**Exuberant endothelial C5b-9 formation in recurrent and de-novo post-transplant TMA**

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**Supplementary material :**

- Supplementary methods;
- Supplementary results;
- Supplementary references;
- Supplementary Table 1: Characteristics and clinical parameters of aHUS patients with recurrencence after kidney transplant at the time of sample collection;
- Supplementary Table 2: Characteristics and clinical parameters of transplanted aHUS patients with no sign of recurrencence at the time of sample collection;
- Supplementary Table 3: Characteristics and clinical parameters of patients with de-novo TMA at the time of sample collection;
- Supplementary Table 4: Characteristics and clinical parameters of aHUS patients in chronic dialysis at the time of sample collection;
- Supplementary Table 5: Characteristics, clinical and complement parameters of stable renal transplant patients at the time of sample collection;
- Supplementary Table 6: Characteristics, clinical and complement parameters of control dialyzed patients at the time of sample collection;
- Supplementary Table 7: Complement parameters and genetic characteristics of aHUS patients with recurrencence after kidney transplant at the time of sample collection;
- Supplementary Table 8: Complement parameters and genetic characteristics of transplanted aHUS patients with no sign of recurrencence at the time of sample collection;
- Supplementary Table 9: Complement parameters and genetic characteristics of patients with de-novo TMA at the time of sample collection;
- Supplementary Table 10: Complement parameters and genetic characteristics of aHUS patients in chronic dialysis at the time of sample collection;
- Appendix - International Registry of Recurrent and Familial HUS-TTP.

***Supplementary Methods***

***Study participants***

We evaluated three groups of patients: 1) 23 aHUS patients recipients of a kidney transplant of which 12 with recurrence and 11 with no sign of recurrence; 2) 22 transplant recipients who had other diseases in the native kidneys of which 15 developed de-novo post-transplant TMA and 7 with stable graft function; and 3) 39 patients on dialysis, including 28 patients with aHUS (4 of them were also studied after transplant) and 11 non-aHUS patients (Table 1 and Supplementary Tables S1-6) .

Patients and controls consented to participate in the "Mario Negri", Biobank "Malattie Rare e Malattie Renali". The patients' main characteristics are shown in Table 1 (individual values are detailed in Suppl. Tables 1-6).

Participants were patients with aHUS or de-novo TMA post-kidney transplant, included in the International Registry of Recurrent and Familial Haemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura (HUS/TTP), who were analysed with the serum-induced C5b-9 formation assay in our laboratories between 2014 and 2023. The Registry was established in 1996 at the Aldo e Cele Daccò Clinical Research Center for Rare Diseases (Ranica, Bergamo) (villacamozzi.marionegri.it/seu).

Inclusion criteria were diagnosis of aHUS, or de novo post-transplant TMA; analysis with the serum-induced C5b-9 formation assay on HMEC-1; kidney transplant or renal replacement therapy; no C5-inhibitors for at least 8 weeks prior to the time of serum collection; informed consent to participate in the "Malattie Rare e Malattie Renali" Biobank.

aHUS was diagnosed in patients who have had one or more episodes of microangiopathic hemolytic anemia and thrombocytopenia, with hematocrit (Ht) <30%, hemoglobin (Hb) <10 g/dL, serum lactate dehydrogenase (LDH) >500 IU/L, undetectable haptoglobin, fragmented erythrocytes in the peripheral blood smear, and platelet count <150,000/μL, associated with acute renal failure (serum creatinine >1.3 mg/dL for adults, >0.5 mg/dL for children below 5 years of age and >0.8 mg/dL for

children aged 5-10, and/or urinary protein/creatinine ratio >200 mg/g; or an increase in serum creatinine or a urinary protein/creatinine ratio >15% compared to baseline levels).[6] Recurrence in the graft in patients with a history of aHUS in the native kidneys was defined as exhibiting hematological and/or histologic markers of TMA: glomerular and/or arteriolar microthrombi, glomerular fibrinoid necrosis, amorphous material and endothelial swelling in glomerular capillaries and arterioles, glomerular fibrin, thickening and double contours of the glomerular basement membrane, and fragmented erythrocytes in microvessels and/or the interstitium.[1]

De-novo post-transplant TMA was defined based on the following criteria: hematological and/or histologic markers of TMA in kidney transplant patients who never had any evidence of TMA before transplantation. Diagnoses were made by the physicians who referred the patients for the C5b-9 assay and were revised by an independent clinician at the Clinical Research Center for Rare Diseases (E.B.). Only patients with a confirmed diagnosis were included in the study.

Patients had not taken C5 inhibitory drugs for at least 8 weeks prior to blood draws.

TTP was ruled out on the basis of ADAMTS13 activity >10% and no anti-ADAMTS13 antibodies. Stx-*E.Coli* infection was ruled out based on negative assays for *stx* and *eae* genes (by PCR) or Shiga-toxin (Vero cell assay) in the stool and/or anti-Shiga-toxin antibodies (ELISA) and/or LPS O157, O26, O111, or O145 (ELISA) in serum.

Full remission was defined as the normalisation of both hematological parameters (Ht >30%; Hb >10 g/dL; LDH <500 IU/L; platelets >150,000/μL) and renal function (serum creatinine <1.3 mg/dL for adults, <0.5 mg/dL for children below 5 years of age and <0.8 mg/dL for children aged 5-10; urinary protein/creatinine ratio <200 mg/g). Hematological remission in patients was defined as the normalisation of hematological parameters but with residual renal dysfunction.

