# Defects in Complement and "Secondary" Hemolytic Uremic Syndrome


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# Defects in Complement and "Secondary" Hemolytic Uremic Syndrome

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The thrombotic microangiopathy (TMA) syndromes are extraordinarily diverse. Most TMAs, ~90%, occur in the setting of coexisting diseases and have been termed secondary hemolytic uremic syndrome (HUS), while a subset of TMAs occur on the background of complement gene variants, i.e., primary atypical HUS.(1) Le Clech *et al*. recently reported the low prevalence of such variants in patients with secondary HUS and poor clinical outcomes.(2)

We screened a cohort of 49 adult patients classified as secondary HUS(1) for complement gene variants (**Table 1**). In total, 13 variants were found in 17 (35%) patients (**Table S1**), 6 of whom were considered pathogenic or likely pathogenic (carriers, *n*=11 [22%]). Patients with (likely) pathogenic variants, however, presented with coexisting diseases not included in the study by Le Clech *et al*.,(2) that is, hypertensive emergency, pregnancy, and *de novo* disease after kidney transplantation. Most of these patients progressed to ESRD and relapses were common, resembling primary atypical HUS. Eleven (73%) out of 15 patients who had been treated with eculizumab (median 13, range 1-70 doses), including 8 patients with no complement gene variants, achieved a renal response (**Figure S2**). Notably, massive serum-induced *ex vivo* C5b9 formation(3) was found in all but one patient with a renal response prior to treatment (tested, *n*=9).

Thus, appropriate screening for complement gene variants should be recommended in patients with "secondary" HUS related to hypertensive emergency(3) or pregnancy.(4) Moreover, serum-induced *ex vivo* C5b9 formation may characterize patients into different groups with potential therapeutic and prognostic implications.

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## SUPPLEMENTARY MATERIAL

**Figure S1**. Individual case report files from 15 patients treated with eculizumab.

Blue bars, renal survival. Red bars, dialysis. CKD, chronic kidney disease. ESRD, end-stage renal disease. F, female. M, male. N, normal *ex vivo* C5b9 formation on the resting endothelium. ND, not determined. SCr, serum creatinine. Y, massive *ex vivo* C5b9 formation on the resting endothelium.

*Methods for *ex vivo* C5b9 formation on the resting endothelium have been described elsewhere.(S7)

¥Deceased.

**Table S1**. Detailed characteristics of the complement gene variants.

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## SUPPLEMENTARY REFERENCES


S1. Merinero H, Garcia S, Garcia-Fernandez J, et al. Complete functional characterization of disease-associated genetic variants in the complement factor H gene. Kidney Int. 2018;93(2):470-81.

S2. Bienaime F, Dragon-Durey M, Regnier C, et al. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. Kidney Int. 2010;77(4):339-49.

S3. Richards A, Kemp E, Liszewski M, et al. Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. Proc Natl Acad Sci U S A. 2003;100(22):12966-71.

S4. Geerlings M, Kremlitzka M, Bakker B, et al. The Functional Effect of Rare Variants in Complement Genes on C3b Degradation in Patients With Age-Related Macular Degeneration. JAMA Ophthalmol. 2017;135(1):39-46.


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S5. Roumenina L, Frimat M, Miller E, et al. A prevalent C3 mutation in aHUS patients causes a direct C3 convertase gain of function. Blood. 2012;119(18):4182-91.

S6. Mohlin F, Nilsson S, Levart T, et al. Functional characterization of two novel non-synonymous alterations in CD46 and a Q950H change in factor H found in atypical hemolytic uremic syndrome patients. Mol Immunol. 2015;65(2):367-76.

S7. Timmermans S, Abdul-Hamid M, Potjewijd J, et al. C5b9 Formation on Endothelial Cells Reflects Complement Defects among Patients with Renal Thrombotic Microangiopathy and Severe Hypertension. J Am Soc Nephrol. 2018;29(8):2234-43.

Supplementary information is available at Kidney International's website.

