Complement gene variants and Shiga toxin producing E. coli -associated hemolytic uremic syndrome

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Supplemental Methods and Patients

Study design

Parental written informed consent was required for entering the study. The study protocol adhered to the Declaration of Helsinki and was approved by the Comité de Protection des Personnes, Ile de France IV (n° IRB 00003835).

Clinical data were prospectively collected at admission, during hospitalisation and at discharge. In April 2017, physicians were asked to document patient's condition at last follow-up.

Caucasian children (< 15 years) with post-diarrheal-HUS were prospectively enrolled in this study from the French Society of Pediatric Nephrology. HUS was defined by the association of at least two of following criteria: mechanical hemolytic anemia (hemoglobin <10g/dL, schizocytosis >1%, lactate deshydrogenase > upper limit of normal (ULN), decreased/undetectable haptoglobin), thrombocytopenia (platelet count < 150 G/L) and acute kidney injury (serum creatinine > ULN for age). Post-diarrheal HUS was defined by prodromal gastro-intestinal symptoms (non bloody or bloody diarrhea, or other gastro-intestinal symptoms (abdominal pain, vomiting)), and Shiga toxin (Stx) producing *E coli* (STEC) infection by specific investigations (see below).

Chronic kidney disease (CKD) stages were defined according to KDIGO[1]. No CKD was defined by estimated (e)GFR ≥ 90 ml/min/1.73m^2 without albuminuria; CKD Stage 1 by eGFR ≥ 90 ml/min/1.73m^2 with albuminuria; Stage 2 by eGFR 60-89 ml/min/1.73m^2 with albuminuria; Stage 3 by eGFR 30-59 ml/min/1.73m^2 with or without albuminuria; Stage 4 by eGFR 15-29 ml/min/1.73m^2 with or without albuminuria; Stage 5 by eGFR < 15 ml/min/1.73m^2 or end stage kidney disease/dialysis. Significant albuminuria or proteinuria were defined by urine albumin/creatinine ratio >30 mg/g or >3mg/mmol or urine protein/creatinine ratio > 200 mg/g or > 20 mg/mmol.

STEC investigations

Investigations for STEC infection included a) Real time polymerase chain reaction (PCR) on stools for Stx1 and Stx2 genes (113 patients) b) Stool culture on selective media for identification and characterization of STEC strains, using PCR for genes coding for 10 frequent STEC serogroups affecting humans in France (O157, O26, O145, O55, O103, O104, O111, O91, O121, and O80) (111 patients) c) Antibody (IgM ± IgA) response to serogroup-specific *E coli* lipopolysaccharides (LPS) (O157, O26, O145, O55, O103, O104, O111, O91, O128) (93 patients)

Stool culture was positive for O157 STEC or other serotypes in 93.5% of Stx positive patients. Serogroup of non-O157 strains in stools was O80 in 8/77 Stx-positive patients (10.3 %), O26 in 5 (6.4%), O104, O121, O145 each in 2, and O2, O5, O98, O103, O111, O177 each in 1, undetermined in 10. Stx-positive patients with O80, O121, O2, O5, O98, O177 STEC in stools (serotypes not included in the serologic screening) had negative anti- LPS serology. In Stx-negative patients, anti-LPS serology was positive for serogroup O157 in 12 patients, O103 in 2, O91, O145 or O111 each in 1.

Patients characteristics

During the prodromal phase, 25 patients received bactericidal antibiotics (mostly amoxicillin/third generation cephalosporin) and 1 patient (Stx negative) received azithromycin. During hospitalization, neurological manifestations occurred in 20 patients, including seizures (12 patients), mental aberration (8), somnolence (8), behaviour disturbances/delirium (5), cranial pairs defect (nystagmus, strabism) (4), coma/decerebration (1). Brain magnetic resonance imaging was documented in 16 patients, showing no abnormalities in 5, ischemic lesions and/or white matter hypersignals in 11. Prolonged hemorrhagic colitis/intestinal symptoms were documented in 27 patients, of whom 13 required parenteral feeding. An additional patient had prolonged cholestasis related to gall

bladder sludge. Cardiac manifestations were pericarditis in 1 patient and cardiogenic shock in another 1.

Plasma infusion (2 in 1 patient, 3 in another patient) and/or plasma exchange (6 patients, who received 1, 1, 2, 6, 10 or 11 sessions, respectively) were administered for neurological manifestations in 7 patients or prolonged hemolysis in 1 patient.

15 patients received eculizumab with a mean of 3 doses (1 to 6), mostly because of neurological manifestations (11/15, 73%). None of the 15 patients carried a pathogenic rare variant. Dialysis was required in 12 of the 15 patients (80%). At median follow-up 5.0 (1- 6) years, documented in 12 patients, 5 (41.6%) had no CKD, 4 (33.3%) had CKD stage 1, 2 (16.6%) had CKD stage 2, and 1 (8.3%) had CKD stage 3b.

