Impact of hypertensive emergency and rare complement variants on the presentation and outcome of atypical hemolytic uremic syndrome

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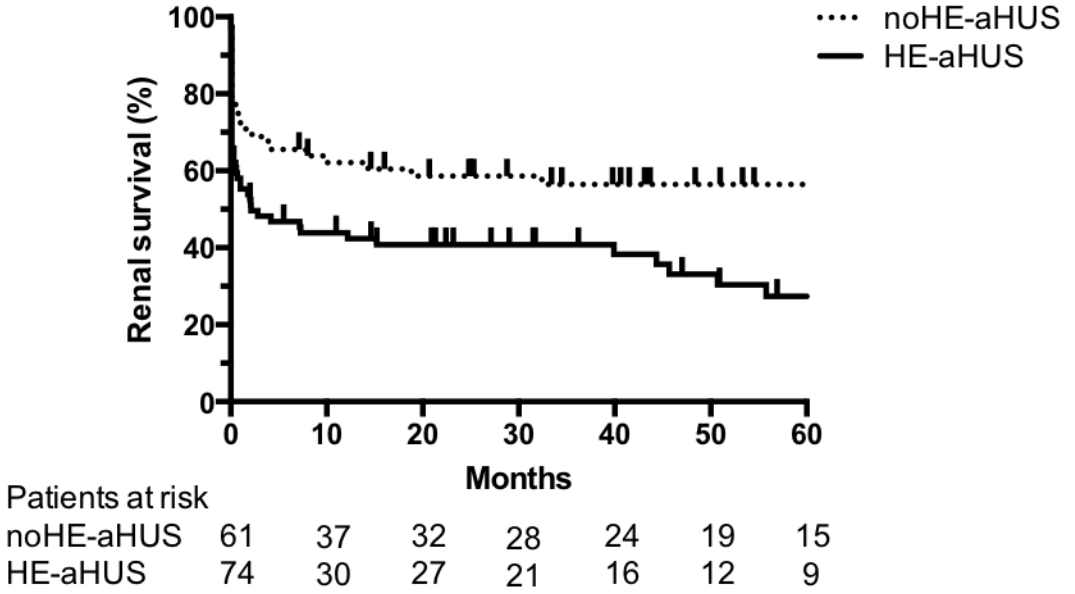
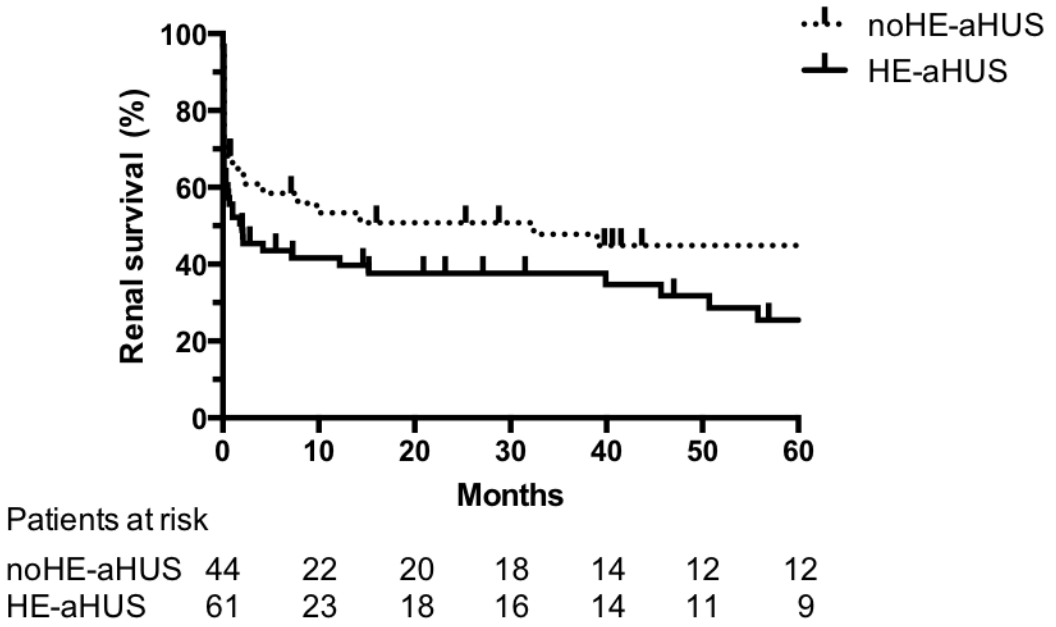
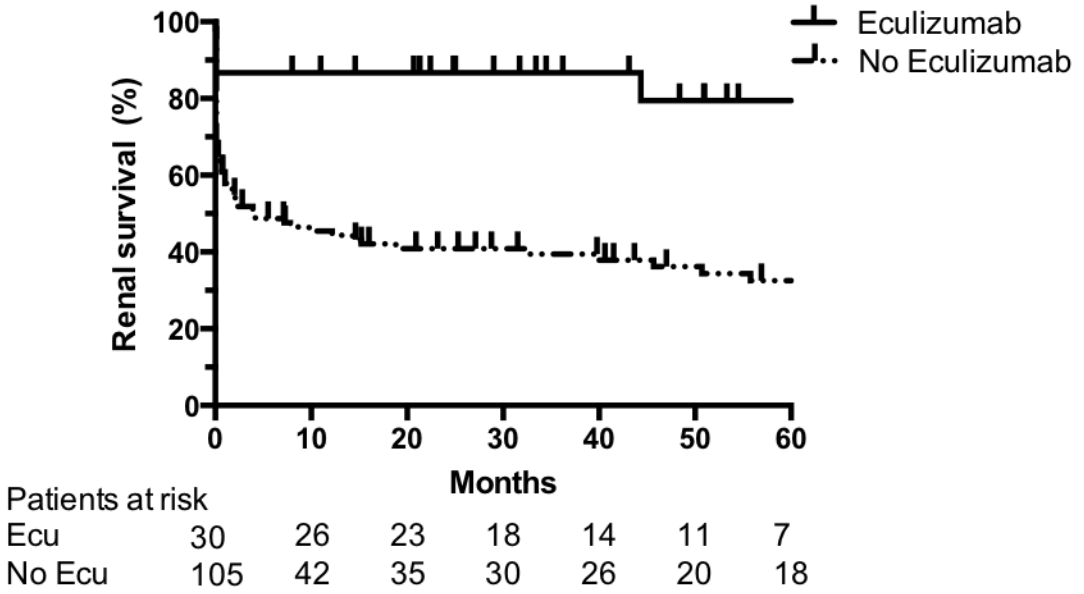


