Supplementary data.

Chronic Thrombotic Microangiopathy in Patients with a C3 Gain of Function Protein: a Case Series.

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Methods S1. Complement work-up.

Patients were screened for rare variants, i.e., variants with a minor allele frequency <1%, and single nucleotide polymorphisms in coding regions of *CFH*, *CFI*, *CD46*, *CFB*, *C3*, *CFHR1-5*, *THBD*, and *DGKE* using DNA sequencing. The classification of variants was based on international standards.[1] Pathogenic variants were defined as those with functional studies supporting a defect in complement regulation, including null variants in genes linked to complement regulation and/or variants that cluster in patients with primary atypical hemolytic uremic syndrome as demonstrated by Osborne *et al*.[2] Likely pathogenic variants were defined as those with functional studies supporting a defect in complement regulation that have been located in a mutational hotspot and/or critical functional domain. In addition, rare variants not fulfilling these criteria have been classified as uncertain significance.

The *CFH-CFHR1-5* genomic region was analyzed for rearrangements by multiplex ligation probe amplification. Factor H autoantibodies were assessed by enzyme-linked immunosorbent assay in selected cases.[3]

Figure S1. Patients with primary atypical hemolytic uremic syndrome included in the Limburg Renal Registry. Patients with chronic features of thrombotic microangiopathy on kidney biopsy not related to C3 p.R161W had a variant in *CFI* (c.452A>G, p.N151S; *n*=1), *CD46* (c.811_816delGACAGT, p.D271_S272del; *n*=1), or no variant (*n*=2) identified.

aHUS, atypical hemolytic uremic syndrome. FHAA, factor H autoantibodies. TMA, thrombotic microangiopathy.

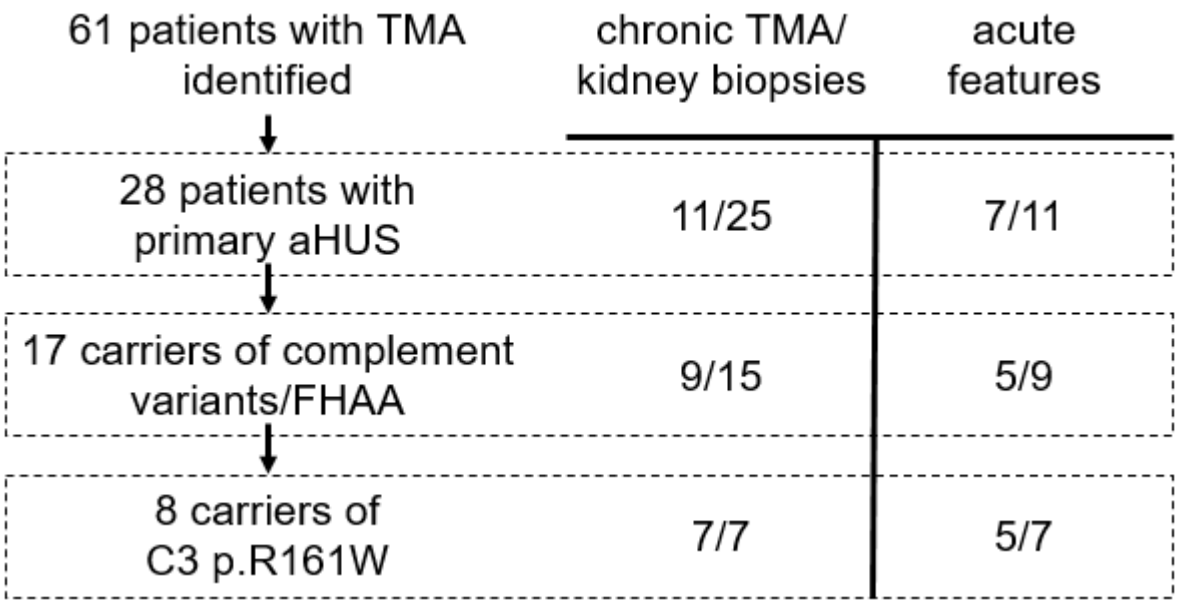

Figure S2. Morphologic features on representative kidney biopsy. Double contour formation of the glomerular basement membrane (arrowheads) and mesangiolysis were appreciated, either with thrombosis (A) or not (B); Jones methenamine silver stain, original magnification 400×. C3c (C) but not immune complex deposits were found along the glomerular basement membrane; fluorescein isothiocyanate labeled anti-C3c (Dako, Heverlee, Belgium), original magnification 400×. Electron lucent material was found in the subendothelial space (D), while electron dense deposits were lacking on electron microscopy; original magnification 1,400×.