The protocol was approved by the Ethical Committee of the Azienda Sanitaria Locale Bergamo, Italy.

***Genetic and biochemical complement abnormalities and anti-CFH autoantibodies***

The screening of coding sequences of aHUS-associated genes (*CFH, CFHR1, MCP, CFI, CFB, C3, THBD* and *DGKE*) and candidate genes (*CFHR2, CFHR3, CFHR4, CFHR5,* and *C5*) was performed using amplicon-based next-generation sequencing (S1). Rare functional variants (missense, nonsense, indel, or splicing variants) with minor allele frequency (MAF) <0.001 in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/) were selected. Stop-gain, frameshift and splicing variants, and missense variants with published functional studies, were categorised as pathogenic variants (PV). The other variants were categorised as likely pathogenic variants (LPV), variants of uncertain significance (VUS), likely benign or benign, using guidelines from the American College of Medical Genetics and Genomics (ACMG) and from the KDIGO conference on aHUS and C3G (S2-S4). We also used the Combined Annotation Dependent depletion tool (CADD), version 1.6, which is a tool for scoring the deleteriousness of single nucleotide variants as well as insertion/deletion variants, and which integrates multiple annotations into one metric (https://cadd.gs.washington.edu/info) (S5). We selected the PHRED-scaled C-score ranking. We searched for genomic abnormalities that affect the *CFH* and *CFHR* genes (S6) using multiplex ligation-dependent probe amplification, as reported (S1). We also genotyped the *CFH* single-nucleotide polymorphisms (SNPs) (c.1-331C>T, rs3753394; c.184G>A, p.V62I, rs800292; c.1204C>T, p.H402Y, rs1061170; c.2016A>G, p.Q672Q, rs3753396; c.2237-543G>A, rs1410996; c.2808G>T, p.E936D, rs1065489) that define the disease risk haplotype *CFH* $_{TGTGGT}$ (known as *CFH*–H3 haplotype) and one SNP in *MCP* (rs7144, c.∗897T>C) that tags the risk *MCP*$_{GGAAC}$ haplotype (S7-S8). Anti-FH autoantibodies were measured in plasma using ELISA (S9).

Complement C3 was measured using nephelometry. Normal ranges (defined as mean±2SD) for C3: 83-180 mg/dL, n=50. SC5b-9 levels were evaluated in EDTA plasma using MicroVue sC5b-9 Plus EIA (SC5b-9 Plus; Quidel). The normal range of plasma sC5b-9 in our laboratory is 127-400 ng/mL; n=50.

***Serum-induced C5b-9 deposition on HMEC-1***

The assay was performed as described previously,[2] with minor modifications. The human microvascular endothelial cells (HMEC-1 cell line) were a gift from Dr Edwin Ades and Francisco J. Candal of the CDC and Dr Thomas Lawley of Emory University, Atlanta, GA. The growth medium we used is MCDB 131 (Gibco, Grand Island, NY) supplemented with 10% foetal bovine serum (Gibco), 10 µg/mL hydrocortisone, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM glutamine (Gibco), and 50 µg/mL endothelial cell growth factor. HMEC-1 (about 75,000 cells) were plated on glass coverslips and used when confluent. Cells were washed 3 times with test medium (HBSS: 137 mM NaCl, 5.4 mM KCl, 0.7 mM $Na_2HPO_4$, 0.73 mM $KH_2PO_4$, 1.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, 28 mM Trizma base pH 7.3, 0.1% dextrose; with 0.5% BSA) and activated with 10 µM ADP (Sigma) for 10 minutes. Thereafter, cells were exposed for 2 hours to serum from patients or healthy subjects, 50% in test medium (HBSS with 0.5% BSA). HMEC-1 were then fixed in 3% paraformaldehyde and stained with rabbit anti-human complement C5b-9 complex antibody (Calbiochem) followed by FITC-conjugated secondary antibody (Jackson Immuno Research Laboratories).

Sera from 10 controls were pooled and the pool was run in each experiment as a reference (100%) for C5b-9 staining. We also analysed single sera from additional controls and calculated the percentages of C5b-9 deposits vs. control serum pool for each to set the normal range (mean±2SD of the percentage of C5b-9 deposits of the single control sera vs. control serum pool: ADP-activated HMEC-1, n=35 control sera, 60-149%; unstimulated HMEC-1, n=22 control sera, 50-150%). We verified the integrity of the cell monolayer after exposure to serum samples in parallel slides in which cells were stained with May-Grunwald Giemsa.

The fluorescent staining on the endothelial cell surface was acquired using the Apotome Axio Imager Z2 (Zeiss) microscope. Fifteen fields, which were systematically digitised along the surface,

were acquired using a computer-based image analysis system. The area occupied by the fluorescent staining was evaluated with automatic edge detection using the built-in specific functions of the software Image J (NIH, Bethesda, MD), and calculated as pixel$^2$ per field analysed.[2] For each sample, 15 fields were analysed; the highest and the lowest values were discarded and the mean of the other 13 fields was calculated. We expressed the results as the percentage of the staining compared to the control serum pool, run in parallel.[2]

***Statistical analysis***

Normality of distributions was visually inspected by box-plot representation and formally assessed with the D'Agostino-Pearson test. Between-groups differences were analysed using the t test, Mann-Whitney U test, or Fisher exact test, as appropriate. All data were analysed using MedCalc 10.0.1 statistical software. P values of less than 0.05 were considered statistically significant. Positive predictive values of serum induced C5b-9 assay were calculated as "true positive values / true positive + false positive values"; negative predictive values were calculated as "true negative values / true negative + false negative values".