Supplemental Figure 1: Renal survival in patients with noHE-aHUS or HE-aHUS, whole cohort



Supplemental Figure 2A: Renal survival in patients with noHE-aHUS or HE-aHUS, without Eculizumab treatment

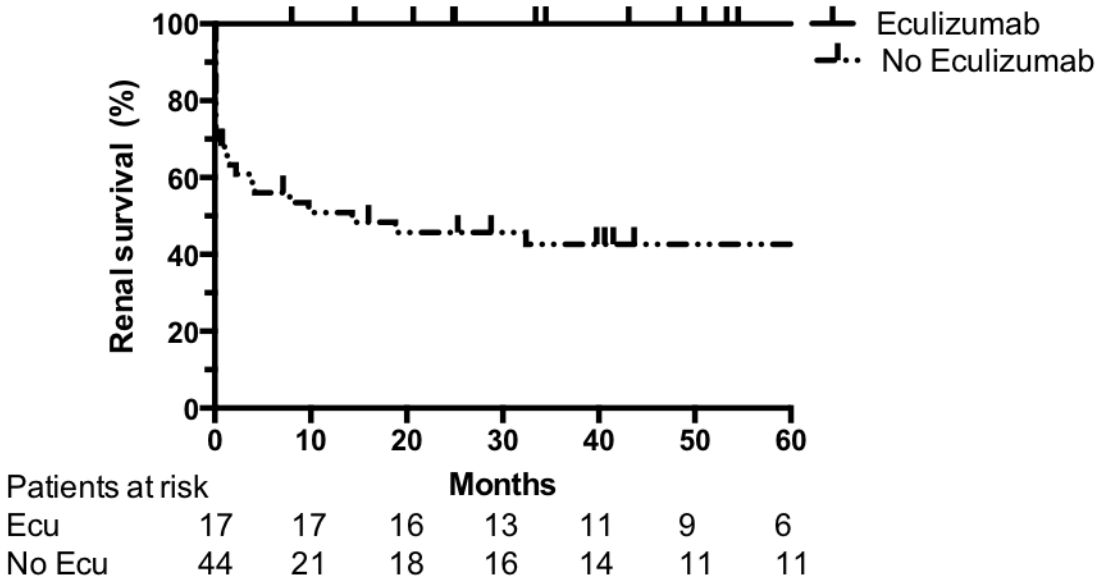
Log rank, P=0.07
Death censored analysis