During hospitalization, 24 children received azithromycin for ≥5 days (a procedure adopted by several French centres to accelerate intestinal decontamination), 13 received bactericidal antibiotics, and 2 received bactericidal antibiotics after azithromycin. Bactericidal antibiotics were prescribed for infectious complications (e.g. catheter- associated).

Nine patients were lost to follow-up (Supplemental Figure 1)

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Supplemental Table 1. Rare variants of uncertain significance (n=10) identified in 13 of 108 patients with post-diarrheal-HUS.

Gene	Variant	Genetic status	Number of patients with the variant	MAF [a] (%)	Functional studies	Polyphen 2 prediction	Previously reported in STEC-HUS	Previously reported in aHUS	Variant categorization
Stx positive - HUS patients									
C3	c.2203C>T p.Arg735Trp	He	3[b]	0.2	Located in the C3a Minor functional changes [2,3]	Probably damaging	No	Yes[2,4]	VUS
C3	c.4369G>C p.Asp1457His	He	1	0.03626	NA	Probably damaging	No	No	VUS
C3	c.1618G>T p.Ala540Ser	He	1	0.005864	NA	Benign	No	No	VUS
CFB	c.978A>C p.Glu326Asp	He	1	0.0766	NA	Benign	No	No	VUS
CFI	c.782G>A p.Gly261Asp	He	1	0.1326	No demonstrated functional alterations[5]	Benign	No	Yes[5,6]	VUS
THBD	c.829G>T p.Gly277Trp	He	1	0.001544	NA	Probably damaging	No	No	VUS
Stx negative - HUS patients									
CFH	c.2867C>T p.Thr956Met	He	1	0.1211	No demonstrated functional alterations[7]	Possibly damaging	Yes[8]	Yes[9]	VUS
C3	c.4855A>C p.Ser1619Arg	He	2[b]	0.1096	Located in the C345C domain No demonstrated functional alterations[3]	Possibly damaging	Yes[10]	Yes[11]	VUS
C3	c.4177C>T p.Arg1393Trp	He	1	0.004121	NA	Possibly damaging	No	No	VUS
C3	c.4319A>C p.Asp1440Ala	He	1[c]	0.02965	NA	Benign	No	No	VUS

a. MAF, minor allele frequency in Exome Aggregation Consortium database http://exac.broadinstitute.org/
b. One of the patients with C3 p.Arg735Trp VUS and one of those with C3 Ser1619Arg VUS also carried a MCP p.Ala353Val pathogenic variant of frequency > 1% in the general population
c. This patient with C3 p.Asp1440Ala VUS also carried a CFH p.Arg1210Cys pathogenic variant (Patient 6, Table 4)

aHUS: atypical hemolytic uremic syndrome; CFB: complement factor B; CFH: complement factor H; CFI: complement factor I; He: heterozygous; MCP: membrane cofactor protein; NA: not available; N: normal; Stx: Shiga toxin; STEC: shiga toxin producing *E.coli*; THBD: thrombomodulin; VUS : variant of uncertain significance

Supplemental Table 2. Pathogenic rare variants identified in French controls (n=1) and in controls from the 1000 Genomes data base (n=7).

Gene	Variant	Genetic status	Number of controls with the variant	MAF [a] (%)	Demonstrated functional alterations	Polyphen 2 prediction	Identified in our cohort of post-diarrheal - HUS	Previously reported in aHUS	Variant categorization
French controls (N=80)									
THBD	c.127G>A p.Ala43Thr	He	2[b]	0.3	Decreased capacity to inactivate C3b[12]	Benign	Yes (1 patient)	Yes[12]	Pathogenic
1000 Genomes (N=503)									
THBD	c.127G>A p.Ala43Thr	He	5	0.343	Decreased capacity to inactivate C3b[12]	Benign	Yes (1 patient)	Yes[12]	Pathogenic
CFI	c.161G>T p.Cys54Phe	He	1	Not found	Low FI level in plasma (FI deficiency)[13]	Probably damaging	No	Yes[c]	Pathogenic
CFH	c.3356A>G p.Asp1119Gly	He	1	0.02	Located in disease-related functional domain[7]	Probably damaging	No	Yes[d]	Pathogenic
MCP	c.565T>G p.Tyr189Asp	He	1	0.00082	Lack of synthesis[14]	Probably damaging	No	Yes[d]	Pathogenic
CFI	c.485G>A p.Gly162Asp	He	1	0.00082	Low circulating FI[13]	Probably damaging	No	Yes [c]	Pathogenic
C3	c.463A>C p.Lys155Gln	He	2	0.3362	Impaired degradation of C3 by FI[3]	Benign	No	Yes[c]	Pathogenic
THBD	c.1502C>T p.Pro501Leu	He	3	0.2276	Decreased capacity to inactivate C3b[12]	Possibly damaging	No	Yes[12]	Pathogenic