**Supplementary results**

Calculations and results of positive and negative predictive values for either recurrent aHUS or de-novo TMA are as follows:

| aHUS Recurrence vs aHUS No Recurrence | | |
|---|---|---|
| | | |
| | aHUS-r | aHUS-nr |
| Positive results | 11 | 1 |
| Negative results | 1 | 10 |
| | | |
| Positive predictive value: 11 / (11+1): **0.916** | | |
| Negative predictive value: 10 / (10+1): **0.909** | | |

| De-Novo TMA vs Stable TX | | |
|---|---|---|
| | | |
| | De Novo | Stable |
| Positive results | 15 | 1 |
| Negative results | 0 | 6 |
| | | |
| Positive predictive value: 15 / (15+1): **0.937** | | |
| Negative predictive value: 6 / (6+0): **1** | | |

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**Supplementary Table 1**. Characteristics and clinical parameters of aHUS patients with recurrence after kidney trasplant at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | phase | time from trasplant (days) | kidney donor | immunosuppression therapy | hystology | eculizumab* (response) | platelets 150-400x10$^3$/μL | LDH 266-500 IU/L | hemoglobin 14-18 g/dL | haptoglobin 30-200 mg/dL | schistocytes | C3 70-152 mg/dL | C4 15-40 mg/dL | serum creatinin 0.55 - 1.25 mg/dL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 1 | M | 45 | aHUS | acute | 3 | cadaver | TA - MMF - ST | TMA | YES (CR) | 108 | 1240 | 9.6 | 67 | n.a. | 102 | 32 | 3.5 |
| patient 2 | M | 26 | aHUS | acute | 1 | cadaver | TA - MMF - ST | TMA | YES (CR) | 52 | 1060 | 13.5 | <30 | YES | 72 | 22 | 9.56 |
| patient 3 | M | 48 | aHUS | acute | 9189 | cadaver | TA - MMF - ST | n.a. | NO | 102 | 165 | 10.1 | 40 | n.a. | 46 | 15 | 2.5 |
| patient 4 | M | 72 | aHUS | acute | 4227 | n.a. | TA - MMF - ST | n.a. | NO | 234 | 622 | 9 | <7 | YES | 62 | n.a. | 4.4 |
| patient 5 | M | 48 | aHUS | acute | 9 | cadaver | ST | n.a. | NO | 160 | 435 | 8.5 | 53 | n.a. | n.a. | n.a. | 4.97 |
| patient 6 | F | 48 | aHUS | acute - post pex | 5 | cadaver | TA - MMF - ST | n.a. | NO | 37 | 1977 | 10.4 | <10 | n.a. | 79 | 18 | 6 |
| patient 7 | F | 29 | aHUS | acute | 69 | n.a. | sirolimus - ST | n.a. | NO | 107 | 309 | 9.4 | 40 | n.a. | n.a. | n.a. | 2.91 |
| patient 8 | F | 35 | aHUS | acute | 2560 | cadaver | TA - MMF -ST | TMA | NO | 170 | 532 | 9 | 95 | n.a. | 77 | 15 | 1.5 |
| patient 9 | M | 56 | aHUS | acute | 121 | cadaver | TA - ST | n.a. | n.a. | 90 | 567 | 8.7 | n.a. | n.a. | n.a. | n.a. | 2.2 |
| patient 10 | M | 61 | aHUS | acute | 2920 | cadaver | TA - ST | n.a. | NO | 161 | 567 | 10.7 | 15 | n.a. | 65 | 12 | 1.9 |
| patient 11 | F | 45 | aHUS | acute | 2561 | cadaver | TA - MMF - ST | TMA | YES (HR) | 275 | 372 | 9.4 | 67 | YES | n.a. | n.a. | 6.3 |
| patient 12 | M | 48 | aHUS | acute | 135 | cadaver | TA - MMF | TMA | NO | 100 | 607 | 9.3 | <1 | YES | 109 | 23 | 3.3 |

n.a. not available. TA, Tacrolimus. MMF, mycophenolate. ST, steroids. Pex, plasma exchange. CR, complete remission. HR, hematological remission.
* treatment started after blood sample collection.

