C5b9 formation on endothelial cells reflect complement defects among patients with renal TMA and severe hypertension

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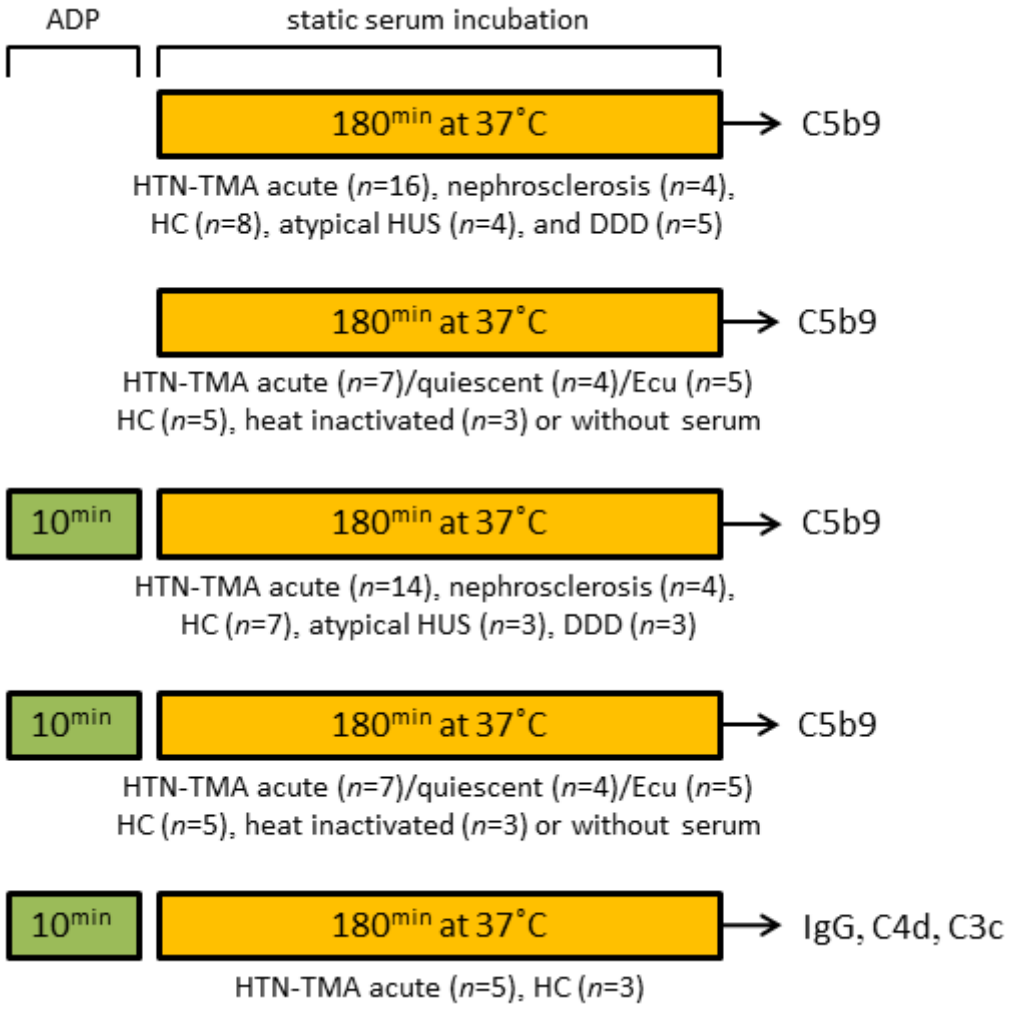
SUPPLEMENTAL CONTENTS

SUPPLEMENTAL METHODS

Item S1. Experimental design of ex vivo complement measurements.

ADP, adenosine 5'-diphosphate. DDD, dense deposit disease. Ecu, eculizumab treated samples. HC, healthy control. HTN-TMA, hypertension-associated TMA. HUS, hemolytic uremic syndrome.