Supplemental Figure 2B : Renal survival with or without Eculizumab treatment, whole cohort

Log rank, P<0.001

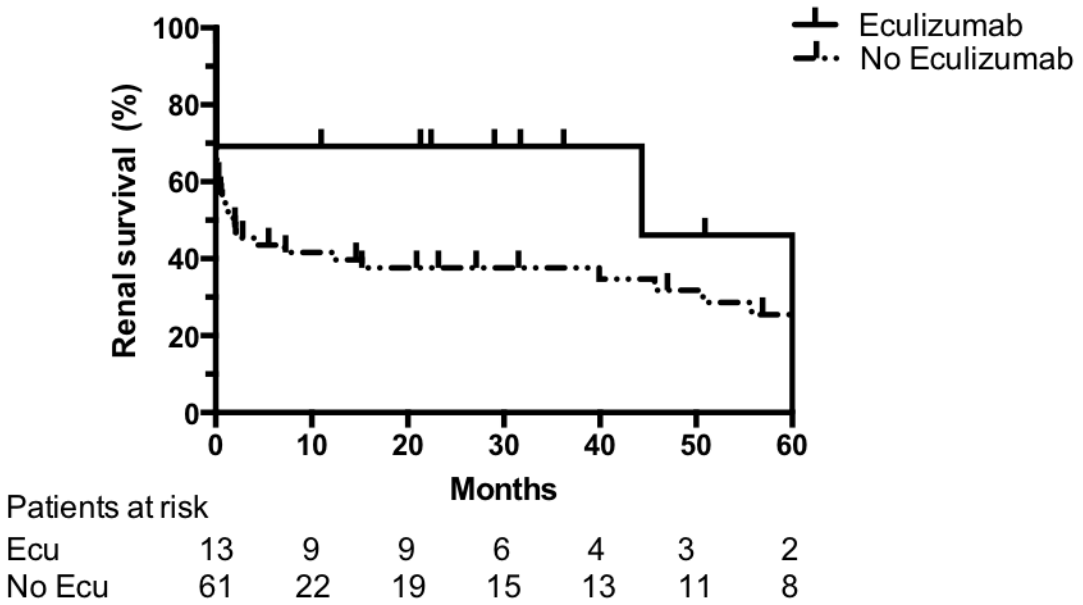
Death censored analysis



Supplemental Figure 2C : Renal survival with or without Eculizumab treatment, noHE-aHUS patients

Log rank, P<0.001

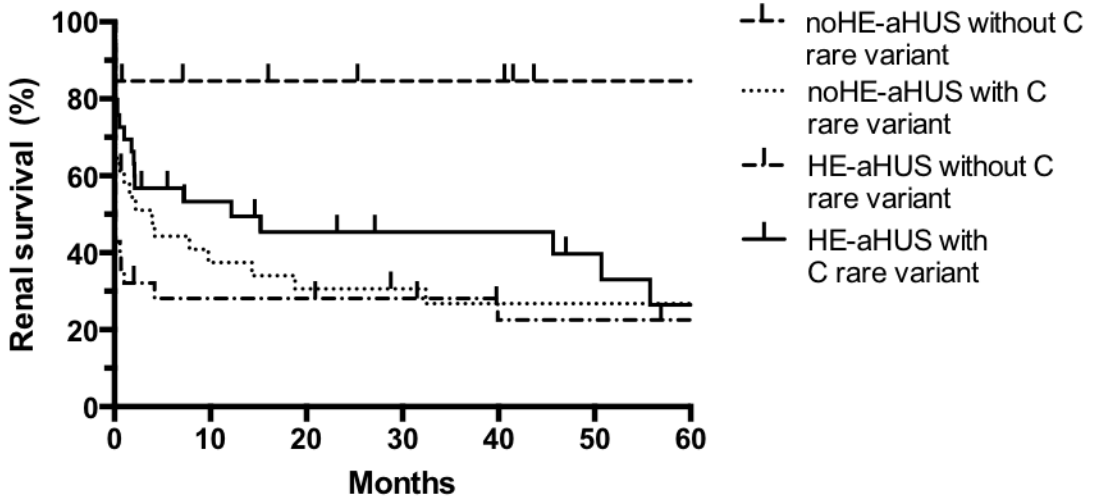
Death censored analysis



Supplemental Figure 2D :: Renal survival with or without Eculizumab treatment, HE-aHUS patients