The ultra rare variants with MAF <0.1 % are p.Asp1119Gly (CFH); p.Tyr189Asp (MCP); p.Gly162Asp (CFI) and p.Cys54Phe (CFI)

a. MAF, minor allele frequency in Exome Aggregation Consortium database http://exac.broadinstitute.org/
b. One French control with THBD p.Ala43Thr pathogenic variant also carried a C3 p.Arg735Trp VUS
c. Author VFB, personal communication: CFI p.Cys 54 Phe and p.Gly162Asp variants and C3 p.Lys155Gln variant found in aHUS patients (French cohort)
d. Atypical HUS mutation database, http://www.fh-hus.org/

Supplemental material is neither peer-reviewed nor thoroughly edited by CJASN. The authors alone are responsible for the accuracy and presentation of the material.

aHUS: atypical hemolytic uremic syndrome; CFH: complement factor H; CFI: complement factor I; He: heterozygous; MCP: membrane cofactor protein; THBD: thrombomodulin; VUS : variant of uncertain significance

Supplemental Table 3. Rare variants identified both in patients with post-diarrheal-HUS and in French controls and European controls from the 1000 Genomes data base

Gene	Variant	Variant categorization	Post-diarrheal –HUS patients (n=108)		French controls (n=80)		HUS patients versus French controls	European controls N=503		HUS patients versus European controls
			Number of HUS patients with the variant	Frequency (%)	Number of controls with the variant	Frequency (%)	P[a]	Number of controls with the variant	Frequency (%)	P[a]
CFH	c.2867C>T p.Thr956Met	VUS	1	0.9	1	1	0.8	1	0.2	0.3
C3	c.2203C>T p.Arg735Trp	VUS	3	3	2	2	0.9	2	0.39	0.04
C3	p.Ser1619Arg	VUS	2	2	1	1	0.7	2	0.39	0.1
THBD	c.127G>A p.Ala43Thr	Pathogenic	1	0.9	2	2	0.4	5	0.99	0.99

a. Fisher exact test

CFH: complement factor H; HUS: hemolytic uremic syndrome; THBD: thrombomodulin; VUS: variant of uncertain significance

Supplemental Table 4. Frequency of homozygous CFH *tgtgt* and MCP *ggaac* haplotypes in 97 patients with post-diarrheal -HUS compared to 80 French controls.

	French controls		Post-diarrheal -HUS patients		HUS patients versus French controls
	Number of controls tested	Number with the haplotype (%)	Number of patients tested	Number with the haplotype (%)	P[a]
CFH *tgtgt* haplotype	80	3 (4)	97	3 (3)	0.8
MCP *ggaac* haplotype	80	5 (6)	97	6 (6)	0.9
CFH *tgtgt*+ MCP*ggaac*	80	0	97	0	0.9

a. Fisher exact test

CFH: complement factor H; HUS: hemolytic uremic syndrome; MCP: membrane cofactor protein

Supplemental Table 5. Clinical characteristics, in hospital-course and outcome of 3 patients with post-diarrheal-HUS and anti-FH antibodies

		In-hospital-course											Outcome		
Patient Gender Age, y	Complement abnormalities	C3[a] mg/L	MCP [a] MFI	sC5b9[a] ng/mL	Stool Stx PCR (STEC serogroup)	Hb[b] g/dL	Plt[b] /mm^3	WBC[b] /mm^3	Screat[b] mg/dL	Dialysis duration days	Extra-renal manifestations	PI/PE and/or eculizumab	F-up y	Sequels[c]	Relapse
1. M 5.2	Anti-FH Ab, 570 AU/mL[d] No CFHR1/R3 deletion	1470	13.7	527	Stx negative (Only O111 serology positive)	5.8	21000	9170	0.97	0	None	No	4.7	CKD2 Proteinuria; eGFR 83 mL/min/1.73m^2	No
2. F 0.5	Anti-FH Ab, 190 AU/mL[d] No CFHR1/R3 deletion	762	11.4	518	Stx2 positive (Not typable STEC in stool)	5.9	25000	ND	1.1	0	None	No	1.0	No CKD	No
3, F 2.9	Anti-FH Ab, 500 AU/mL[d] C3 VUS p.Ser1619Arg No CFHR1/R3 deletion	1050	15.8	456	Stx negative (Only O157 serology positive)	6.4	95000	27300	0.7	0	None	No	4.8	No CKD	No

a. Normal range: C3: 615-1250 mg/L; MCP: 13-19 MFI; sC5b9 :< 420 ng/mL; Conversion factor for serum creatinine from mg/dL to µmol/L : x 88.4
b. At admission
c. CKD stages according to KDIGO 2012[1]. http://www.kdigo.org/clinical_practice_guidelines/ pdf/CKD/KDIGO_2012_CKD_GL.pdf. For definition of CKD stages, see Supplemental Methods and Patients
d. Anti-FH antibody titre: Positive threshold is 100 AU/mL. Patients with anti-FH antibody-associated aHUS (outside of STEC infection) have titres >1000 AU/mL. · Patient 1 had persistent anti-CFH antibody (620 AU/mL) at 4.7 years follow-up. Patient 2 had persistent anti-FH antibody (268 AU/ml) at 2 months follow-up (not documented subsequently). Anti-FH antibodies titre was not documented during follow-up in patient 3. None of the 3 patients carried a homozygous CFHR1/R3 deletion, contrary to approximately 90% of patients with anti-FH antibodies-associated aHUS.