SUPPLEMENTAL RESULTS

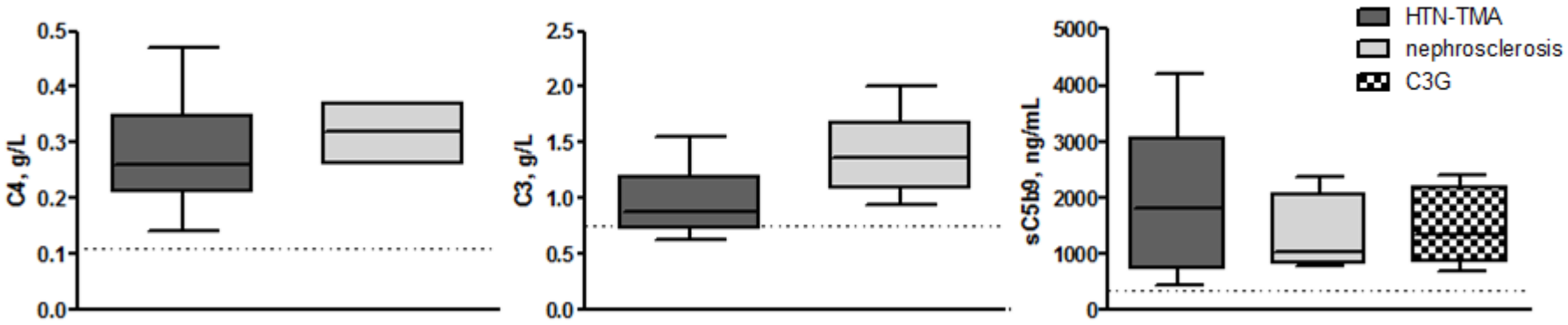
Item S2. Baseline characteristics.

	TMA (*n*=17)	**Nephrosclerosis** (*n*=5)	***P* value**
M/F	9/8	5/0	N/a
Age, years	38 (34 – 45)	63 (52 – 68)	0.01
SBP, mmHg	212 (184 – 232)	185 (175 – 195)	NS
DBP, mmHg	126 (120 – 142)	100 (80 – 123)	0.03
Systemic hemolysis, %	5, 29	0	N/a
Creatinine, µmol/L	835 (471 – 1,141)	193 (122 – 521)	0.01
Dialysis, %	14, 82	0	N/a
Proteinuria, g/d	1.6 (0.7 – 3.1)	2.1 (0.4 – 3.3)	NS
Low C4 (*n*/*N*)	1/16	0/5	N/a
Low C3 (*n*/*N*)	5/16	0/5	N/a

DBP, diastolic blood pressure. N/a, not applicable. NS, not significant. SBP, systolic blood pressure. TMA, thrombotic microangiopathy.

Item S3. Routine complement tests. C4, C3, and sC5b9 levels do not differ between severely hypertensive patients with thrombotic microangiopathy and those with nephrosclerosis on renal biopsy.

C3G, C3 glomerulopathy. HTN-TMA, hypertension-associated TMA.



Item S4. Ex vivo C5b9 formation in disease controls. Absolute values in pixels and % of control of serum-induced C5b9 formation on resting and ADP-activated HMEC-1 in patients with atypical HUS and dense deposit disease.

	C5b9 formation on resting HMEC-1			**C5b9 formation on ADP-activated HMEC-1**		
	absolute value, pixels	control, pixels	% of the control	absolute value, pixels	control, pixels	% of the control
Atypical HUS, rare variants in complement genes and/or FHAA						
1, DEAP-HUS	34,148	10,349	**330***	25,412	7,843	**324***
2, *C3*-R161W	27,170	9,918	**274***	34,514	13,019	**265***
3, *C3*-R161W, *CFB*-K565E	40,967	9,057	**452***	33,689	10,401	**324***
4, None	17,237	4,707	**366***	ND	N/a	N/a
Dense deposit disease						
1	5,531	4,707	118	4,026	4,945	81
2	4,970	4,707	106	ND	N/a	N/a
3	7,543	12,551	60	9,704	5,872	165
4	15,719	12,551	125	7,940	5,872	135
5	9,202	12,551	73	9,390	5,872	160

DEAP, deficiency of plasma proteins and factor H autoantibody positive. FHAA, factor H autoantibody. HUS, hemolytic uremic syndrome. N/a, not applicable. ND, not determined. **P* value <0.05.