Log rank, P=0.19

Death censored analysis

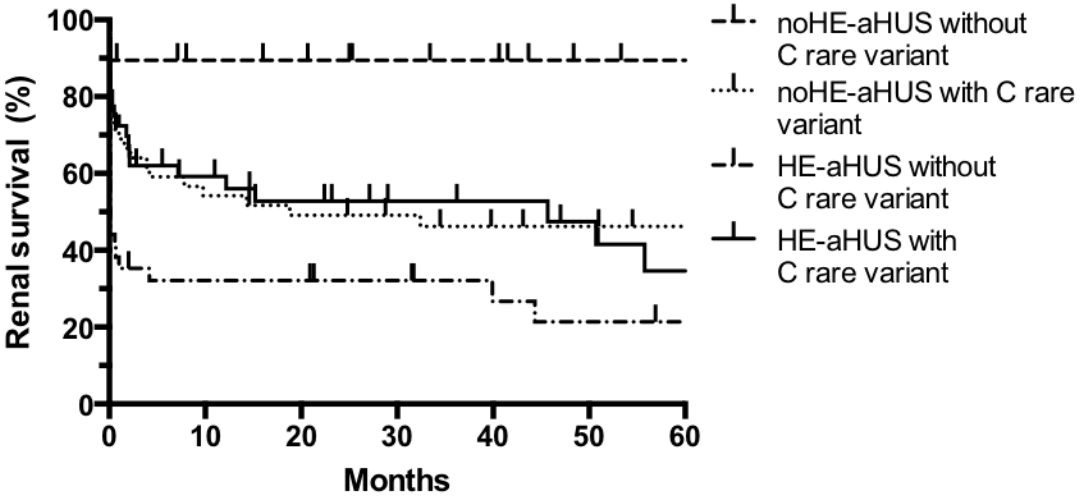


Patients at risk

noHE-aHUS without C rare variant	13	10	9	8	8	5	5
noHE-aHUS with C rare variant	31	12	10	9	7	7	7
HE-aHUS without C rare variant	28	8	8	7	5	5	4
HE-aHUS with C rare variant	33	15	12	9	9	7	5

Supplemental Figure 2E : Renal survival according to Complement rare variant and Hypertensive Emergency, without Ecu treatment

Death censored analysis Log rank, P=0.01



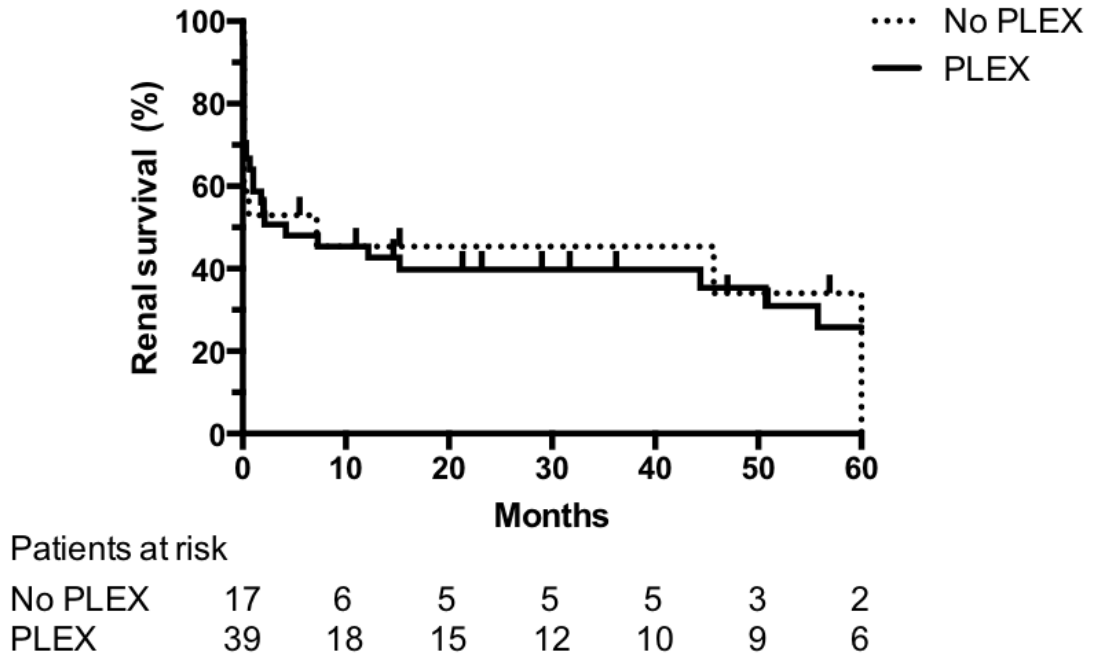
Patients at risk

noHE-aHUS without C rare variant	19	15	13	11	9	6	5
noHE-aHUS with C rare variant	42	23	20	18	15	14	12
HE-aHUS without C rare variant	34	11	10	9	6	5	4
HE-aHUS with C rare variant	40	20	17	12	11	7	6