Ab: antibody; aHUS: atypical hemolytic uremic syndrome; AU: arbitrary unit; FH: complement factor H;CFHR: Complement factor H-related protein; CKD: chronic kidney disease; eGFR: estimated glomerumar filtration rate; F: female; F-up: follow-up; Hb: haemoglobin; M: male; MCP: membrane cofactor protein; MFI: Mean Fluorescence Intensity; PCR: polymerase chain reaction; PI: plasma infusion; PE: plasma exchange; Plt: platelet count; Screat: serum creatinine; STEC: Shigatoxin –producing *E coli*; Stx: shiga toxin;; WBC: white blood cell

Supplemental Table 6. Plasma levels of CH50, C3, C4, FH, FI, and sC5b9, MCP expression and anti-FH antibodies at the acute phase of post-diarrheal-HUS. Median delay of blood sampling after admission was 4 days (Q1;Q3:1;6) (from admission to 13 days post-admission) for the total cohort. Median delay of blood sampling after admission was 2.5 days (Q1;Q3 1; 4.8) and 5.5 days (Q1;Q3 ; 2.3; 9) in Stx positive and Stx negative-HUS patients respectively. Plasma samples collected under PI/PE (n=3) or eculizumab (n=6) or after day 14 (n=12) were excluded from the analysis.

(Normal range)	C3 (615-1250 mg/L)		C4 (90-320 mg/L)		FH (70-140%)		FI (70-140%)		MCP (13-19 MFI)		sC5b9 (< 420 ng/mL)		Anti-FH antibody (> 100 AU)	
Stx status	Stx pos	Stx neg	Stx pos	Stx neg	Stx pos	Stx neg	Stx pos	Stx neg	Stx pos	Stx neg	Stx pos	Stx neg	Stx pos	Stx neg
N patients	61	29	61	29	59	30	59	30	47	27	58	27	55	27
Median (Q1;Q3)	1025 (886;1150)	1025 (979;1345)	194 (147;265)	251 (193;287)	109 (92;125)	115 (96;124)	127 (108;137)	120 (115;135)	12 (10;14)	13 (11;15)	498 (381;761)	456 (339;632)		
< LLN, N (%)	0	0	2(2)	0	3 (5)	1 (3)	1 (2)	0	27 (57)[a]	12 (44)[a]	0	0		
> ULN, N (%)	8 (13)	8 (27)	12 (19)	4 (14)	3 (5)	1 (3)	10 (17)	4 (11)	7 (14)	0	38 (66)[a]	14 (52)[a]	1 (2)	2 (7)
Within normal limits, N (%)	53(87)	21 (72)	47(77)	25 (86)	53 (90)	28 (93)	48 (81)	26(87)	13 (28)	15 (55)	20 (34)	13 (48)		

a. sC5b9 level was above the upper limit of normal in 61% (52/85) of patients with post- diarrheal/Shiga toxin positive or negative-HUS and MCP expression below the lower limit of normal in 53% (39/74)

AU: arbitrary unit; FH: factor H; FI: factor I; HUS: hemolytic uremic syndrome; LLN: lower limit of normal; MCP: membrane cofactor protein; MFI: Mean Fluorescence Intensity; N: number of patients; PE: plasma exchange; PI: plasma infusion; Stx: Shiga toxin; ULN: upper limit of normal

Supplemental Table 7. In-hospital course and outcome of 3 patients with post-diarrheal-HUS and C3 plasma levels close to the lower limit of normal at admission.