**Supplementary Table 2**. Characteristics and clinical parameters of transplanted aHUS patients with no sign of recurrence at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | phase | time from trasplant (days) | kidney donor | immunosuppression therapy | hystology | platelets 150-400x$10^3$/µL | LDH 266-500 IU/L | hemoglobin 14-18 g/dL | schistocytes | C3 83-180 mg/dL | C4 15-40 mg/dL | serum creatinin 0.55-1.25 mg/dL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 13 | F | 29 | aHUS | remission | 1090 | cadaver | TA - MMF | n.a. | 172 | n.a. | 11.6 | n.a. | n.a. | n.a. | 0.76 |
| patient 14 | F | 39 | aHUS | remission | 2289 | cadaver | TA - MMF - ST | n.a. | 169 | 201 | 13 | n.a. | 105 | 27 | 2.07 |
| patient 15 | F | 18 | aHUS | remission | 1585 | n.a. | n.a. | n.a. | n.a. | 115 | n.a. | n.a. | 84 | 25 | n.a. |
| patient 16 | F | 33 | aHUS | remission | 1717 | cadaver | TA - MMF - ST | n.a. | 440 | n.a. | 12 | n.a. | n.a. | n.a. | 1 |
| patient 17 | F | 60 | aHUS | remission | 2057 | cadaver | TA - MMF - ST | n.a. | 215 | n.a. | 12.2 | n.a. | 112 | 54 | 2.23 |
| patient 18 | F | 56 | aHUS | remission | 7 | cadaver | TA - MMF - ST | n.a. | 155 | n.a. | 13 | n.a. | n.a. | n.a. | 1.41 |
| patient 19 | F | 44 | aHUS | remission | 506 | cadaver | TA -MMF - ST | n.a. | 355 | n.a. | 12.4 | n.a. | n.a. | n.a. | 1.53 |
| patient 20 | M | 75 | aHUS | remission | 4383 | cadaver | TA - MMF - ST | n.a. | 150 | n.a. | 15 | n.a. | 125 | 41 | 1.5 |
| patient 21 | M | 45 | aHUS | remission | 1167 | cadaver | TA -MMF | MPGN | 275 | 372 | 12.4 | NO | 21 | n.a. | 2 |
| patient 22 | M | 13 | aHUS | remission | 2728 | cadaver | TA - MMF - ST | chronic rejection | 222 | 327 | 6.9 | n.a. | 106 | 54 | 3.85 |
| patient 23 | F | 43 | aHUS | remission | 1040 | cadaver | n.a. | n.a. | 275 | 327 | 15 | n.a. | 78.2 | 35.4 | 0.8 |

n.a. not available. TA, Tacrolimus. MMF, mycophenolate. ST, steroids. MPGN, membrano-proliferative-glomerulonephritis.

**Supplementary Table 3**. Characteristics and clinical parameters of patients with de-novo TMA at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | phase | time from trasplant (days) | kidney donor | immunosuppression therapy | hystology | eculizumab* response | platelets 150-400x$10^3$/µL | LDH 266-500 IU/L | hemoglobin 14-18 g/dL | haptoglobin 30-200 mg/dL | schistocytes | C3 70-152 mg/dL | C4 15-40 mg/dL | serum creatinin 0.55 - 1.25 mg/dL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 59 | F | 63 | pyelonephritis | acute | 4 | cadaver | TA - MMF - ST | TMA | YES (HR) | 150 | 620 | 11 | 97 | n.a. | 104 | 26 | 5.6 |
| patient 60 | M | 65 | IgA N | acute | 96 | cadaver | TA - MMF - ST | TMA | NO | 93 | 628 | 9.3 | <20 | n.a. | 84 | 21 | 1.76 |
| patient 61 | F | 64 | Good-pasture | acute | 1 | cadaver | TA - MMF - ST | TMA | YES (CR) | 133 | 1162 | 13.6 | 45 | YES | n.a. | n.a. | 7 |
| patient 62 | M | 59 | ADPKD | acute | 12 | cadaver | TA - MMF - ST | TMA | YES (HR) | 178 | 751 | 7.7 | <30 | YES | n.a. | n.a. | 7.4 |
| patient 63 | F | 66 | hypertension | acute | 4 | cadaver | TA - MMF - ST | n.a. | YES (HR) | 36 | 1746 | 7.9 | <30 | n.a. | n.a. | n.a. | 9 |
| patient 64 | M | 59 | diabetic N | acute | 8 | cadaver | TA - MMF - ST | n.a. | YES (CR) | 79 | 317 | 8.7 | <30 | YES | n.a. | n.a. | 4.28 |
| patient 65 | M | 58 | obstructive N | acute | 8 | cadaver | MMF - ST | n.a. | NO | 90 | 1249 | 9.3 | <20 | n.a. | 104 | 27 | 3.48 |
| patient 66 | M | 81 | tubulo-interstitial N | acute | 6047 | cadaver | TA - MMF - ST | n.a. | YES (NR) | 98 | 506 | 9.4 | <5 | YES | 59 | 11 | 1.4 |
| patient 67 | M | 51 | IgA N | acute | 6595 | cadaver | TA - ST | n.a. | n.a. | 110 | n.a. | n.a. | n.a. | n.a. | 72 | 26 | 4.55 |
| patient 68 | M | 60 | ANCA-vasculitis | acute | 3 | cadaver | TA - MMF - ST | TMA | YES (CR) | 70 | 1790 | 7.7 | <30 | n.a. | n.a. | n.a. | 11 |
| patient 69 | F | 46 | lupus N | acute | 5 | cadaver | TA - MMF - ST | TMA | YES (CR) | 76 | 369 | 7.5 | <30 | YES | 81 | 35 | 5.93 |
| patient 70 | M | 60 | ADPKD | acute | 3 | cadaver | TA - MMF - ST | TMA | YES (HR) | 71 | 951 | 9 | 49 | YES | n.a. | n.a. | 9.4 |
| patient 71 | M | 57 | PAH / CRI | acute | 1497 | n.a. | n.a. | n.a. | NO | 95 | 624 | 10 | <1 | n.a. | 79.5 | 20.5 | 5.3 |
| patient 72 | M | 73 | IgA N | acute | 3 | cadaver | TA - MMF - ST | n.a. | YES (CR) | 39 | 627 | 8.3 | <30 | YES | n.a. | n.a. | 4.35 |
| patient 73 | M | 55 | diabetic N | acute | 3 | cadaver | TA -MMF - ST | TMA | n.a. | 63 | n.a. | 11.9 | <30 | n.a. | n.a. | n.a. | 12.4 |