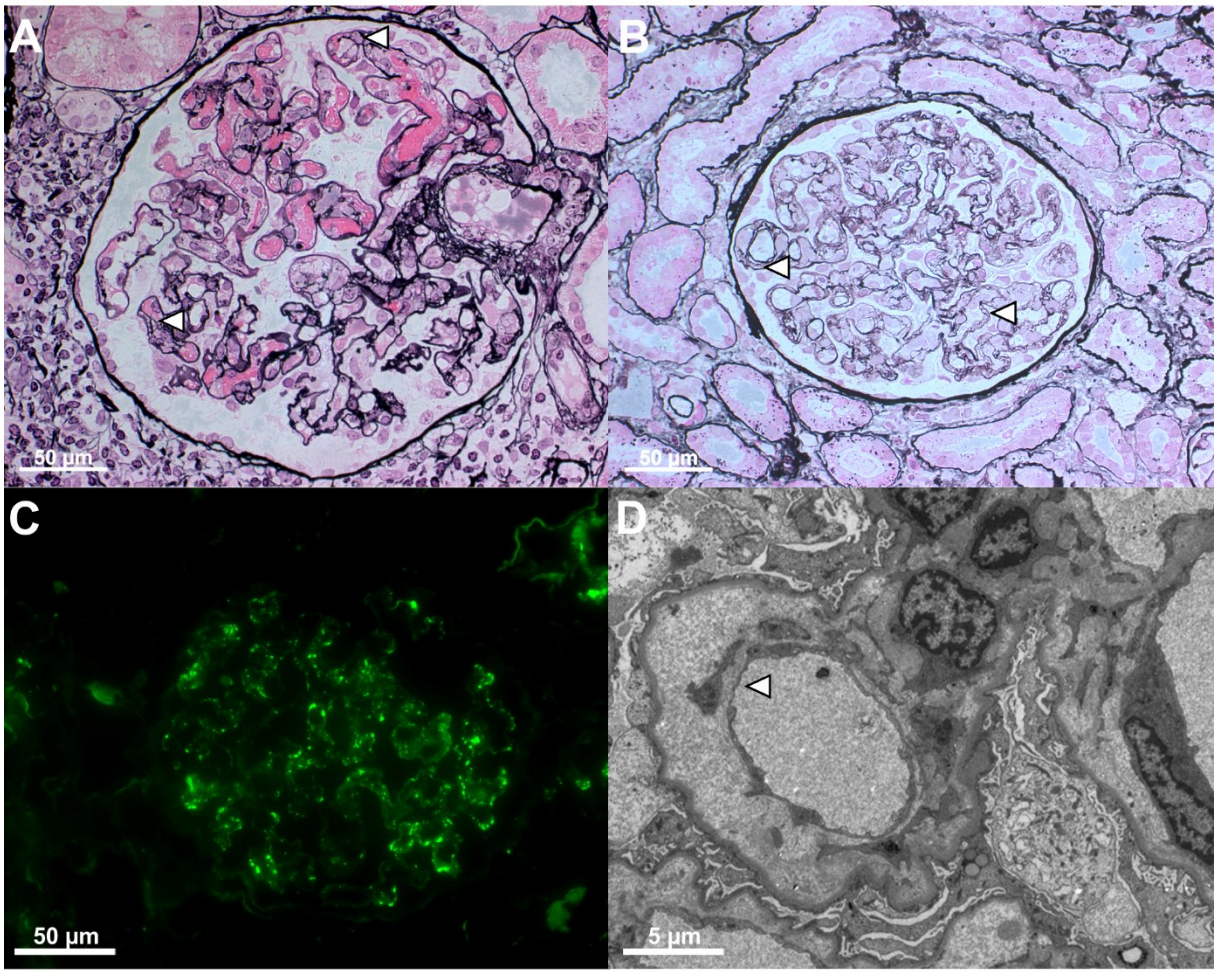

Figure S3. Disease course after kidney transplantation.

Arrowheads (red), recurrent aHUS. IST, immunosuppression (i.e., rituximab and steroids). T1, donor kidney 1. T2, donor kidney 2. T3, donor kidney 3. X, graft loss.