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**Table 1**. Main characteristics of the 49 patients, either with a pathogenic variant or not.

| | Pathogenic variant(s) | | |
|---|---|---|---|
| | Yes, *n*=11 | No, *n*=38 | ***P* value*** |
| M/F | 4/7 | 20/18 | 0.50 |
| Conditions | | | |
| Hypertensive emergency | 9 | 17 | **0.04** |
| Pregnancy | 1 | 5 | 1.00 |
| Kidney transplantation | 1 | 3 | 1.00 |
| Autoimmunity | 0 | 3 | 1.00 |
| Cancer | 0 | 2 | 1.00 |
| Others | 0 | 8 | 0.18 |
| Hemolysis | 7 | 23 | 1.00 |
| Platelets, G/L | 191 (±69) | 113 (±80) | **<0.01** |
| Normal platelet counts | 7 | 12 | 0.08 |
| Creatinine, µmol/L | 1,089 (586-1,388) | 515 (307-699) | **<0.01** |
| Dialysis at presentation, *n*/*N* | 10 | 23 | 0.08 |
| Outcome, *N* | 11 | 36 | |
| Follow-up, months | 116 (36-152) | 16 (7-32) | **<0.01** |
| ESRD | 8 | 12 | **0.04** |
| Relapse | 5 | 1 | **<0.01** |
| Death | 1 | 5 | 1.00 |

ESRD, end-stage renal disease. F, female. M, male.

**P* value as calculated by the unpaired sample t, Mann-Whitney u, or Fisher's exact test as appropriate.

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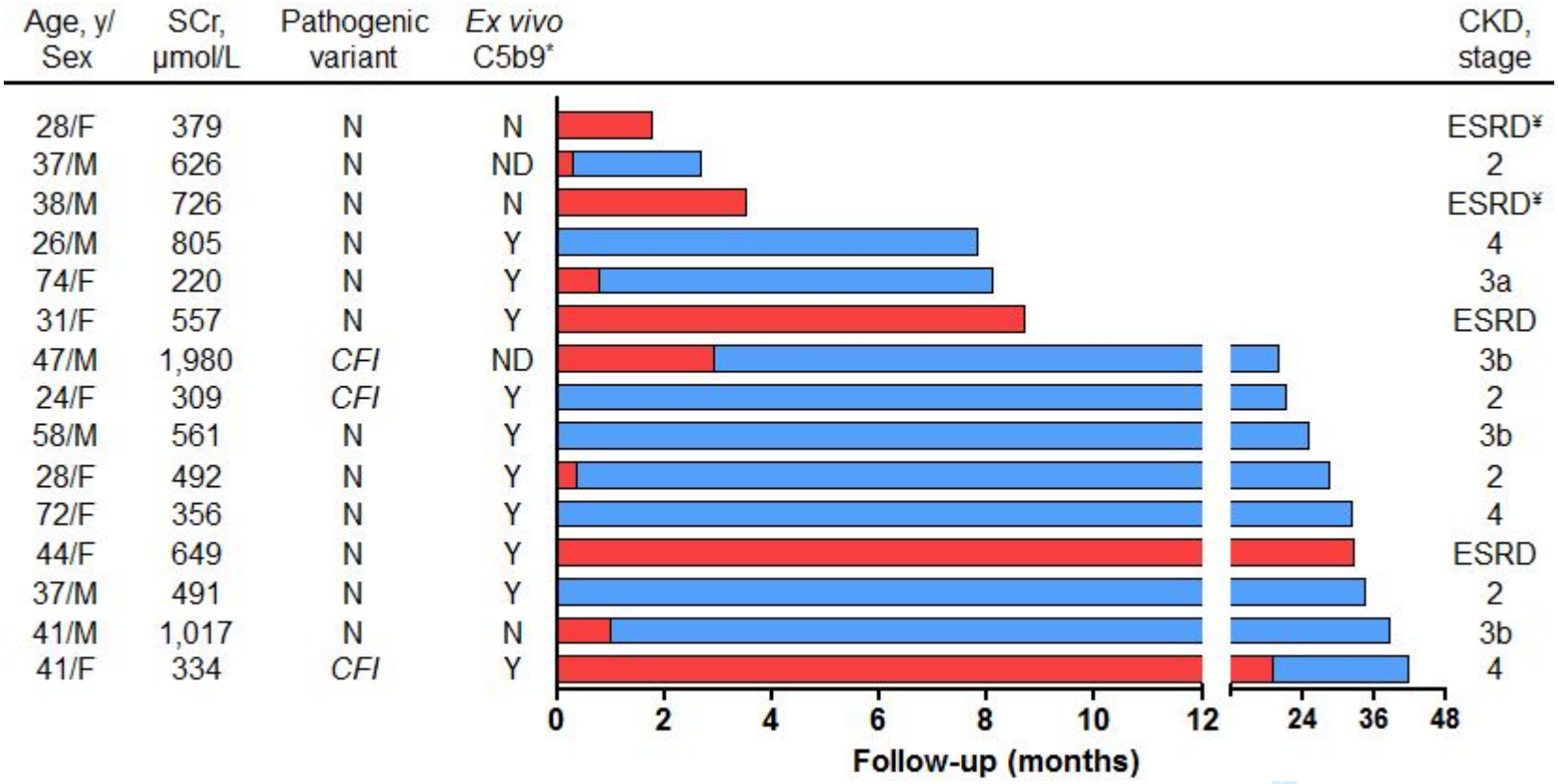
**Figure S1**. Individual case report files from 15 patients treated with eculizumab.