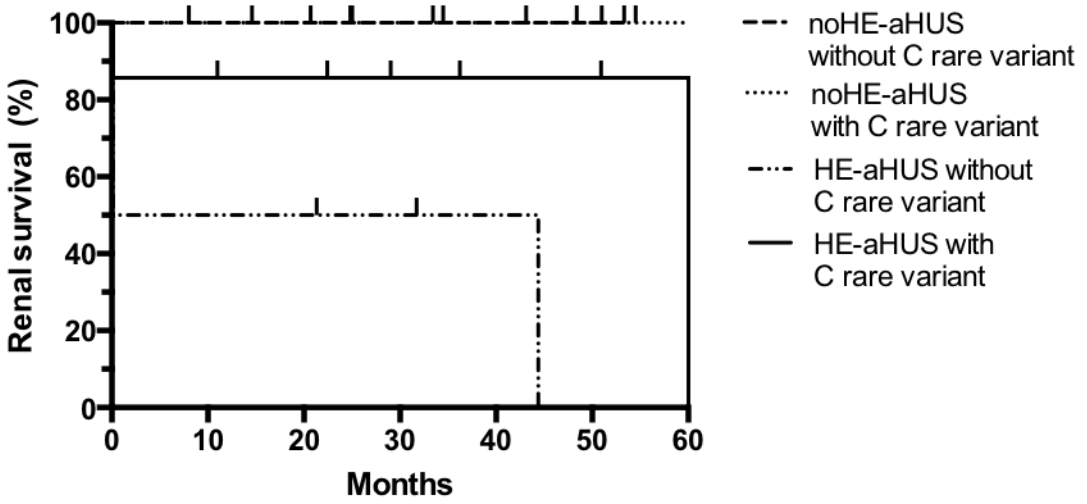
Supplemental Figure 2F : Renal survival according to Complement rare variant and Hypertensive Emergency,
Whole cohort

Log rank, P=0.0004

Death censored analysis



Supplemental Figure 3: Renal survival according to PLEX , whole cohort



Patients at risk

noHE-aHUS without C rare variant	6	6	5	4	3	2	2
noHE-aHUS with C rare variant	11	11	11	10	10	8	8
HE-aHUS without C rare variant	6	3	3	2	2	0	0
HE-aHUS with C rare variant	7	6	6	4	3	2	2

Supplemental Figure 4: Renal survival with Eculizumab according to C rare variant and HE

Impact of Hypertensive Emergency and genetics on presentation and outcome of atypical Hemolytic Uremic Syndrome

Supplemental tables and supplemental figure legends

Supplemental Table 1

Distribution and pathogenicity of C rare variants identified in noHE-aHUS and HE-HUS patients

	HE-aHUS (n=45)	noHE-aHUS (n=44)	Controls (n=11)	Controls vs HE-aHUS p	noHE-aHUS vs HE-aHUS p
CFH, n (%)	20 (44)	24 (54)	2 (18)	0.1	0.4
P variant, n (% of CFH rare variant)	16 (80)	22 (91)	0	0.06	0.39
MCP, n (%)	2 (4)	3 (7)	0	0.8	0.7
P variant, n (% of MCP rare variant)	2 (100)	3 (100)	0	>0.9	>0.9
CFI, n (%)	14 (31)	6 (14)	2 (18)	0.4	0.06
P variant, n (% of CFI rare variant)	9 (64)	3 (50)	0	0.18	0.6
CFB, n (%)	2 (4)	2 (4.5)	0	0.8	>0.9
P variant, n (% of CFB rare variant)	0 (0)	2 (100)	0	0.3	>0.9
C3, n (%)	6 (13.3)	9 (20)	6 (54)	0.002	0.4
P variant, n (% of C3 rare variant)	3 (50)	8 (88)	1 (16.6)	0.5	0.2
THBD, n (%)	1 (2)	0 (0)	1(9)	0.3	>0.9
P variant, n (% of THBD rare variant)	0 (0)	0 (0)	1 (100)	>0.9	>0.9
Total P variant, n (% of rare variant)	**30 (66.6)**	**40 (91)**	**2 (18)**	**0.04**	**0.007**
Total VUS, n (% of rare variant)	15 (33.3)	4 (9)	9 (82)	0.0004	0.01

CFB: C factor B; CFH, C factor H; CFI: C factor I; MCP, membrane cofactor protein; THBD: thrombomodulin; P: pathogenic; VUS: variants of uncertain significance.

