Life-threatening atypical haemolytic uremic syndrome and cardiomyopathy related to novel CFHR3 mutation in a patient with lupus.

Prianka Puri[1*], Lavinia Petrescu[2], Matthew Cook[2,3], Mitali Fadia[1,4], Krishna Karpe[3], Simon Jiang[1,3]

[1]Department of Nephrology, The Canberra Hospital, Canberra, Australia
[2]Department of Immunology, The Canberra Hospital, Canberra, Australia
[3]Centre for Personalised Immunology, Australian National University, Canberra, Australia
[4]Department of Anatomical and Histopathology, The Canberra Hospital, ACT, Australia

Abstract

A 50-year-old Bangladeshi female with a background of Lupus, presented with biopsy proven thrombotic microangiopathy. She underwent empiric plasma exchange with limited efficacy, subsequently her ADAMTS-13 activity retuned to be normal excluding thrombotic thrombocytopenic purpura. She was then treated with prednisolone, and Rituximab for a possible Lupus flare, with minimal response. Due to ongoing haemolysis and progressive kidney injury a diagnosis of atypical haemolytic uremic syndrome (aHUS) was made and Eculizumab therapy commenced. During her admission she developed new onset non-ischemic cardiomyopathy. It was thought her cardiomyopathy was related to her aHUS, and she underwent immunophenotyping and whole exome. This identified a novel homozygous missense mutation in CFHR3 (Ch1:196748315 A/G). Peripheral flow cytometry displayed an expansion in plasma blast and anergic B cells suggesting her immunophenotype displayed a significant antibody response. After 12-months of Eculizumab treatment it was noted her haematological parameters normalized, and a subsequent echocardiogram showed resolution of her heart dysfunction, coinciding with the resolution of her aHUS.

Conclusion: This case highlights a novel CFHR3 mutation that may confer risk for developing aHUS and related cardiomyopathy. There is future consideration for familial genetic screening for the patient's offspring, given disease severity.

Keywords: A typical Haemolytic Uremic Syndrome (aHUS), Thrombotic microangiopathy, Eculizumab, Complement Factor (CFH).

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Background

Atypical haemolytic uremic syndrome (aHUS) is a form of Thrombotic Microangiopathy (TMA) characterised by abnormal alternate complement pathway activation [1,2]. It can arise as a result of variation in complement regulating genes or from auto antibodies that impede complement regulation [1,3]. To date over190 gene variants have been identified in complement factor-H (CFH) and CFH-related genes contributing to complement mediated diseases [4]. aHUS associated with these variants is thought to have poor prognosis and high relapse rates [1,5]. CFH regulates the alternative complement pathway by C3 convertase dissociation, uncoupling C3b from complement factor I (CFI) [1,6]. This process is mediated by the N-terminus of CFH [1]. The C-terminus of CFH contains vital binding sites for C3b and endothelial surfaces, which allows CFH to bind and exert its regulatory function. CFH, CFHR3 and CFHR5 are members of the human complement Factor H gene cluster located at 1q31.3 (GRCh370) [4].

Manifestations of aHUS include renal, haematological and less commonly cardiac [1,2]. Cardiac involvement is reported in up to 9.5% of patients displaying cardiac dysfunction and is associated with high mortality rates up to 25% of patients with cardiac aHUS [7-9]. Cardiac manifestations are often delayed from time of presentation and can occur months after a diagnosis of aHUS due to the cumulative effect of complement over activation [7].

In this case we describe a patient with complicated aHUS resulting in End-Stage Renal Failure (ESRF) with possible cardiac involvement from her disease. She has an associated a novel homozygous CFHR3 Single Nucleotide Variant (SNV) which we believe is pathogenic.

Case Report

A52-year-old female of Bangladeshi ethnicity presents with acute kidney injury, anaemia and thrombocytopaenia on a background of a 15-year history of Systemic Lupus Erythematosus (SLE), complicated by musculoskeletal and cutaneous involvement. The patient was consistently serologically active with elevated titreds DNAs, persistently suppressed C3/C4 levels, with normal renal function and no evidence if lupus nephritis Table 1. Disease activity was controlled with hydroxychloroquine, azathioprine and sulfasalazine.