n.a. not available. TA, Tacrolimus. MMF, mycophenolate. ST, steroids. N, nephropathy. ADPKD, autosomal dominant polycystic kidney disease. PAH/CRI, pulmonary arterial hypertension/chronic renal insufficiency.

* treatment started after blood sample collection. CR, complete remission. HR, hematological remission. NR, no remission.

**Supplementary Table** 4. Characteristics and clinical parameters of aHUS patients in chronic dialysis at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | dialysis | platelets 150-400x$10^3$/µL | LDH 266-500 IU/L | hemoglobin 14-18 g/dL | haptoglobin 30-200 mg/dL | C3 70-152 mg/dL | C4 15-40 mg/dL | serum creatinin 0.55 - 1.25 mg/dL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 24 | F | 74 | aHUS | HD | 302 | 488 | 14.6 | n.a. | 105 | 35.4 | 3.81 |
| patient 25 | F | 39 | aHUS | HD | 163 | 653 | 10.4 | n.a. | n.a. | n.a. | 4.59 |
| patient 22 | M | 14 | aHUS | HD | 150 | 611 | 8 | n.a. | 67 | 34 | 4.15 |
| patient 26 | F | 35 | aHUS | HD | 302 | 303 | 11.5 | n.a. | 47.7 | 27.5 | 8.9 |
| patient 27 | M | 51 | aHUS | PD | 188 | 489 | 13.5 | 232 | 111 | 41 | 14.9 |
| patient 28 | F | 43 | aHUS | HD | 177 | 277 | 8 | 91 | 79 | 25 | 9.95 |
| patient 29 | F | 27 | aHUS | HD | 228 | 309 | 12.7 | n.a. | 45 | 20 | 11 |
| patient 30 | F | 54 | aHUS | HD | 105 | 339 | 9.75 | n.a. | 72 | 32 | 4.87 |
| patient 31 | M | 41 | aHUS | HD | 189 | 409 | 8 | n.a. | n.a. | n.a. | 12 |
| patient 15 | F | 11 | aHUS | PD | 254 | 339 | 9.2 | n.a. | n.a. | n.a. | 3.5 |
| patient 17 | F | 52 | aHUS | PD | 291 | 352 | 11.1 | n.a. | 112 | 54 | 11 |
| patient 32 | F | 71 | aHUS | HD | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| patient 33 | F | 43 | aHUS | PD | 164 | 394 | 12.2 | n.a. | 86 | n.a. | 3.2 |
| patient 34 | F | 43 | aHUS | HD | 260 | 345 | 14.5 | n.a. | 58 | 27 | 8.4 |
| patient 35 | F | 46 | aHUS | HD | 168 | 447 | 12.8 | n.a. | n.a. | n.a. | 8.8 |
| patient 36 | M | 57 | aHUS | HD | 130 | 418 | 13.2 | n.a. | 51 | n.a. | 9 |
| patient 37 | F | 37 | aHUS | HD | 328 | 453 | 11.4 | n.a. | 63 | 33 | 8.11 |
| patient 38 | F | 49 | aHUS | HD | 238 | n.a. | 11.6 | n.a. | n.a. | n.a. | 11.6 |
| patient 39 | F | 48 | aHUS | HD | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| patient 40 | M | 51 | aHUS | HD | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| patient 41 | M | 52 | aHUS | HD | 175 | 401 | 13.6 | n.a. | 111 | n.a. | 6.6 |
| patient 42 | M | 53 | aHUS | n.a. | 176 | n.a. | 13.6 | n.a. | 67 | 47 | 7.58 |
| patient 43 | F | 37 | aHUS | HD | 269 | n.a. | 9.6 | n.a. | 48.6 | 25 | 11 |
| patient 20 | M | 60 | aHUS | PD | n.a. | n.a. | n.a. | n.a. | 125 | 41 | 8 |
| patient 44 | F | 23 | aHUS | HD | 209 | n.a. | 11.9 | n.a. | 36.4 | 28.3 | 5.7 |
| patient 45 | M | 2 | aHUS | PD | 395 | n.a. | 12.6 | n.a. | n.a. | n.a. | 11.4 |
| patient 46 | F | 46 | aHUS | HD | 179 | 136 | 10.6 | 37 | n.a. | n.a. | n.a. |
| patient 47 | F | 25 | aHUS | HD | 166 | 397 | 9.3 | n.a. | 67 | 19 | n.a. |

n.a. not available. HD, hemodialysis. PD, peritoneal dialysis.