Supplemental Table 2

Rare variants identified in patients with noHE-aHUS

Gene	Variant		MAF (%)	Functional consequences	Number of patients	Variant categorization
C3	c.193A>C	p.Lys65Gln	0.005766	Impair binding CFH, MCP	1	P
C3	c.481C>T	p.Arg161Trp	0.0008240	Impair binding MCP	4[2]	P
C3	c.3280G>T	p.Ala1094Ser	na	Impair binding CFH, MCP	1	P
C3	c.3284T>G	p.Ile1095Ser	na	Impair binding CFH, MCP	1	P
C3	c.4390C>G	p.His1464Asp	na	None	1	VUS
C3	c.463A>C	p.Lys155Gln[1]	0.3362	Decreased cofactor activity	11	P
CFB	c.836A>G	p.Asp279Gly	na	Gain of function	2	P
CFH	c.1022G>A	p.Arg341His[2]	0.001653		1	VUS
CFH	c.3557A>C	p.Lys1186Thr[3]	na		1	VUS
CFH	c.3628C>T	p.Arg1210Cys	0.0001730	Alter the C3b/polyanions –binding site	2	P
CFH	c.3572C>T	p.Ser1191Leu	na	Alter the C3b/polyanions –binding site	1	P
CFH	c.3607C>T	p.Arg1203Trp	0.008238		1	VUS
CFH	c.3047A>G	p.Tyr1016Cys	na		1	VUS
CFH	c.2056G>A	p.Val686Met	na	low FH level (CFH deficiency)	1	P
CFH	c.1867T>A	p.Cys623Ser	na	low FH level (CFH deficiency)	1	P
CFH	c.481G>T	p.Ala161Ser	0.004124		1	P
CFH	c.1750A>T	p.Lys584Stop [1]	na	low FH level (CFH deficiency)	1	P
CFH	large deletion		na	low FH level (CFH deficiency)	1	P
CFH	c.3493+1	IVS19+1	na	low FH level (CFH deficiency)	1	P
CFH	c.2596+2T>C	IVS14+2	na	low FH level (CFH deficiency)	1	P
CFH	c.32T>C	p.Met11Thr	na		1	VUS
CFH	c.2431T>C	p.Cys811Arg	na	low FH level (CFH deficiency)	1	P
CFH	c.1292G>A	p.Cys431Tyr	na	low FH level (CFH deficiency)	1	P

CFH	c.1734del	p.Val579PheFs *15	na	low FH level (CFH deficiency)	1	P
CFH	c.2242_2245delGATA	p.Asp748Asnfs *10	na	low FH level (CFH deficiency)	1	P
CFH	c.2857_2859delGAA	p.Glu953del3nt	na	low FH level (CFH deficiency)	1	P
CFH		Hybrid CFH/CFHR1			4	P
CFI	c.1019T>C	p.Ile340Thr[3]	0.004120		1	VUS
CFI	c.355G>A	p.Gly119Arg	0.05290	Decrease FI level (FI deficiency)	2	P
CFI	c.269G>A	p.Ser90Asn	na		1	VUS
CFI	c.1367G>T	p.Trp456Leu	na		1	VUS
CFI	c.1246A>C	p.Ile416Leu	0.001113		1	P
MCP	c.565T>G	p.Tyr189Asp	na	Decrease MCP expression (MCP deficiency)	1	P
MCP	c.653G>A	p.Arg218His	0.00002474		1	VUS
MCP	c.287-2A>G	IVS3-2	na	Decrease MCP expression (MCP deficiency)	1	P

Allele frequency given by Exome aggregation consortium

[1] Combined with CFH p.Lys584*; [2] Combined with C3 p.Arg161Trp; [3] Combined with CFI p.Ile340Thr