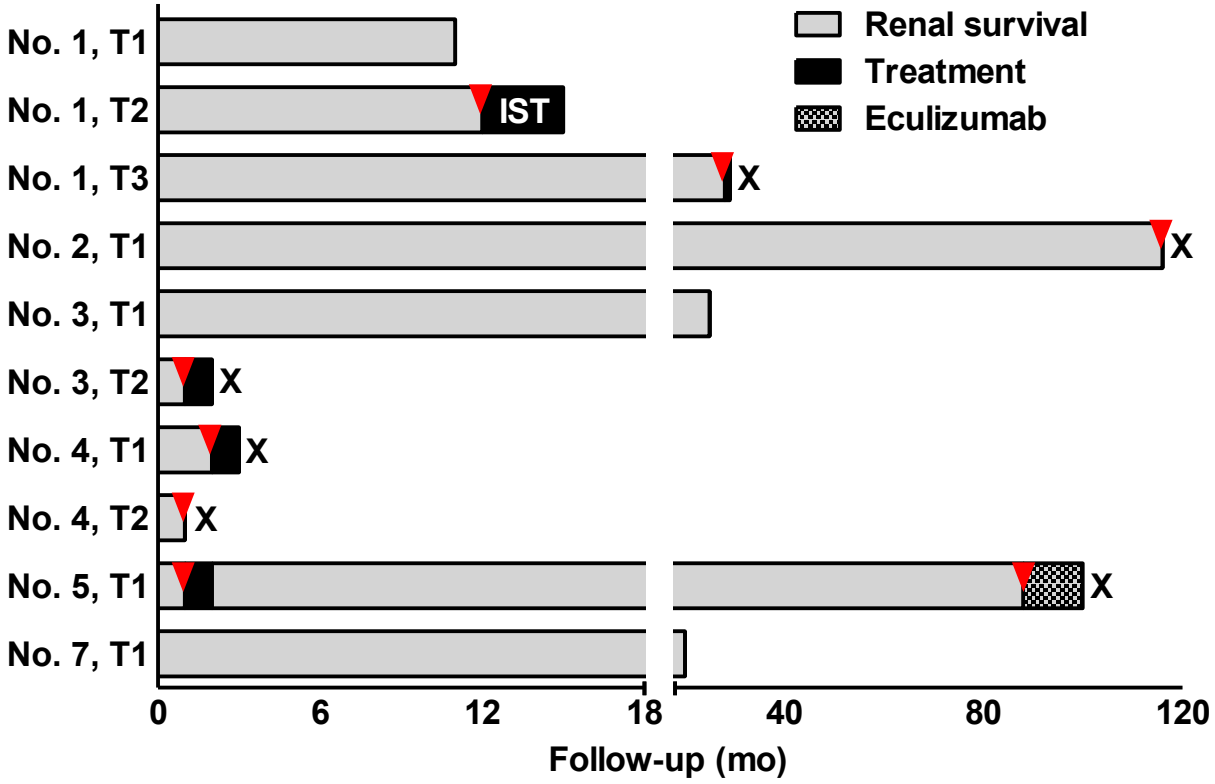

Table S1. Patients' characteristics at the time of recurrent primary atypical hemolytic uremic syndrome.
CMV, cytomegalovirus reactivation. DCD, donation after cardiac death. LR, living-related donation. LUR, living-unrelated donation. MAHA, microangiopathic hemolytic anemia. TAC, tacrolimus.

Patient./ TX	**Donor type**	**Platelets**, G/L	**MAHA**	**LDH**, U/L	**Creatinine**, mg/dL	**Proteinuria**, g/d	**Precipitant(s)**
No. 1/T2	DCD	334	–	366	8.5	5.1	TAC
No. 1/T3	DCD	158	–	560	5.2	3.7	TAC
No. 2/T1	DCD	205	–	159	6.3	3.0	TAC
No. 3/T2	LR	253	–	200	8.7	Anuria	TAC
No. 4/T1	DCD	142	+	1,274	5.6	1.4	TAC
No. 4/T2	LUR	116	+	793	4.5	3.3	TAC
No. 5/T1(1)	LR	127	–	367	2.3	0.5	TAC, CMV
No. 5/T1(2)	LR	237	–	255	5.5	4.1	TAC

References

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424.

2. Osborne A, Breno M, Borsa N, et al. Statistical validation of rare complement variants provides insight into the molecular basis of atypical hemolytic uremic syndrome and C3 glomerulopathy. J Immunol 2018; 200: 2464-2478.

3. Dragon-Durey M, Loirat C, Cloarec S, et al. Anti-factor H autoantibodies associated with atypical hemolytic uremic syndrome. J Am Soc Nephrol 2005; 16: 555-563.