion, confirming published data (12,25). Interestingly, we found genetic abnormalities—mainly in *CFH*—also in patients with pregnancy-associated aHUS, post-transplant aHUS, and other systemic or renal diseases, whereas no patients with secondary aHUS had anti-CFH autoantibodies. In addition, the majority of patients with secondary forms carried one or two polymorphic variants in *CFH* that have been shown to predispose to aHUS (21 and present data). Altogether, these findings provide evidence that secondary forms of sporadic aHUS are genetically determined and indicate that genetic screening—at least in *CFH*—should be performed also in these cases.

An important observation in our study is that serum C3 levels were normal in three sporadic aHUS patients with *C3*



N. at risk

CFH	64	50	49	49	31	28	21	17	16	12	8
CFI	10	10	10	10	8	7	7	6	6	6	4
C3	12	12	12	12	7	6	6	6	6	6	5
THBD	13	9	9	9	5	5	4	4	3	3	3
MCP	17	17	17	17	16	16	12	12	10	8	7
CFH Ab	8	8	8	8	3	2	1	1	0	0	0
Non mut	129	116	113	110	47	38	32	26	19	17	17

Comparison	HR	95% C.I.	P value
CFH vs CFI	N.E.	N.E.	0.03
CFH vs C3	N.E.	N.E.	0.02
CFH vs THBD	1.07	0.37-3.09	0.90
CFH vs CFH Ab	N.E.	N.E.	0.11
CFH vs MCP	N.E.	N.E.	0.004*
CFH vs non mut	3.89	1.89-8.00	0.0001*
THBD vs CFI	N.E.	N.E.	0.06
THBD vs C3	N.E.	N.E.	0.04
THBD vs CFH Ab	N.E.	N.E.	0.09
THBD vs MCP	N.E.	N.E.	0.015
THBD vs non mut	3.74	1.19-11.71	0.016

N.E.: Not Estimable
(no events in the CFI, C3 and MCP groups)

Figure 3. Cumulative Kaplan-Meier estimates of the rates of death. Fractions of patients still alive at any time point according to the presence of mutations in *CFH*, *CFI*, *C3*, *THBD*, *MCP*, or CFH autoantibodies or without mutations (Non mut) are shown. The *CFH* and *THBD* groups were chosen as reference groups. When feasible, hazard ratios and 95% confidence intervals were calculated by means of Cox proportional hazards regression model and shown in the figure. *P* values were calculated by means of the log-rank test after Bonferroni's correction. The most relevant comparisons are shown in the table. *Statistically significant after Bonferroni correction. °Follow-up < 120 months.

mutations. In published series, *C3* mutation screening was limited to patients with low C3 levels (8,26); our data suggest that it should be performed in all patients.

Five percent of patients carried mutations in the very recently discovered HUS-associated gene *THBD* (10), one in combination with a *CFH* mutation. Of note, cells expressing the aHUS-associated *THBD* variants have diminished capacity to inactivate C3b (10). This finding documents a functional link between complement and coagulation, opening new perspectives for candidate gene research in aHUS.

We confirmed that mutations in *CFB* are rare in aHUS (5,7,9); including the one described here, only five mutations have been reported.

In ~26% of familial cases, we could not identify any mutation. Therefore, the search for new gene abnormalities should continue.

In the majority of patients, the disease became manifest during infancy. However, age of onset showed a large range, even within the same family. Moreover, we found unaffected carriers among relatives of both familial and sporadic cases, which confirms previous findings (5,15,27) that mutations in complement genes are predisposing rather than directly causal. Indeed, in 70% of patients, aHUS onset was associated with a triggering/underlying condition. Viral or bacterial infections triggered disease in young children (<10 years). A second peak of onset was observed at ~25 to 40 years of age, often in association with pregnancy, a condition of complement activation (28). Four percent of patients developed *de novo* HUS after transplantation (18). Alternative pathway activation in these circumstances can mainly be attributed to ischemia/reperfusion injury (29).