		In-hospital course											Outcome		
Patient Gender Age, y	Rare variant or anti-FH Ab	C3[a] mg/L	MCP[a] MFI	sC5b9[a] ng/mL	Stool Stx PCR (Stool STEC serogroup)	Hb[b] g/dL	Plt[b] /mm^3	WBC[b] /mm^3	Screat [ab] mg/dL	Dialysis duration days	Extra-renal manifestations	PI/PE and/or eculizumab	F-up y	Sequels[c]	Relapse
M, 6.7	No	663	6.1	901	Stx1 and Stx2 positive (O157)	6.0	34000	38000	3.3	43	CNS Pancolitis Pancreatitis Cardiogenic shock	2 PE Eculizumab (6 doses)	5.7	CKD3 Proteinuria; eGFR 42 mL/min/1.73m^2	No
M, 4.5	No	619	13	518	Stx2 positive (undetermined)	10.8	29000	7500	1.5	4	CNS	11 PE	6.0	CKD1 Proteinuria; eGFR 160 mL/min/1.73m^2	No
M, 1.8	No	626	9.9	452	Stx2 positive (O157)	7.8	140000	25200	2	22	CNS Diabetes	1 PI, 1 PE Eculizumab (9 doses)	5.7	No CKD Persistent diabetes	No

a. Normal range: C3: 615-1250 mg/L; MCP: 13-19 MFI; sC5b9 :< 420 ng/mL; Conversion factor for serum creatinine from mg/dL to µmol/L to: x 88.4
b. At admission
c. CKD stages according to KDIGO 2012[1] . ,http://www.kdigo.org/clinical_practice_guidelines/ pdf/CKD/KDIGO_2012_CKD_GL.pdf. For definition of CKD stages, see Supplemental Methods and Patients

Ab: antibody; FH: factor H; CNS: central nervous system; eGFR: estimated glomerular filtration rate; f-up: follow-up; Hb: haemoglobin; LPS: lipopolysaccaride; M: male; MCP: membrane cofactor protein; MFI: Mean Fluorescence Intensity; PCR: polymerase chain reaction; PI: plasma infusion; PE: plasma exchange; Plt: platelet count; Screat: serum creatinine; STEC: Shiga toxin producing E coli; Stx: Shiga toxin; WBC: white blood cell; y: year

Supplemental Table 8. Summary of the clinical course of 17 patients reported in the literature, who had post-diarrheal-HUS and carried a complement rare variant[a].

Patients	Age at HUS (y)	Stx in stool	Stool culture or anti-LPS serology	Outcome	Complement Variant[b]	Genetic categorization[c]	MAF [d] (%)	Demonstrated functional alterations	Author, year
Pediatric onset									
Stx positive - HUS patients									
1	10	Stx positive	Stool culture negative	Severe renal failure + lethargy/confusion until initiation of PE at day 12 Full recovery and no relapse after PE discontinuation at day 50 (follow-up 1 year)	CFH p.Gln950His	Pathogenic	0.36	Moderately decreased binding to GAG and/or C3b (Hemolytic assay)[16]	McCoy et al, 2014[17]
2	0.7	Stx positive	*E. Coli* in stool	Relapse of HUS (STEC-negative) at age 3 Remission at age 3.5	MCP p.Phe242Cys + CFH p.Gly1194Asp	Pathogenic + VUS	Not found 0.003	Decrease MCP expression (MCP deficiency)[18] NA for the CFH variant	Noris et al, 2010[18] and communication
3	16	Stx1/Stx2 positive	O157 in stool	No recovery of renal function Post-LRD (mother) transplant recurrence (7 months post-transplant) at age 18; graft loss	MCP c.286+2T>G Also carried by the mother	Pathogenic	0.003	Decreased MCP expression (MCP deficiency) . Affects splicing[14]	Alberti et al, 2013[19]
4	1.5	Stx2 positive	*E. Coli* in stool	Very low C3 level at the acute phase Full recovery and no relapse under eculizumab at 1.5 y follow-up	CFH p.Trp701X	Pathogenic	Not found	Decreased FH in plasma, predicted deleterious effect[20]	Caillaud et al, 2016[20]
5	1.6	Circulating Stx positive	O26 serology positive	Full recovery and no relapse at 6 y follow-up	MCP c.286+2T>G	Pathogenic	0.003	Decreased MCP expression (MCP deficiency)[14]	Ardissino et al, 2016[21] and communication