Supplemental Table 3

Rare variants identified in patients with HE-HUS

Gene	Variant		MAF (%)	Functional consequences	Number of patients	Variant categorization
C3	c.3475G>A	p.Glu1159Lys	0.0008238		1	P
C3	c.3343G>A	p.Asp1115Asn	na	Impair MCP binding	1	P
C3	c.3470T>C	p.Ile1157Thr	na	Impair CFH, MCP binding	1	P
C3	c.463A>C	p.Lys155Gln	0.3362	Decreased cofactor activity	1	P
C3	c.2203C>T	p.Arg735Trp[1]	0.2	Located in the C3a	1	VUS
CFB	c.724A>C	p.Ile242Leu	0.0009882		1	VUS
CFB	c.1363G>A	p.Val455Ile[2]	na		1	VUS
CFH	c.3196A>T	p.Lys1066*	na	low FH level (CFH deficiency)	1	P
CFH	c.2461C>T	p.His821Tyr	0.009246		1	VUS
CFH	c.3590T>C	p.Val1197Ala	0.0008237	Alter the C3b/polyanions –binding site	2	P
CFH	c.118C>T	p.Gln40*	na	low FH level (CFH deficiency)	1	P
CFH		CFH-CFHR1 Hybrid gene	na		4	P
CFH	c.1548T>A	p.Asn516Lys	0.04046		2	VUS
CFH	c.1789T>C	p.Cys597Arg	na	low FH level (CFH deficiency)	1	P
CFH	c.2169delT	p.Phe723Leufs*3	na	low FH level (CFH deficiency)	1	P
CFH	c.2635G>A	p.Gly879Arg	na	low FH level (CFH deficiency)	1	P

CFH	c.2933G>A	p.Trp978*	na	low FH level (CFH deficiency)	1	P
CFH	c.2198G>A	p.Cys733Tyr	na	low FH level (CFH deficiency)	1	P
CFH	c.2017T>C	p. Cys673Arg	na	low FH level (CFH deficiency)	1	P
CFH	c.3628C>T	p.Arg1210Cys	0.01730	Alter the C3b/polyanions –binding site	1	P
CFH	c.1607G>T	p.Cys536Phe	na	low FH level (CFH deficiency)	1	P
CFI	c.548A>G	p.His183Arg	0.0006260		1	VUS
CFI	c.355G>A	p.Gly119Arg	0.05290	CFI deficiency	1	P
CFI	36G>C	p.Val46Leu	na	CFI deficiency	1	P
CFI	c.1322A>G	p.Lys441Arg	0.3421	CFI deficiency	1	P
CFI	c.559C>T	p.Arg187Ter	na	CFI deficiency	1	P
CFI	c.1246A>C	p.Ile416Leu	0.1113	CFI deficiency	2	P
CFI	c.1234G>A	p.Val412Met	0.01073		1	VUS
CFI	c.452A>G	p.Asn151Ser	0.0008237	CFI deficiency	1	P
CFI	c.559C>T	p.R187X	0.001647	CFI deficiency	2	P
CFI	c.1376A>C	p.Tyr459Ser	na		1	VUS
CFI	c.1207G>A	p.Asp403Asn[3]	0.002480		1	VUS
CFI	c.782G>A	p.Gly261Asp[4]	0.1326		1	VUS
CFI	c.485G>A	p.Gly162Asp[5]	0.0008278	CFI deficiency	1	P

MCP	c.516A>C	p.Lys172Asn	na		1	VUS
MCP	c.565T>G	p.Tyr189Asp	na	MCP deficiency	1	P
THBD	c.229G>A	G77S Htz	0,006		1	VUS

Allele frequency given by Exome aggregation consortium

[1] Combined with CFI p.R187X; [2] Combined with CFI p.Tyr459Ser; [3] Combined with C3 p.Lys155Gln [4] Combined with CFH p.Cys536Phe; [5]Combined with CFH p.Asn516Lys

Supplemental Table 4

Pathogenic rare variants (n=1) and rare variants of undetermined significance (n=10), identified in 11 of 80 (13.7%) French controls (N1 to N11)

Controls	Gene	Variant	Genetic status	Number of French controls with the variant	Allele frequency given by Exome aggregation consortium[d] (%)	Polyphen 2 prediction	Variant categorization
N 1	C3	c.4855A>C p.Ser1619Arg	He	1[a]	0.1096	Possibly damaging	VUS
N1	C3	c.533 A>G p.Asp178Gly[a]	He	1[a]	0.001657	Benign	VUS
N2 and N3	THBD	c.127G>A p.Ala43Thr	He	2[b]	0.3	Benign	Pathogenic
N2 and N4	C3	c.2203C>T p.Arg735Trp	He	2[b]	0.2	Probably damaging	VUS
N5	CFH	c.2867C>T p.Thr956Met	He	1	0.1211	Damaging	VUS, benign
N6	CFH	c.3028G>A p.Ala1010Thr	He	1	0.0008	Benign	VUS
N7	CFI	c.1322A>G p.Lys441Arg	He	1	0.36	Benign	VUS
N8	CFI	c.1657C>T p.Pro553Ser	He	1	0.1384	Benign	VUS
N9	C3	c.1855 G>A p.Val619Met	He	1[c]	0.02914	Possibly damaging	VUS
N10	C3	c.1909G>C p.Gly637Arg	He	1	0.02153	Probably damaging	VUS
N11	C3	c.2437A>G p.Lys813Glu	He	1	Not found	Probably damaging	VUS