Blue bars, renal survival. Red bars, dialysis. CKD, chronic kidney disease. ESRD, end-stage renal disease. F, female. M, male. N, normal *ex vivo* C5b9 formation on the resting endothelium. ND, not determined. SCr, serum creatinine. Y, massive *ex vivo* C5b9 formation on the resting endothelium. *Methods for *ex vivo* C5b9 formation on the resting endothelium have been described elsewhere.(S7) ¥Deceased.

## REFERENCES

S7. Timmermans S, Abdul-Hamid M, Potjewijd J, et al. C5b9 Formation on Endothelial Cells Reflects Complement Defects among Patients with Renal Thrombotic Microangiopathy and Severe Hypertension. J Am Soc Nephrol. 2018;29(8):2234-43.

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**Table S1**. Detailed characteristics of the complement gene variants.

| Gene | *n* | Variant | Consequence | MAF, % | SIFT | PolyPhen2 | Defect | Coexisting disease |
|---|---|---|---|---|---|---|---|---|
| ***Pathogenic or likely pathogenic variants*** | | | | | | | | |
| *CFH* | 1 | c.2558G>A | C853Y | 0 | Deleterious | Probably damaging | Quantitative deficiency(S1) | HT |
| *CFI* | 2 | c.148C>G | P50A | <0.02 | Deleterious | Probably damaging | Quantitative deficiency(S2) | HT[1], KTX[2] |
| *CFI* | 1 | c.452A>G | N151S | <0.01 | Deleterious | Probably damaging | Quantitative deficiency(S2) | HT |
| *CD46* | 1 | c.811_816delGACAGT | ΔD271/S272 | 0 | N/a | N/a | Quantitative deficiency(S3) | HT[3] |
| *C3* | 1 | c.463A>C | K155Q | 0.3-0.4 | Tolerated | Benign | Gain of function(S4) | HT |
| *C3* | 5 | c.481C>T | R161W | 0 | Deleterious | Probably damaging | Gain of function(S5) | HT, P-HUS[4] |
| ***Benign variants or those of uncertain significance*** | | | | | | | | |
| *CFH* | 3 | c.2850G>T | Q950H | 0.1-0.4 | Deleterious | Possibly damaging | Loss of function?(S6) | HSCT, HT[3], KTX[2] |
| *CFH* | 2 | c.3148A>T | N1050Y | 2.2 | Tolerated | Benign | Not characterized | KTX, P-HUS |
| *CFI* | 1 | c.772G>A | A258T | <0.03 | N/a | N/a | Not characterized | P-HUS |
| *CFB* | 1 | c.11693A>G | K565E | 1.6 | Deleterious | Benign | Not characterized | P-HUS[4] |
| *CFHR2* | 1 | c.791A>G | Y264C | 1.2 | Deleterious | Probably damaging | Not characterized | S. *pneumoniae* |
| *CFHR4* | 1 | c.799+3A>C | K268Q | 0 | Deleterious | Probably damaging | Not characterized | HELLP |
| *THBD* | 1 | c.1433C>T | T478I | 0 | Tolerated | Benign | Not characterized | HT[1] |

HELLP, hemolysis, elevated liver enzymes, low platelets. HSCT, hematopoietic stem cell transplantation. HT, hypertensive emergency. KTX, kidney transplantation. MAF, minor allele frequency according to the 1000 Genomes Project (1000GP), Exome Aggregation Consortium (ExAC), and Exome Variant Server (EVS). N/a, not applicable. P-HUS, pregnancy associated hemolytic uremic syndrome. PolyPhen2, Polymorphism Phenotyping V2. SIFT, Sorting Intolerant From Tolerant. [1, 2, 3, 4], indicate cases with combined mutations.

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S1. Merinero H, Garcia SP, Garcia-Fernandez J, et al. Complete functional characterization of disease-associated genetic variants in the complement factor H gene. Kidney Int. 2018;93(2):470-81.

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