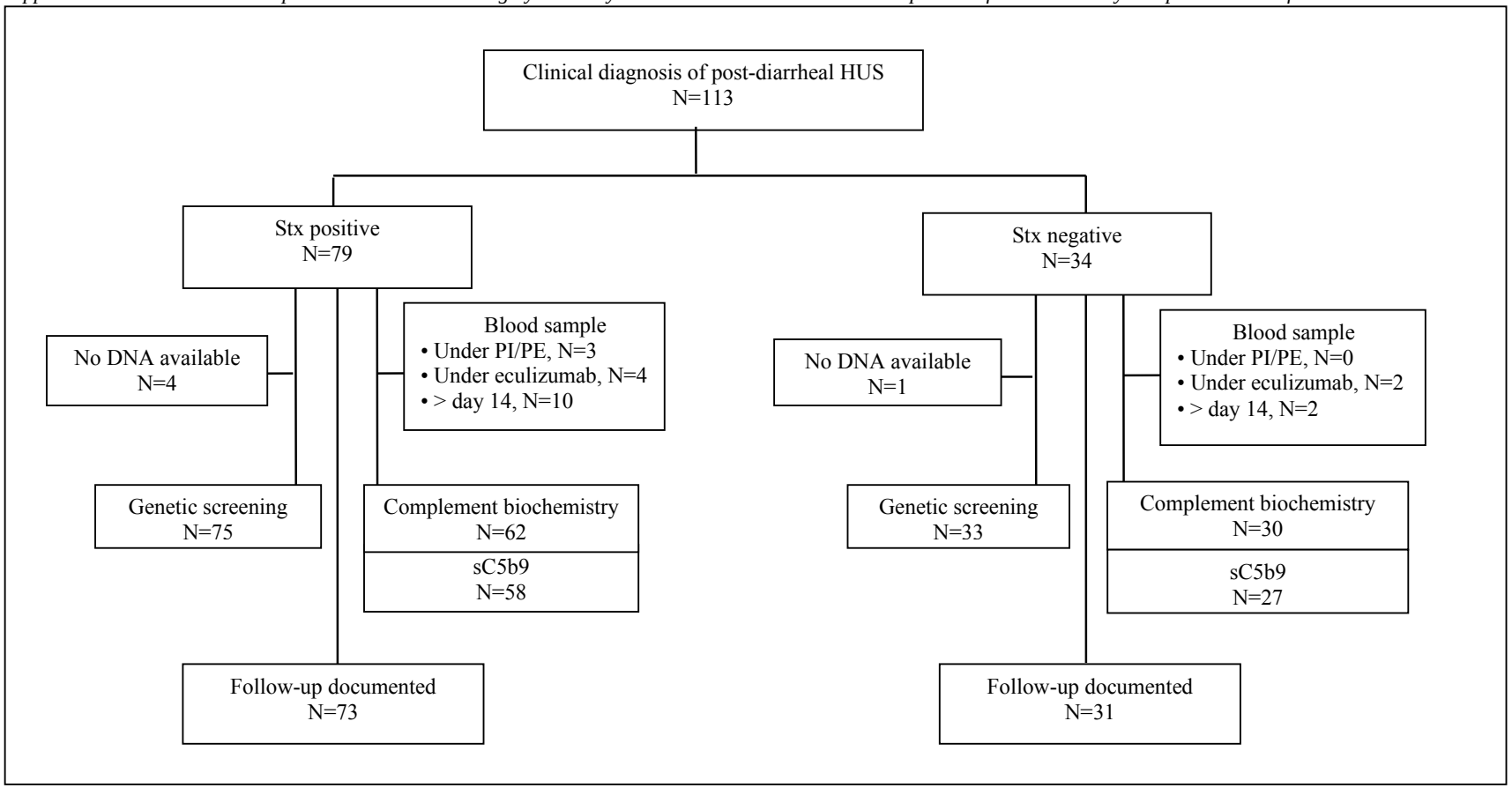
6	10.4	Circulating Stx positive	ND	Full recovery and no relapse at 6.2 y follow-up	MCP p.Thr383Ile	VUS	0.06	NA	Ardissino et al, 2016[21] and communication
Stx non documented - HUS patients									
7	1.5	ND	O157 in stool	Relapses of HUS (not post-diarrheal, Stx/STEC negative) starting 1 y after STEC-HUS Sister with one episode of Stx/STEC negative HUS	MCP p.Tyr155Asp + c.857-2 A>C Both variants also carried by the sister	Both variants pathogenic	Not found 0.000823	Decrease MCP expression (MCP deficiency)[14] Predicted deleterious effect (affects splicing)[14]	Sellier-Leclerc et al, 2007[22]
8	2	ND	O157 in stool	No relapse at 2.4 y follow-up	C3 p.Lys155Glu	Pathogenic	0.3362	Impaired C3 degradation by FI[3]	Westra et al, 2017[8, d]
9	0.7	ND	O157 in stool	Relapse of HUS one month after the first episode No relapse but CKD under PE (3.5y), then eculizumab (3y)	CFH/CFHR3 hybrid	Pathogenic	Not found	Functional CFH deficiency[23]	Challis et al, 2016[23]
10	6.2	ND	O157 in stool	No relapse at 2.6 y follow-up	CFH p.Thr956Met	VUS	0.12	No demonstrated functional alterations[7]	Westra et al, 2017[8, e]
11	16	ND	No diarrhea O157 IgM serology positive	No recovery of renal function Post-DD transplant recurrence (day 10) at age 17.5, recovery under eculizumab (follow-up 3y)	C3 p.Ser1619Arg	VUS	0.1096	No demonstrated functional alterations[3]	Dowen et al, 2017[10]
12	2.7	ND	O5 in stool	No relapse at 3.25 y follow-up	C3 p.Arg1219His	VUS	0.01	NA	Westra et al, 2017[8, e]
13	9.3	ND	O26 in stool	No relapse at 1.9 y follow-up	CFH p.Ser58Ala	Pathogenic	0.02	Decrease in vitro FH production [7]	Westra et al, 2017[8, e]
14	14	ND	O104 in stool	No relapse at 4y follow-up	C3 p.Val159Glu	VUS	Not found	NA	Ahlenstiel-Grunow et al, 2016[24, f]
15	3	ND	ND	ND	CFI p.Pro553Ser	VUS	0.06	No demonstrated functional alterations[13]	Ahlenstiel-Grunow et al, 2016[24, f]
Adult onset									
16	26	ND	Anti-Stx and O157 serology	No recovery of renal function Post-DD transplant recurrence (1y post-transplant) at age 33; graft loss	CFI p.Val412Met	Pathogenic	0.011	Variant located in serine protease domain, responsible of	Alberti et al, 2013[19]