She was admitted after a 2-month history of reduced mobility, weight loss, and anorexia. During the admission she developed an acute kidney injury and became progressively anaemic and thrombocytopenic. Investigations revealed marked schistocytes

Table 1. *Serum and urine investigations over the course of the patient's aHUS.*

Admission	Jan-18	16-May-18	22-Jun-18	31-Dec-18	31-Jan-19	24-Apr-19	04-Jun-19	31-Aug-19
Presenting complaint	**Baseline**	**Left flank pain, haematuria, nausea and Vomiting**	**Post Eculizumab for aHUS requiring RRT**	**Vomiting, diagnosed with intracranial toxo-plasmosis**	**Fluid overload remaining on dialysis**	**Prior to cessation of eculizumab. remaining on dialysis**	**Post cessation of eculizumab. remaining on dialysis**	**Outpatient follow up. remaining on dialysis**
Bloods (units, normal range)								
Haematology								
Hb (g/L, 115-160)	102	87	91	118	90	104	113	102
Platelets (× 10^9/L,150-400)	150	79	34	92	107	187	143	190
WCC (× 10^9/L,4.0-11.0)	3.7	9	5.3	1.2	6.9	5.8	4.2	12
Reticulocytecount (%, 0.5-2.0)	0.9		4.19	0.97				3.39
ESR (mm/Hr, 1-30)	52		17		46			67
Blood film	Tear drop, microcytosis, schistocytes	Hyper-segmented neutrophils, anisocytosis	Anisocytosis, schistocytes	WCC dysplastic features, schistocytes	Aniso-cytosis, schistocytes	Anisocytosis	Normal morphology	Normal morphology, mild Anisocytosis
Chemistry								
Creatinine (umol/L, 45-90)	160	337	402	165	206	356	281	326
Est. GFR (>90)	32	13	25	31	24	12	16	13
Ferritin (ug/L, 138-652)	574	657				5471		551
Transferrin sat (%, 18-46)	20	15				21		19
CRP (mg/L, <6)	0.7	9	7	3.9	1.4	8.2	0.8	12.3
Synthetic Liver Function								
Albumin (g/L, 33-50)	43	34	25	40	33	38	35	35
Globulin (g/L, 10-42)	32	20	17	33	26	25	23	25
ALT (U/L, <33)	10	38	43	19	16	200	51	16
Bilirubin (umol/L, 2-20)	9	12		6	6	11	7	6
Haemolytic Panel								
Haptoglobin (g/L, 0.3-2.0)	1	<0.1	<0.1	0.4	<0.1	<0.1	0.2	1.2
LDH (U/L, 120-250)	274	540	1025	629	609	606	216	157
D-dimer (mg/L 0.00-0.50)				1.5	0.84			
Fibrinogen (g/L, 1.5-4.0)								
Direct Coomb'sTest			Negative		Negative		Negative	
Autoimmune								
dsDNA (IU/mL, <35)	66	<10	27	31	<10	12	<10	<10
C3 (g/L, 0.75-1.61)	0.5	0.49	0.46	1.07	0.77	0.51	0.85	0.91
C4 (g/L, 0.13-0.40)	0.16	0.22	0.09	0.56	0.44	0.28	0.34	0.42
CH50 (U/mL, >520)						<300		
Protein/Creatinine Ratio (mg/ mmoL,<25 mg/mmoL)	21	886	559	461	741	149		

on blood film, undetectable haptoglobins, increased lactate dehydrogenase (LDH) but negative Direct Coomb's Test (DCT). Her base line immunosuppression was ceased, and she was empirically treated for thrombotic Thrombocytopenic Purpura (TTP) with Plasma Exchange (PLEX), with no response. Her ADAMTS-13 level retuned within normal range, and PLEX was ceased, she was trialled on Rituximab and prednisolone however her Microangiopathic Haemolytic Anaemia (MAHA) persisted Table 1. The patient underwent a renal biopsy demonstrating TMA without evidence of proliferative lupus nephritis Figure 1A-1C.

Her stool Shiga toxin was negative, and a subsequent diagnosis of aHUS was made. She was given the quadrivalent and sero-group B meningococcal vaccinations and commenced on Eculizumab (900 mg), along with ongoing oral prednisolone.

Over the course of the next few months the patient remained on fortnightly Eculizumab infusions (1200 mg) and developed multiple infective complications of immunosuppression requiring intensive care admissions. Her renal function further declined,and she became haemodialysis dependant. A Trans-Thoracic Echocardiogram (TTE) at this time demonstrated normal Left Ventricular (LV) function (Figure 2A).

