

Chronic thrombotic microangiopathy in patients with a C3 gain of function protein

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The syndromes of thrombotic microangiopathy (TMA) are rare and occur in patients with severe endothelial damage caused by various mechanisms [1]. The TMAs converge to a final common pathway, inducing microvascular thrombosis with platelet consumption, haemolysis and ischaemic damage, often affecting the kidneys. Endothelial damage can occur on the background of complement dysregulation as demonstrated in primary atypical haemolytic uraemic syndrome (aHUS) [2]. Most cases of primary aHUS present with acute TMA, while a small subset of patients present with chronic disease. Half the patients have rare variants in complement genes, encoding proteins that either regulate or activate complement and/or autoantibodies that inhibit complement regulation [1]. The genotype–phenotype correlation has clinical significance [3].

The aetiology and disease course of patients with chronic TMA remain poorly understood [3]. Smith-Jackson *et al.* [4] demonstrated that a gain of function change in C3 (i.e. p.D1115N) drives murine TMA with heavy proteinuria and chronic rather than acute TMA on kidney biopsy. The arginine to tryptophan substitution at amino acid 161 (i.e. p.R161W) in C3 has been identified in 8 (29%) of 28 patients with primary aHUS in the Limburg Renal Registry (Supplementary data, Figure S1) but not in 3 asymptomatic relatives; the variant's minor allele frequency is <0.004% according to the Genome Aggregation Database and Exome Variant Server. C3 p.R161W results in a gain of function protein and has been linked to nephrotic-range proteinuria in more than half the patients [5], suggesting chronic damage to podocytes. *In vivo* and clinical observations therefore suggest that C3 gain of functional proteins may cause chronic TMA. Human morphological data, however, are not available.

Herein we evaluated this premise in seven patients with primary aHUS and C3 p.R161W included in the Limburg Renal Registry; one patient was excluded because no kidney tissue

sections were available [6, 7]. No rare variants in *CFH*, *CFI*, *CD46*, *CFB*, *THBD* and *DGKE* were found using DNA sequencing (Supplementary data, Methods S1). *CFH*-H3 but not *MCP*_{GGAAC} was found in 3 (43%) patients. The homozygous deletion of *CFHR1*–*CFHR3* was identified in one patient with no factor H autoantibodies. We therefore analysed the effect of C3 p.R161W in isolation. Our observations add to the understanding of the aetiology and disease course of patients with chronic TMA.

Baseline characteristics and outcome data have been depicted in Table 1, corroborating previous observations from the French cohort [5]. Patients presented with proteinuric kidney failure (mean serum creatinine 11.4 ± 5.4 mg/dL), either with nephrotic-range proteinuria ($n = 4$) or not. Normal platelet counts were found in 5 (71%) patients. C3 but not C4 levels were low in 6 (86%) patients. Kidney tissue sections showed double contour formation of the glomerular basement membrane, mesangiolytic and global foot process effacement (FPE), either with fibrin thrombi ($n = 5$) or not (Supplementary data, Figure S2). Moderate to severe interstitial fibrosis and tubular atrophy was present. These morphologic features thus suggest a smouldering rather than acute onset of disease. C3c but not immune complex deposits were found along segments of the glomerular basement membrane on immunofluorescence microscopy ($n/N = 5/7$; Supplementary data, Figure S2). Electron microscopy showed subendothelial electron-lucent material but not electron-dense deposits ($n/N = 4/4$; Supplementary data, Figure S2), excluding C3 glomerulopathy [8]; global FPE was found in three cases. Two patients presented with extrarenal manifestations. Patient 1 had seizures and left ventricular hypertrophy and Patient 5 had dilated cardiomyopathy at presentation.

Plasma exchange was started in five patients. Plasma exchange was associated with a complete clinical remission for at