			positive					C3b inactivation by FI[19] Decreased plasma FI level[g]	
17	41	ND	Post-diarrheal HUS Stx/STEC ND	No recovery of renal function	CFH p.Lys1188del	Pathogenic	Not found	Deletion located in disease related functional domain[25]	Edey et al, 2008[25]

a. Notice that the MCP p.Ala353Val pathogenic variant, first identified in a case of fulminant STEC-HUS[15], has been found in more than 1% of the general population (MAF 1.532% in Exome Aggregation Consortium database http://exac.broadinstitute.org/) and therefore is not classified as a rare variant in our study. We found it in 3 of the 80 French controls (4%) and 3 of 75 patients with Stx positive-HUS (4%), the latter without kidney damage at last follow-up.
b. All variants were heterozygous
c. Some variant categories may be different from those indicated in original articles[8,24], according to results of more recent functional studies
d. MAF, minor allele frequency in Exome Aggregation Consortium database http://exac.broadinstitute.org/
e. Westra et al[8] identified P/LP variants or VUS of CFH or C3 in 4/25 (16%) STEC-HUS children
f. Ahlenstiel-Grunow et al[24] identified VUS in CFI or C3 in 2/16 (12.5%) STEC-HUS children. A third patient carried a C1s variant
g. Author VFB, personal communication: CFI p.Val412Met variant associated with decreased FI plasma level

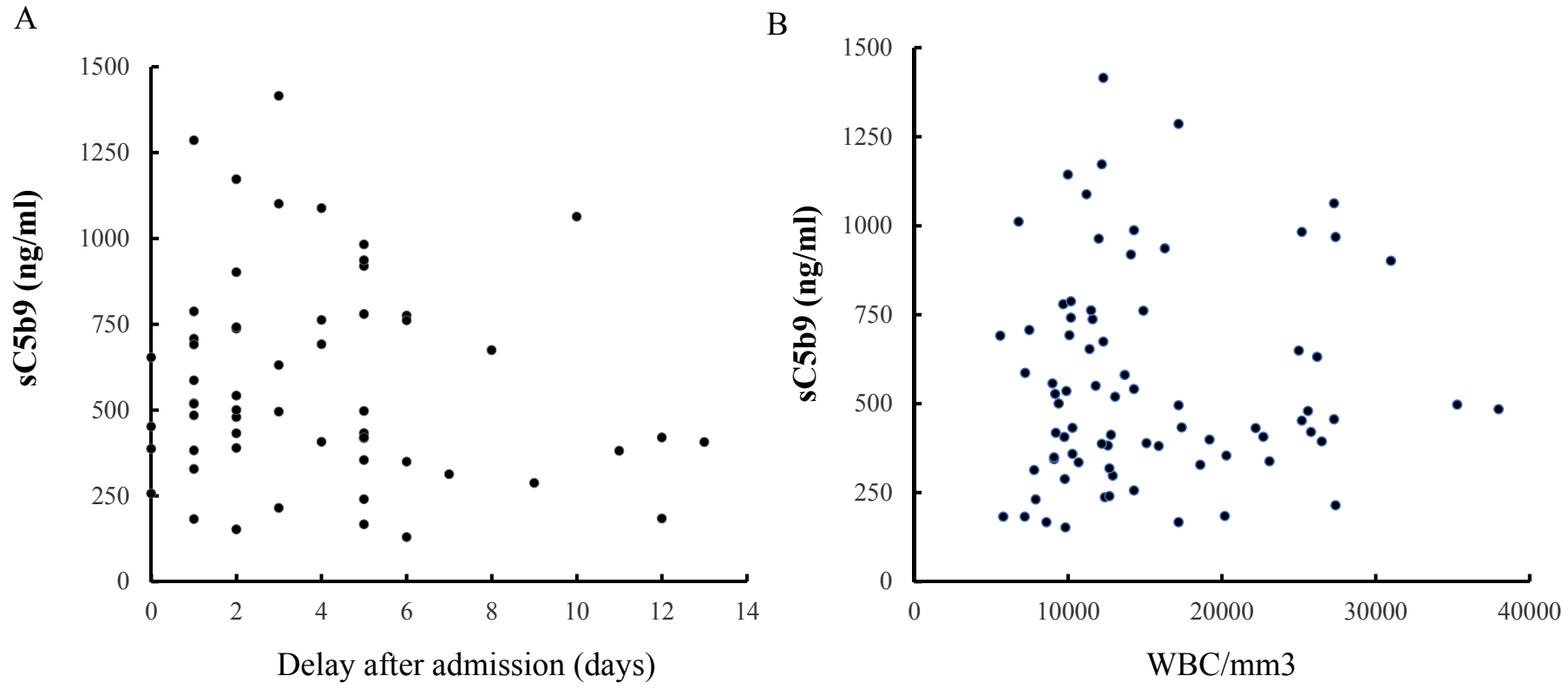
aHUS: atypical hemolytic uremic syndrome; CFH: complement factor H; CFHR: CFHR: Complement factor H-related protein; CFI: complement factor I; CKD: chronic kidney disease; DD: deceased donor; HUS: hemolytic uremic syndrome; LPS: lipopolysaccharide; LRD: living related donor; MCP: membrane cofactor protein; MLPA: Multiplex ligation-dependent probe amplification; NA: no functional studies available; PE: plasma exchange; STEC: Shiga toxin-producing E coli; Stx: Shiga toxin; VUS: variant of uncertain significance; y: year