She underwent a repeat renal biopsy which showed no lupus nephritis, however demonstrated chronic TMA and fibrosis Figure 3.

Six months into admission the patient developed pulmonary oedema despite two months of stable dialysis. A repeat echocardiogram showed a dilated LV, moderate to severe LV hypertrophy and LV dysfunction Figure 2B. Her coronary angiogram showed no evidence of coronary artery disease, and her cardiomyopathy was attributed to aHUS microvascular changes. She subsequently underwent flow cytometric immunophenotyping and whole exome sequencing [10]. This identified a novel homozygous missense SNV in CFHR3 (Ch1:196748315 A/G) resulting in a change from an isoleucine to a valine at amino acid position 28. This residue resides within the anchoring domain essential to the alternative complement regulatory functions of CFHR3. The SNV was predicted to be damaging with a Combined Annotation Dependant Depletion (CADD) score of 13.95 and a Polyphen-2 score of 0.96.

Peripheral flow cytometry prior to treatment displayed a reduction in naive CD4+ T-cells, increased CD4+ T-effector

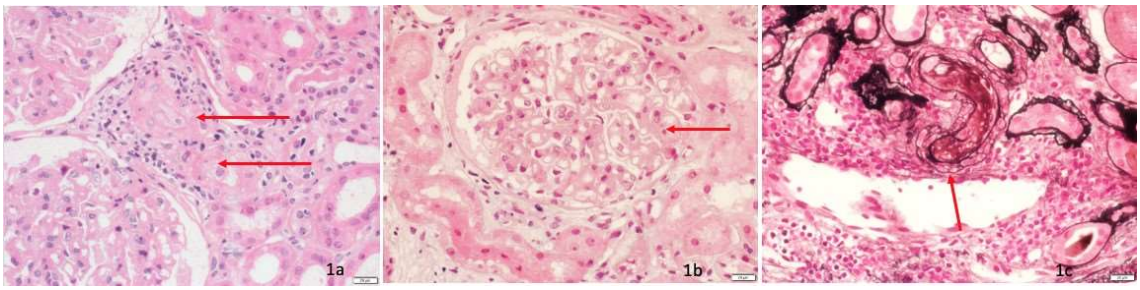

Figure 1. *Light Microscopy of a renal biopsy undertaken on the 31.8.18, showing no active lupus nephritis but ongngooing thrombotic microangiopathy, no other causes of acute kidney injury identified. 1a) Interlobar artery showing mucoid intima and narrowed lumen; 1b) Glomerular capillary loops show thick loops on H and E and double contountours; 1c) Artery showing luminal thrombus, consistent with thrombotic microangiopathy, using Periodic Schiff-Methenamine (PAS-M) stain.*

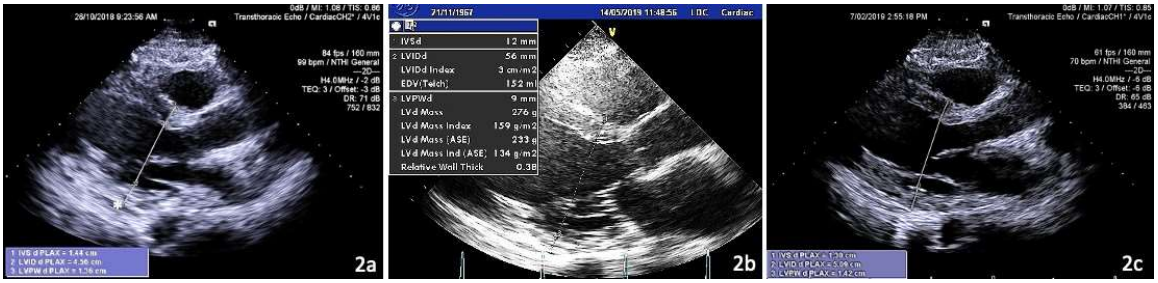

Figure 2. *Showing serial transthoracic echocardiograms of the patient over time. 2a) Baseline transthoracic echocardiogram, normal left ventricular size (LV diastolic diameter 3.8 cm-5.2 cmm), and moderate to severe increase in left ventricular mass (Intetterventricularseptal thickness 0.6 cm-0.9 cm).*Small pericardial effusion (June 2018); 2b) Presentation of symptomatic heart failure. Midly dilated left ventricular size and severe global left ventricular dysfunction, mild id increase in left ventricular mass (December 2019); 2c) Outpatiieent study. Left ventricular size returning to within normal limits, normal left ventricicular function, increase in left ventricular mass (October 2019)*