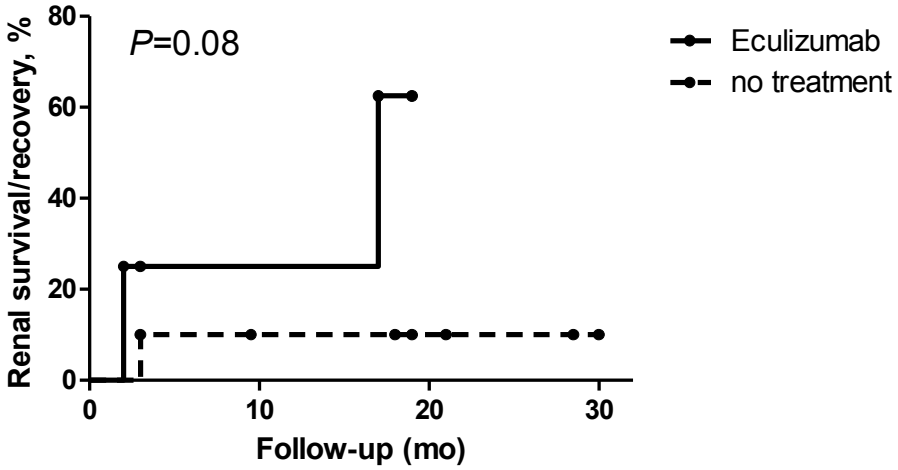
Item S5. Characteristics of the genetic complement defects.

No.	Mutation(s)	Consequence	MAF, %	SIFT	PolyPhen2	Defect
1	None	N/a	N/a	N/a	N/a	N/a
2	*CFI* c.148C>G	P50A	≤0.01	Deleterious	Probably damaging	Quantitative deficiency(1)
	THBD c.1433C>T	T478I	0	Tolerated	Benign	Not characterized
3	None	N/a	N/a	N/a	N/a	N/a
4	None	N/a	N/a	N/a	N/a	N/a
5	None	N/a	N/a	N/a	N/a	N/a
6	None	N/a	N/a	N/a	N/a	N/a
7	None	N/a	N/a	N/a	N/a	N/a
8	*C3* c.481C>T	R161W	0	Deleterious	Probably damaging	Gain of function(2)
9	*CFI* c.452A>G	N151S	<0.01	Deleterious	Probably damaging	Quantitative deficiency(1)
10	None	N/a	N/a	N/a	N/a	N/a
11	*CFH* c.2558G>A	C853Y	0	Deleterious	Probably damaging	Not characterized
12	*C3* c.481C>T	R161W	0	Deleterious	Probably damaging	Gain of function(2)
13	*CD46* c.811_816delGAGACT	ΔD237/S238	0	N/a.	N/a.	Quantitative deficiency(3)
	CFH c.2850G>T	Q950H	0.36 – 0.44	Deleterious	Probably damaging	Functional defect?(4)
14	*C3* c.481C>T	R161W	0	Deleterious	Probably damaging	Gain of function(2)
15	None	N/a	N/a	N/a	N/a	N/a
16	*C3* c.481C>T	R161W	0	Deleterious	Probably damaging	Gain of function(2)
17	None	N/a	N/a	N/a	N/a	N/a

MAF, minor allele frequency according to the Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS/) and Exome Aggregation Consortium databases (ExAC, http://exac.broadinstitute.org/). N/a, not applicable. PolyPhen2, Polymorphism Phenotyping V2. SIFT, Sorting Intolerant From Tolerant.

Item S6. Renal survival. Kaplan Meier regarding the cumulative incidence of renal survival and/or recovery in eculizumab treated (*n*=5) and treatment naive patients (*n*=12).

Mo, months.



References

1. Bienaime F, Dragon-Durey M-A, Regnier CH, Nilsson SC, Kwan WH, Blouin J, et al. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. KidneyInt. 2010;77(4):339-49.
2. Roumenina LT, Frimat M, Miller EC, Provot F, Dragon-Durey MA, Bordereau P, et al. A prevalent C3 mutation in aHUS patients causes a direct C3 convertase gain of function. Blood. 2012;119(18):4182-91.
3. Richards A, Kemp EJ, Liszewski MK, Goodship JA, Lampe AK, Decorte R, 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.
4. Mohlin FC, Nilsson SC, Levart TK, Golubovic E, Rusai K, Muller-Sacherer 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.