In ~3% of cases, aHUS was diagnosed in patients with other renal diseases. The most common was membranoproliferative glomerulonephritis (MPGN), diagnosed in five patients, four of them carrying mutations in complement genes (Supplemental Table 2). Genetically determined dysregulation of the alternative pathway of complement plays a role in MPGN, and mutations in *CFH*, *CFI*, and *MCP* have been reported in patients with type I and type II MPGN (30). In addition, cases have been described where biopsies first suggested MPGN, and in a later phase aHUS, or *vice versa* (31). These findings suggest a close pathogenetic link between aHUS and MPGN.

The majority of patients with *CFH*, *CFI*, *C3*, and *THBD* mutations or anti-CFH autoantibodies lost renal function or died

Table 5. Outcomes after plasma treatment in aHUS patients with mutations in *CFH, CFI, C3, THBD, MCP*, or CFH autoantibodies and in patients without mutations

Mutation	CFH	CFI	C3	THBD	MCP	CFH Antibodies	None
Plasma treated episodes	**90** (52 patients)	**8** (7 patients)	**14** (10 patients)	**8** (6 patients)	**29** (14 patients)	**12** (7 patients)	**103** (84 patients)
Remission	57 (63%)	2 (25%)[a]	8 (57%)[a]	7 (88%)	28 (97%)[b]	9 (75%)	71 (69%)[a]
Complete remission	5 (5%)	1 (12.5%)[a]	6 (43%)[a,b]	5 (62%)[b]	26 (90%)[b]	3 (25%)	30 (29%)[a,b]
Partial remission	52 (58%)	1 (12.5%)	2 (14%)[b]	2 (25%)	2 (7%)[b]	6 (50%)[a]	41 (40%)[a]
ESRF–death	33 (37%)	6 (75%)[a]	6 (43%)[a]	1 (13%)	1 (3%)[b]	3 (25%)	32 (31%)[a]
ESRF	25 (28%)	6 (75%)[a]	6 (43%)[a]	—	1 (3%)	3 (25%)	31 (30%)
Death	8 (9%)	—	—	1 (13%)	—	—	1 (1%)

Complete remission is defined as normalization of both hematologic parameters and of renal function (see Table 3). Partial remission is defined as normalization of hematologic parameters with renal sequelae (see Table 3). In this table, we included only the episodes for which information about plasma treatment was available. CFH group includes two patients with CFH mutations and CFH autoantibodies and three patients with the *CFH/CFHR-1* hybrid gene.

[a] $P < 0.0024$ after Bonferroni correction compared with the group with MCP mutations.

[b] $P < 0.0024$ after Bonferroni correction compared with the group with CFH mutations.

Figure 4. Outcome of kidney transplantation. The outcome at 1 year of 64 transplanted kidneys in genotyped patients of the International Registry of HUS/TTP is reported, according to the absence or presence of a mutation. Plasma prophylaxis was used in three patients with *CFH* mutation, in one patient with *C3* mutation, and in one patient with *CFI* mutation (*), all with good outcomes. Plasma was given to treat aHUS recurrences in 20 grafts. Remission was achieved in only three (°). The numbers of transplanted organs are shown above each column.

during the presenting episode or progressed to ESRF as a consequence of relapses. Finding that patients with mutations in the C-terminal region of CFH had increased mortality and higher incidence of ESRF than patients with mutations in other regions of CFH emphasizes the pivotal role of the CFH C terminus in protecting endothelial cells from complement attack (32). Of note, all *CFH* mutations in familial cases caused either alteration or loss of the C terminus, whereas *CFH* mutations in sporadic cases were more broadly distributed, which could explain the better outcome in sporadic *versus* familial cases.

MCP mutation carriers had a good prognosis, which is consistent with previously published data (15,27). Recurrences were very frequent, but their effect on outcome was mild, with 90% of patients remaining alive and dialysis free in the long term.

Of relevance, patients with *CFH* or *THBD* mutations had a higher mortality rate than other patients. Patients with *CFH* mutations had a higher incidence of cardiovascular disease (CVD) (Table 2), which may partly be explained by the higher percentage of patients on dialysis, but a causal link between chronic complement activation and CVD has also been suggested (33).