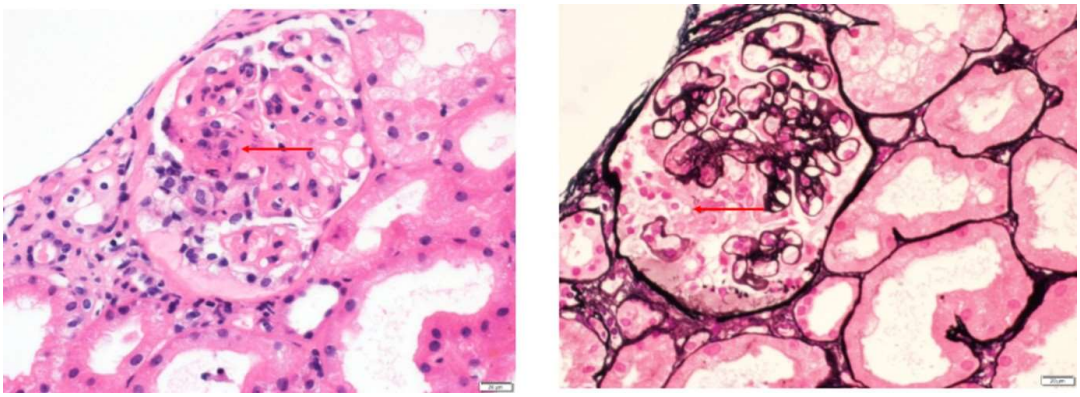

Figure 3. *Light Microscopy of a renal biopsy undertaken on the 30.4.19, whilst the patient had commended Eculizumab therapy: Glomeruli show focal segmental endothelial proliferation, nuclear debris and mesangiolysis. No active thrombotic microangiopathy or lupus nephritis.*

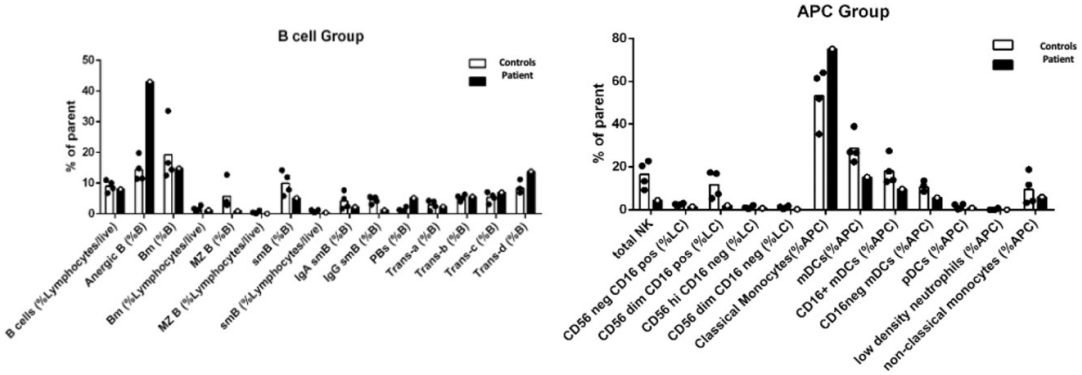

Figure 4. *Immunophenotype assays of peripheral blood, for patient and healthy contro; B Cell subsets and total percentage of all Antigen Presenting Cells (APC). Assay undertaken prior to Eculizumab therapy.*

memory cells and T-regulatory cells compared to healthy controls (HC) Figure 4. The associated expansion of her T effector cells, the patient's plasmablasts and anergic B cells suggested her immunophenotype displayed a significant antibody response in the absences of T-cell interaction Figure 4.

After 12-months of Eculizumab treatment the patient went into remission, with resolution of her MAHA. Her renal function did not recover, with repeat kidney biopsy demonstrating extensive fibrosis and glomerular obsolescence. A subsequent TTE showed resolution of her heart dysfunction, which coincided with resolution of her aHUS (Figure 2C).

Discussion

The SNV identified in our patient affects C-terminus of CFHR3 which is important for protein anchoringto cell surfacesand subsequent C3b convertase inhibition [1,4]. Heterozygous CFHR3 SNVs can be dominant negatives, dimerising with, and impairing wildtype CFHR3-mediated complement regulation [2,3]. Homozygous CFHR3 mutations occur less commonly, detected in only 15%-20% of aHUS patients, these patients have a quantitative reduction in CFHR3 levels and persistently low C3 titres [2]. Despite Eculizumabtherapy there is an elevated risk of disease relapse in the presence of an identifiable pathogenic mutations [2,11]. Prognosis post-transplant is poor in patients with CFH mutations with disease reoccurrence reported in up to 90%of patients, and graft failure being greater than 50% [1,5].