Table 1. Patients' characteristics and clinical outcome

Patient no.	1	2	3	4	5	6	7
Year of presentation	1986	2003	2003	2005	2007	2009	2015
Sex/age (years)	M/39	F/32	F/18	F/38	M/37	M/20	F/28
Precipitant(s)	HE	P	None	HE	HE	None	HE
Platelets (g/L)	179	212	20	228	100	345	228
MAHA	-	+	+	+	+	+	-
LDH (U/L)	680	4,106	1,000	1,800	2,125	1,251	298
Creatinine (mg/dL)	12.3	15.7	10.0	19.6	6.6	3.2	12.0
Proteinuria (g/day)	Oliguria	>3.5	0.4	Anuria	6.4	4.5	5.5
C3 (g/L)	0.74	0.69	0.72	0.69	0.88	0.72	0.72
CFH-H3/MCP _{GGAAC}	+/-	-/-	+/-	-/-	+/-	-/-	-/-
Kidney biopsy							
Pattern on light microscopy	CresGN, ischaemia	DC, thrombi	DC, thrombi	DC, thrombi	DC, mes., thrombi	DC, mes.	DC, mes., thrombi
Electron lucent material	+	ND	ND	ND	+	+	+
FPE (%)	80	ND	ND	ND	>50	80	40
Neurologic disease	+	-	-	-	-	-	-
Cardiac disease	+	-	-	-	+	-	-
Plasma exchange	-	+	+	+	+	+	+
Follow-up (years)	30.6	15.4	16.0	11.4	11.8	3.0	4.6
Outcome	ESKD, deceased	ESKD	ESKD	ESKD	ESKD	CKD G1	ESKD
Donor kidney(s)	3	1	2	2	1	0	1
Graft failure (aHUS)	3 (2)	1 (1)	2 (1)	2 (2)	1 (1)	N/A	0

CresGN, crescentic glomerulonephritis; DC, double contour formation of the glomerular basement membrane; F, female; HE, hypertensive emergency; M, male; MAHA, microangiopathic haemolytic anaemia; mes., mesangiolytic; N/A, not applicable; ND, not determined; P, pregnancy; +, positive; -, negative.

least 3 years (i.e. chronic kidney disease Stage G1) in Patient 6. The other patients [$n = 6$ (86%)] required dialysis and did not recover kidney function.

C3 p.R161W has been linked to a high risk of recurrent primary aHUS [3]. Six patients received a total of 10 kidney donors. Eight of 10 kidney donors were transplanted before eculizumab's approval by the European Medicines Agency. Both other recipients (Patients 1 and 7) were transplanted with a transplantation protocol not using eculizumab prophylaxis [9]. Five (83%) of six recipients developed TMA on allograft biopsy (episodes, $n = 8$) with normal platelet counts in 5 (63%) episodes (Supplementary data, Figure S3 and Table S1). Four episodes presented early, that is, <12 months after kidney transplantation, with microangiopathic haemolysis ($n = 4$), low platelet counts ($n = 2$) and acute TMA on donor kidney biopsy. Remarkably, four episodes presented 'late' with nephrotic-range proteinuria, normal platelet counts and morphologic features consistent with chronic TMA and C3c deposits ($n/N = 3/4$), identical to native kidney biopsies; thrombosis was not found. Neither donor-specific alloantibodies nor C4d deposits were present along the peritubular capillaries and thus chronic transplant glomerulopathy was considered unlikely.

In all cases, including Patient 5 who had been treated with C5 inhibition, that is, eculizumab, graft loss developed. However, patient 5 presented with a creatinine of 5.5 mg/dL.

In the current study we demonstrated that primary aHUS linked to C3 p.R161W can present with chronic morphologic features of TMA, characterized by nephrotic-range proteinuria, normal platelet counts and a poor prognosis. The clinical and pathological observations on kidney biopsy are consistent with the murine data [4]. The affinity of C3 p.R161W for CD46 (i.e. membrane cofactor protein) is decreased [5, 10], identical to C3 p.D1115N [11]. This might explain the chronic morphologic

features of TMA, identical to some patients with pathogenic variants in CD46 [12]. Also, cardiac and neurologic manifestations appeared prevalent in patients carrying C3 p.R161W [5].

C5 inhibition rescued affected mice despite the presence of chronic TMA [4]. In clinical practice, the use of therapeutic complement inhibition for the treatment of chronic TMA is debatable [3]. Patient 5, who relapsed 'late' after kidney transplantation, progressed to graft loss despite eculizumab. The donor kidney's capacity to recover is limited as compared with native kidneys [13]. Early recognition is therefore of utmost importance. Proteinuria >1 g/day, although aspecific, may be a marker of smouldering disease after kidney transplantation.

In conclusion, C3 p.R161W and probably other C3 gain of function proteins may present with proteinuria related to chronic TMA, often with normal platelet counts. Recognition of these patients at an early stage of disease may improve the prognosis, and particularly kidney survival, in the era of therapeutic complement inhibition.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt online](http://ndt.online).

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AUTHORS' CONTRIBUTIONS

S.T. and P.P. contributed to the research idea and study design and analysed and interpreted the data. S.T. and M.A.H.

were involved in data acquisition. P.P. supervised the research. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for his/her own contributions and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work appropriately investigated and resolved.

CONFLICT OF INTEREST STATEMENT

The results presented in this article have not been published previously in whole or part.

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