Plasma exchange and plasma infusion are considered first-line therapies in aHUS (1,27,34), but the reported clinical response varies from complete remission to no response and immediate ESRF, depending on the underlying genetic defect (15,27). Plasma treatment could theoretically be beneficial in patients with mutations in circulating complement regulators (27). This hypothesis is supported by the finding that, in our patients with *CFH* mutations, complete or partial remission

was achieved in the majority of plasma-treated episodes. On the other hand, results were less favorable for patients with *CFI* mutations, despite the fact that CFI is a plasma complement regulatory protein as well. Data on treatment of patients with *C3* mutations are scarce (8). Plasma treatment could remove mutant hyperactive C3 and also provide regulatory plasma proteins to counteract complement activation induced by mutant C3. In fact, in our series, response to plasma treatment in patients with *C3* mutations was comparable to that of patients with *CFH* mutations. In patients with *MCP* mutations, plasma therapy did not affect outcome, which is consistent with the fact that MCP is not a circulating protein (35).

Contrary to other reports (34,36), we found no difference in response to plasma infusion *versus* plasma exchange. The rationale behind using plasma exchange instead of infusion is that plasma exchange also removes mutant circulating molecules (37) and CFH autoantibodies and allows administration of higher volumes of plasma without the risk of fluid overload.

Of note, results of response to plasma in patient subgroups and the comparison between plasma infusion/exchange are limited by the retrospective nature of our analyses and by different approaches to plasma treatment in different centers (including volume of plasma, delay between diagnosis, and treatment). Recent expert opinion papers (23,34) recommended, as a practical point, empiric plasma exchange in episodes of aHUS, since genetic information is usually not available when the patient is presenting with aHUS.

These findings and previous data emphasize that kidney transplantation alone in aHUS is severely compromised by the risk of recurrence (27,38), especially in patients with *CFH* and *CFI* mutations and to a lesser degree in patients with *C3* mutations. Because CFH, CFI, and C3 are plasma proteins synthesized predominantly by the liver, kidney transplantation alone does not correct the defect. As reported previously, simultaneous liver–kidney transplantation prevented recurrences in patients with *CFH* mutations but had a high mortality rate (23,24,39,40).

Kidney graft outcome was favorable in patients with *MCP* mutations, none of whom had disease recurrence in the graft, as expected, considering that MCP is a transmembrane protein highly expressed in the kidney.

Of note, two patients with *THBD* mutations developed HUS after kidney transplant (one *de novo* and one recurrence), which is unexpected, because thrombomodulin is an endothelial transmembrane protein like MCP. However, a soluble thrombomodulin form (sTM) circulates in plasma and possesses similar functional activities as membrane-bound thrombomodulin. Treatment with sTM attenuated ischemia–reperfusion renal injury in the rat (41). One could speculate that, because of dysfunctional sTM, in *THBD*-mutated recipients, the grafts were not sufficiently protected against complement activation and prothrombotic stimuli triggered by ischemia–reperfusion injury.

Plasma prophylaxis has been proposed as a strategy to prevent disease recurrence (42,43). In our series, three patients with *CFH* mutations, one with a *CFI* mutation, and one with a *C3* mutation received plasma prophylaxis after transplant and had no recurrence. However, these patients are plasma dependent, which calls for alternative, more specific strategies. In contrast, plasma was minimally effective at treating ongoing recurrences in transplanted patients with *CFH*, *CFI,* or *C3* mutations, because remission was achieved in only 1 of 10 plasma-treated patients.

Screening for all genetic aHUS susceptibility factors is a time-consuming procedure; however, results of this study emphasize the clinical importance of such screening, because patients on dialysis with single mutations in *MCP* would safely benefit from a kidney transplant. Finally, showing the complement abnormalities underlying aHUS opens perspectives for specific treatment of the disease with complement inhibitors. Eculizumab, a human anti-C5 monoclonal antibody, induced remission of aHUS in recent case reports (44–48) and could represent the future for treatment of acute episodes and prevention of recurrences in the graft.

Appendix

International Registry of Recurrent and Familial HUS/TTP

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Disclosures

None.

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