Cardiac manifestations of aHUS are less common and reported in up to 10% of patients with aHUS [3]. CFHR mutations have been detected in a portion of patients with cardiac aHUS, both in case series and individual case studies [3,12,13]. Presentation of cardiac disease has been reported in paediatric cases, with acute myocardialinfarction and arrhythmias carrying the highest morality risk from this group of patients [14]. Of note CFHR mutations have been suggested to confer greatest cardiovascular risk, and patients with CHFR mutations also showed the highest rate of progression to ESRF in the group [2,14].

In our case study we note that 12-months of treatment with Eculizumab improved cardiac function and haematological parameters. Unfortunately, she became dialysis dependent due to significant renal damage which occurred from her aHUS. We hypothesized her non-ischemic cardiomyopathy maybe related to her aHUS, and as we observed resolution of her disease, we noted improvement in her cardiac function [9]. Since the discontinuation of Eculizumab her haemoglobin and blood counts remain stable Table 1, her repeat echocardiogram performed three months after cessation revealed stable cardiac function Figure 2C.

Conclusion

This case highlights a novel mutation in CFHR3 (Ch1:196748315 A/G), our patient had significant renal involvement and what may appear to have been cardiac involvement from her aHUS. In the absence of a cardiac biopsy this case remain hypothesis generating, however a case series carrying the same mutation may assist in proving causality. There is a consideration for family genetic screening given the patient has children and displayed severe disease phenotype.

References

1. Noris M, Remuzzi G. Atypical hemolyticuremic syndrome. N Engl J Med. 2009;361-17:1676-87.
2. Noris M, Caprioli J, Bresin E, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5(10):1844-59.
3. Abarrategui-Garrido C, Martínez-Barricarte R, López-Trascasa M, et al. Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. Blood. 2009;114-19:4261-71.
4. Ding Y, Zhao W, Zhang T, et al. A haplotype in CFH family genes confers high risk of rare glomerular nephropathies. Sci Rep. 2017;7:6004.
5. Grenda R, Jarmużek W, Rubik J, et al. Favorable four-yr outcome after renal transplantation in a patient with complement factor H antibody and CFHR1/CFHR3 gene mutation-associated HUS. Pediatr Transplant. 2015.
6. Legendre CM, Licht C, Muus P, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368:2169-81.
7. Hofer J, Rosales A, Fischer C, et al. Extra-renal manifestations of complement-mediated thrombotic microangiopathies. Front Pediatr. 2014.
8. Vilalta R, Lara E, Madrid A, et al. Long-term eculizumab improves clinical outcomes in atypical hemolytic uremic syndrome. Pediatr Nephrol Berl Ger. 2012;27(12):2323-26.
9. Sallee M, Daniel L, Piercecchi MD, et al. Myocardial infarction is a complication of factor H-associated atypical HUS. Nephrol Dial Transplant. 2010;25(6):2028-32.
10. Ellyard JI, Tunningley R, Lorenzo AM, et al. Non-parametric heat map representation of flow cytometry data: Identifying cellular changes associated with genetic immunodeficiency disorders. Front Immunol. 2019.
11. Iatropoulos P, Noris M, Mele C, et al. Complement gene variants determine the risk of immunoglobulin-associated MPGN and C3 glomerulopathy and predict long-term renal outcome. MolImmunol. 2016;71:131-42.
12. Noris M, Remuzzi G. Cardiovascular complications in atypical haemolytic uraemic syndrome. Nat Rev Nephrol. 2014;10(3):174-80.
13. Gami AS, Hayman SR, Grande JP, et al. Incidence and prognosis of acute heart failure in the thrombotic microangiopathies. Am J Med. 2005;1(18)5:544-47.
14. Zhang T, Lu J, Liang S, et al. Comprehensive analysis of complement genes in patients with atypical hemolytic uremic syndrome. Am J Nephrol. 2016;43(3):160-69.

***Correspondence to:**
Prianka Puri
Department of Nephrology
The Canberra Hospital
Australia
E-mail: pp473@uowmail.edu.au