a. This control with C3 p.Ser1619Arg (No decreased capacity to regulate the activity of the alternative pathway was observed with this variant) also carried a C3 VUS
b. This control with THBD p.Ala43Thr pathogenic variant also carried a C3 VUS
c. This control with a C3 VUS also carried a MCP p.Ala353Val pathogenic variant of frequency >1% in control populations
d. http://exac.broadinstitute.org/

CFH: complement factor H; CFI: complement factor I; He: heterozygous; THBD: thrombomodulin; VUS: variant of uncertain significance

Supplemental Table 5

A. Univariate analysis of prognosis factors associated with ESRD (whole cohort)

Variables	Hazard Ratio	p
Age	1	0.79
Sex	1.5	0.077
Neurologic impairment	1.7	0.06
Cardiac dysfunction	1.19	0.6
Hypertensive Emergency	2.1	0.0024
Dialysis at onset	2.57	0.081
Hemoglobin level	0.952	0.45
Platelets account	2.12	0.085
C rare variant	0.909	0.68
Plasmapheresis	0.812	0.51
Eculizumab	0.208	0.00022

B. Multivariate analysis of prognosis factors associated with ESRD (whole cohort)

Variables	Hazard Ratio	p
Sex	1.55	0.2
Neurologic impairment	1.41	0.37
Hypertensive Emergency	1.15	0.7
Dialysis at onset	3.8	0.006
Platelets account	1.96	0.26
Eculizumab	0.2	0.003

Supplemental Table 6

Univariate analysis of prognosis factors associated with ESRD (HE-aHUS patients)

Variables	Hazard Ratio	p
Age	0.997	0.82
Sex	1.15	0.63
Neurologic impairment	1.11	0.79
Cardiac dysfunction	0.655	0.29
Dialysis at onset	1.61	0.28
Hemoglobin level	0.897	0.14
Platelets account	1.32	0.62
C rare variant	0.597	0.07
Plasmapheresis	1.07	0.85
Eculizumab	0.554	0.18

Supplemental Figure legends

Supplemental Figure 1. Renal survival in patients with noHE-aHUS or HE-aHUS, including patients treated with Eculizumab

Analysis of renal survival in the whole cohort including patients treated with Eculizumab, Log rank test, p=0,001. Follow-up was not available in 2 patients with HE-aHUS. HE: hypertensive emergency, aHUS: atypical hemolytic uremic syndrome.

Supplemental Figure 2. Death-censored renal survival analyses

A Renal survival in patients with noHE-aHUS or HE-aHUS, without Eculizumab treatment. Log rank test, p=0.07

B Renal survival with or without Eculizumab treatment in the whole cohort, p<0.001

C Renal survival with or without Eculizumab treatment in noHE-aHUS patients, p<0.001

D Renal survival with or without Eculizumab treatment in HE-HUS patients, p=0.19

E Renal survival according to Complement rare variant and Hypertensive Emergency in patients without Eculizumab treatment, p=0,01

F Renal survival according to Complement rare variant and Hypertensive Emergency in the whole cohort, p=0,0004

Log rank test. Follow-up was not available in 2 patients with HE-aHUS. HE: hypertensive emergency, aHUS: atypical hemolytic uremic syndrome. C: Complement.

Supplemental Figure 3. Renal survival in patients with HE-aHUS treated with or without plasma exchange or plasma infusion

Analysis of renal survival without ESRD or death in patients with HE-aHUS according to plasma infusion or plasma exchange (PLEX), Log rank test, p=0,95. HE: hypertensive emergency, aHUS: atypical hemolytic uremic syndrome.

Supplemental Figure 4. Renal survival with Eculizumab according to C rare variant and hypertensive emergency

Analysis of renal survival without ESRD or death in patients treated with Eculizumab, Log rank test, p<0,001; HE-aHUS without C variant versus HE-aHUS with C variant, Log Rank test, p=0,06. HE: hypertensive emergency, aHUS: atypical hemolytic uremic syndrome. C: complement