Supplemental Figure 1. Flow diagram of patients with post-diarrheal hemolytic uremic syndrome included in the study.



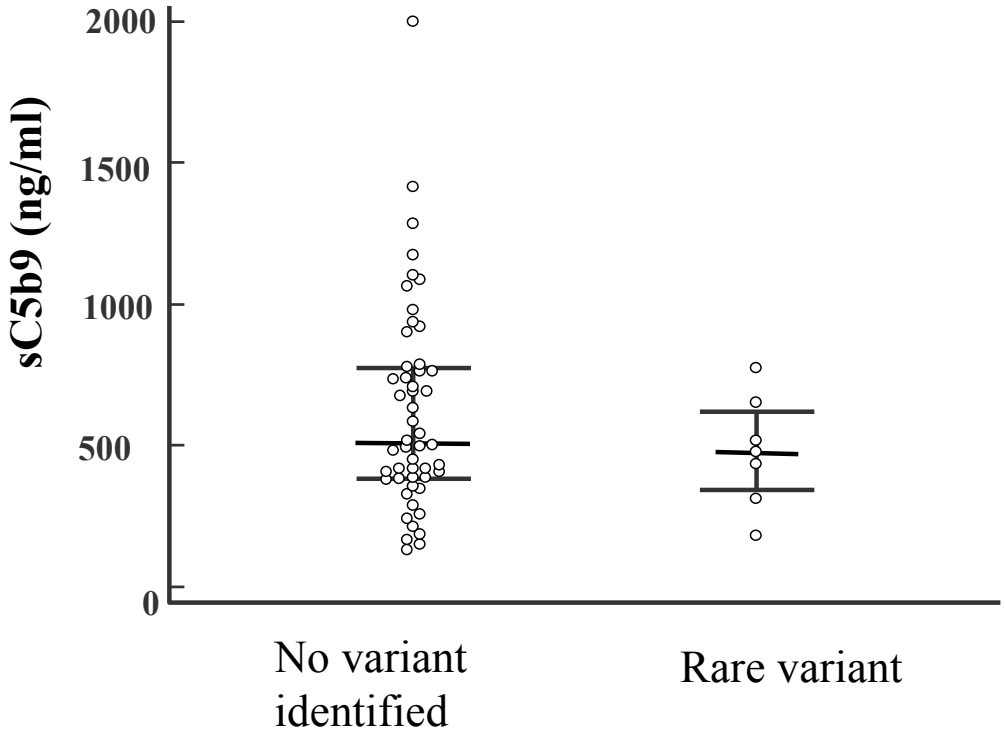
DNA: deoxyribonucleic acid; HUS: hemolytic uremic syndrome; PE: plasma exchange; PI: plasma infusion; Stx: Shiga toxin

Supplemental Figure 2. sC5b-9 plasma level according to delay after admission (A) and white blood cell count (B).



The level of sC5b-9 was not correlated (A) with the delay in blood sampling within the first 14 days of admission (r2: -0.1877; 95% CI: 0.4254 – 0.07417; p=0.16) or (B) with white blood cell count (r2: -0.03; 95% CI: 0.1855 – 0.2590; p=0.73)

Supplemental Figure 3. sC5b-9 level during the acute phase in Shiga toxin positive-HUS patients with (n=7) or without (n=49) rare variant identified.



The median (Q1; Q3) level of sC5b9 was 479 ng/ml (373; 586) and 500 ng/ml (385; 771) in patients with a rare variant and no variant, respectively (p=0.7).