**Supplementary Table 5**. Characteristics, clinical and complement parameters of stable renal transplant patients at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | time from trasplant (days) | Kidney donor | immunosuppression therapy | platelets 150-400x$10^3$/µL | hemoglobin 14-18 g/dL | serum creatinin 0.55-1.25 mg/dL | C5b-9 on HMEC-1 unstimulated (50-150%) | C5b-9 on HMEC-1 ADP-activated (60-149)% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 74 | M | 81 | FSGS | 182 | cadaver | TA - MMF | 200 | 13.4 | 2.29 | 106 | 109 |
| patient 75 | F | 59 | proteinuric N | 730 | cadaver | TA - MMF | 343 | 14.9 | 1.21 | 100 | 113 |
| patient 76 | M | 49 | IgA N | 730 | cadaver | TA - MMF | 191 | 12.9 | 1.16 | 95 | 97 |
| patient 77 | M | 61 | ADPKD | 1460 | cadaver | TA - MMF | 227 | 13.4 | 1.33 | 101 | 108 |
| patient 78 | M | 56 | hypertensive N | 2190 | cadaver | TA - MMF | 206 | 14.5 | 1.58 | 113 | 122 |
| patient 79 | M | 36 | IgA N | 6 | living | TA - MMF - ST | 196 | 10.7 | 1.03 | 217 | 221 |
| patient 80 | M | 39 | IgA N | 182 | cadaver | TA - MMF | 139 | 13.4 | 1.39 | 98 | 99 |

TA, Tacrolimus. MMF, mycophenolate. ST, steroids. FSGS, focal segmental glomerulosclerosis. N, nephropathy. ADPKD, autosomal dominant polycystic kidney disease.

**Supplementary Table 6**. Characteristics, clinical and complement parameters of control dialyzed patients at the time of sample collection.

| patient (n°) | gender | age (years) | native kidney disease | dialysis | platelets 150-400x$10^3$/µL | LDH 266-500 IU/L | hemoglobin 14-18 g/dL | C3 70-152 mg/dL | C4 15-40 mg/dL | plasma sC5b-9 140-280 ng/mL | serum creatinin 0.55 - 1.25 mg/dL | C5b-9 on HMEC-1 unstimulated (50-150%) | C5b-9 on HMEC-1 ADP-activated (60-149)% | rare gene variants |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 48 | M | 48 | ADPKD | HD | 179 | 320 | 13 | 111 | 23 | n.a. | 15.22 | 110 | 148 | n.a. |
| patient 49 | M | 26 | proteinuric N | PD | 264 | 436 | 12.4 | 149 | 28 | n.a. | 9.4 | 115 | 116 | n.a. |
| patient 50 | M | 47 | ADPKD | HD | 166 | n.a. | 12.3 | n.a. | n.a. | n.a. | 13.7 | 95 | 99 | n.a. |
| patient 51 | M | 32 | CRI | HD | 267 | 353 | 11.9 | n.a. | n.a. | n.a. | 14.74 | 91 | 121 | n.a. |
| patient 52 | F | 27 | ADPKD | PD | 359 | 269 | 9 | 113 | 25 | n.a. | 12.91 | 65 | 78 | n.a. |
| patient 53 | M | 46 | fabry disease | PD | 256 | 257 | 11.8 | 93 | 19 | n.a. | 10.3 | 98 | 130 | n.a. |
| patient 54 | M | 46 | MPGN | PD | 250 | 353 | 11.7 | 96 | 22 | n.a. | 10.36 | 91 | 102 | n.a. |
| patient 55 | M | 28 | nephroangio sclerosis | HD | 277 | 277 | 12 | 64 | 43 | n.a. | 17.47 | 105 | 119 | NO |
| patient 56 | M | 39 | CRI | PD | 268 | 440 | 13.4 | 119 | 35 | 175 | 7.24 | 113 | 138 | NO |
| patient 57 | M | 62 | ADPKD | HD | 240 | n.a. | 11.4 | 78 | 27 | n.a. | 7.73 | 94 | 159 | NO |
| patient 58 | F | 59 | horse kidney | HD | 251 | n.a. | 11.2 | n.a. | n.a. | n.a. | 8 | 115 | 127 | NO |

n.a. not available. N, nephropathy. ADPKD, autosomal dominant polycystic kidney disease. MPGN, membrano-proliferative-glomerulonephritis. CRI, chronic renal insufficiency. HD, hemodialysis. PD, peritoneal dialysis.

**Supplementary Table 7**. Complement parameters and genetic characteristics of aHUS patients with recurrence after kidney transplant at the time of sample collection.

| patient (n°) | plasma | C5b-9 on HMEC-1 | | rare gene variants | | | | | | | risk haplotypes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | sC5b-9 140-280 ng/mL | unstimulated (50-150%) | ADP-activated (60-149)% | | gene | nucleotide change | aminoacid change | gnomAD frequency | CADD | classification | CFH-H3 (n) | MCPggaac (n) | total (n) |
| patient 1 | n.a. | 161 | 151 | YES | CFI | c.905-3T>C | - | $1.4x10^{-4}$ | 13 | VUS | 2 | 0 | 2 |
| patient 2 | n.a. | 298 | 365 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 3 | 102 | 173 | 182 | n.a. | - | - | - | - | - | - | n.a. | n.a. | n.a. |
| patient 4 | n.a. | 196 | 197 | YES | MCP | c.286+2T>G | - | $1x10^{-4}$ | 14.5 | PV | 1 | 2 | 3 |
| patient 5 | n.a. | 255 | 283 | NO | - | - | - | - | - | - | 0 | 2 | 2 |
| patient 6* | n.a. | 84 | 86 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 7 | n.a. | 204 | 219 | NO | - | - | - | - | - | - | 0 | n.a. | 0 |
| patient 8 | 96 | 209 | 212 | NO | - | - | - | - | - | - | 2 | 1 | 3 |
| patient 9 | 1043 | 165 | 180 | NO | - | - | - | - | - | - | 1 | 2 | 3 |
| patient 10 | n.a. | 187 | 187 | NO | - | - | - | - | - | - | 2 | 1 | 3 |
| patient 11 | 269 | 182 | 204 | YES | MCP<br>CFI | c.286+2T>G<br>c.G950A | -<br>p.R317Q | $1x10^{-4}$<br>$5.8x10^{-5}$ | 14.5<br>15 | PV<br>VUS | 2 | 2 | 4 |
| patient 12 | 242 | 169 | 178 | YES | MCP | c.C1148T | p.T383I | $1.9x10^{-3}$ | 4 | VUS | 2 | 1 | 3 |

n.a. not available. VUS, variant of uncertain significance. PV, pathogenic variant.

**Supplementary Table 8**. Complement parameters and genetic characteristics of transplanted aHUS patients with no sign of recurrence at the time of sample collection.

| patient (n°) | plasma | C5b-9 on HMEC-1 | | rare gene variants | | | | | | | risk haplotypes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | sC5b-9 140-280 ng/mL | unstimulated (50-150%) | ADP-activated (60-149)% | | gene | nucleotide change | aminoacid change | gnomAD frequency | CADD | classification | CFH-H3 (n) | MCPggaac (n) | total (n) |
| patient 13 | n.a. | 105 | 173 | YES | CFI | c.A779G | p.Q260R | $2.4x10^{-5}$ | 1 | VUS | 0 | 0 | 0 |
| patient 14 | n.a. | 145 | 180 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 15 | 219 | 123 | 159 | NO | - | - | - | - | - | - | 0 | 1 | 1 |
| patient 16 | n.a. | 107 | 138 | NO | - | - | - | - | - | - | 0 | 2 | 2 |
| patient 17 | 295 | 78 | 256 | NO | - | - | - | - | - | - | 1 | 1 | 2 |
| patient 18 | n.a. | 135 | 172 | NO | - | - | - | - | - | - | n.a. | n.a. | n.a. |
| patient 19 | n.a. | 95 | 198 | NO | - | - | - | - | - | - | 2 | 1 | 3 |
| patient 20 | 676 | 132 | 264 | YES | CFI | c.C148G | p.P50A | $1x10^{-3}$ | 24.9 | VUS | 0 | 1 | 1 |
| patient 21 | 2092 | 131 | 161 | YES | CFI | c.G1075T | p.D359Y | $8.7x10^{-6}$ | 24.4 | VUS | 0 | 0 | 0 |
| patient 22 | n.a. | 133 | 135 | YES | C3<br>*CFHR1/CFH hybrid* | c.G2284A<br>reverse hybrid *CFHR1* $_{1\text{-}5}$-*CFH* $_{23}$ | p.V762I<br>- | 0.0002<br>- | 0.25<br>- | VUS<br>PV | 0 | 2 | 2 |
| patient 23 | n.a. | 215 | 225 | NO | - | - | - | - | - | - | 1 | 0 | 1 |

n.a. not available. VUS, variant of uncertain significance. PV, pathogenic variant.

**Supplementary Table 9.** Complement parameters and genetic characteristics of patients with de-novo TMA at the time of sample collection.

| patient (n°) | plasma sC5b-9 140-280 ng/mL | C5b9 on HMEC-1 unstimulated (50-150%) | C5b9 on HMEC-1 ADP-activated (60-149)% | rare gene variants | gene | nucleotide change | aminoacid change | gnomAD frequency | CADD | classification | risk haplotypes CFH-H3 (n) | MCPggaac (n) | total (n) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 59 | n.a | 171 | 247 | NO | - | - | - | - | - | - | 0 | 1 | 1 |
| patient 60 | n.a | 196 | 230 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 61 | 235 | 181 | 213 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 62 | 252 | 245 | 262 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 63 | n.a | 175 | 201 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 64 | 974 | 197 | 242 | NO | - | - | - | - | - | - | 0 | 1 | 1 |
| patient 65 | n.a | 378 | 384 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 66 | n.a | 188 | 235 | YES | CFH | c.C1511T | p. T504M | $3x10^{-4}$ | 6.3 | VUS | 0 | 1 | 1 |
| patient 67 | n.a | 303 | 311 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 68 | 831 | 193 | 209 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 69 | n.a | 230 | 268 | YES | CFH | c.C3626T | p. S1209L | - | 9 | VUS | 1 | 0 | 1 |
| patient 70 | 833 | 301 | 352 | YES | C3 | c.G1909C | p. G637R | $5x10^{-4}$ | 23.5 | LPV | 1 | 1 | 2 |
| patient 71 | n.a | 241 | 244 | NO | - | - | - | - | - | - | 0 | 2 | 2 |
| patient 72 | 277 | 232 | 273 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 73 | 358 | 194 | 225 | NO | - | - | - | - | - | - | 1 | 0 | 1 |

n.a. not available.

**Supplementary Table 10**. Complement parameters and genetic characteristics of aHUS patients in chronic dialysis at the time of sample collection.

| patient (n°) | plasma sC5b-9 140-280 ng/mL | C5b 9 on HMEC-1 unstimulated (50-150%) | C5b 9 on HMEC-1 ADP-activated (60-149)% | rare gene variants | gene | nucleotide change | aminoacid change | gnomAD frequency | CADD | classification | risk haplotypes CFH-H3 (n) | risk haplotypes MCPggaac (n) | risk haplotypes total (n) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| patient 24 | 233 | 97 | 246 | YES | CFH | c.C3628T | p.R1210C | 0.0003 | 11.8 | PV | 1 | 1 | 2 |
| patient 25 | 380 | 129 | 193 | YES | CFH | c.A232G | p.R78G | - | 15.8 | PV | 0 | 1 | 1 |
| patient 22 | 127 | 114 | 213 | YES | C3<br>*CFHR1/CFH hybrid* | c.G2284A<br>reverse hybrid *CFHR1* $_{1\text{-}5}$-*CFH* $_{23}$ | p.V762I<br>- | 0.0002<br>- | 0.25<br>- | VUS<br>PV | 0 | 2 | 2 |
| patient 26 | n.a | 120 | 154 | YES | C3 | c.G181A | p.D61N | $1x10^{-4}$ | 19 | LPV | 2 | 1 | 3 |
| patient 27 | 260 | 145 | 201 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 28 | n.a | 111 | 173 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 29 | 1436 | 78 | 229 | YES | C3 | c.1774C>T | p.R592W | $1.5x10^{-5}$ | 34 | LPV | 2 | 2 | 4 |
| patient 30 | n.a | 102 | 199 | n.a. | - | - | - | - | - | - | n.a. | n.a. | n.a. |
| patient 31 | n.a | 139 | 235 | NO | - | - | - | - | - | - | 0 | 1 | 1 |
| patient 15 | 183 | 148 | 504 | NO | - | - | - | - | - | - | 0 | 1 | 1 |
| patient 17 | 295 | n.a. | 409 | NO | - | - | - | - | - | - | 1 | 1 | 2 |
| patient 32 | n.a | n.a. | 336 | YES | CFI | c.G1234A | p.V412M | 0.0013 | 25 | VUS | 0 | 2 | 2 |
| patient 33 | 444 | 128 | 266 | YES | C3 | c.A3187C | p.S1063R | $3.7x10^{-5}$ | 23.4 | LPV | 0 | n.a. | n.a. |
| patient 34 | n.a | 104 | 155 | NO | - | - | - | - | - | - | 1 | 0 | 1 |
| patient 35 | n.a | 142 | 164 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 36 | n.a | n.a. | 403 | YES | CFH | c.G3587T | p.E1172X | 0.0002 | - | PV | 0 | n.a. | n.a. |
| patient 37 | n.a | n.a. | 590 | YES | CFH | c.C3572T | p.S1191L | - | 18.6 | PV | 0 | 2 | 2 |
| patient 38 | 91 | 173 | 177 | YES | *CFHR1/CFH hybrid* | reverse hybrid *CFHR1* $_{1\text{-}4}$-*CFH* $_{22\text{-}23}$ | - | - | - | PV | 1 | 0 | 1 |
| patient 39 | n.a | 155 | 276 | NO | - | - | - | - | - | - | 1 | 1 | 2 |
| patient 40 | n.a | 191 | 197 | YES | C3 | c.4973T>C | p.V1658A | - | 1.45 | VUS | n.a. | n.a. | n.a. |
| patient 41 | 236 | n.a. | 605 | YES | *CFHR1/CFH hybrid* | reverse hybrid *CFHR1* $_{1\text{-}5}$-*CFH* $_{23}$ | - | - | - | PV | 1 | 2 | 3 |
| patient 42 | 467 | 249 | 286 | NO | - | - | - | - | - | - | 1 | 1 | 2 |
| patient 43 | n.a | n.a. | 1219 | YES | CFH | c.3493+1G>C | - | - | 24.3 | PV | 2 | 0 | 2 |
| patient 20 | 676 | n.a. | 250 | YES | CFI | c.C148G | p.P50A | $1x10^{-3}$ | 24.9 | VUS | 0 | 1 | 1 |
| patient 44 | 294 | 137 | 170 | YES | C3 | c.T544C | p.S182P | - | 7.12 | VUS | 0 | 0 | 0 |
| patient 45 | n.a | 120 | 157 | NO | - | - | - | - | - | - | 0 | 0 | 0 |
| patient 46 | n.a | 140 | 189 | YES | CFI | c.C148G | p.P50A | $1x10^{-3}$ | 24.9 | VUS | 1 | 1 | 2 |
| patient 47 | n.a | 193 | 207 | NO | - | - | - | - | - | - | 1 | 1 | 2 |

n.a. not available. PV, pathogenic variant. LPV, likely pathogenic variant. VUS, variant of uncertain significance.

## *International Registry of Recurrent and Familial HUS/ TTP*

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