Atypical haemolytic-uraemic syndrome due to heterozygous mutations of CFH/CFHR1-3 and complement factor H 479

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Introduction

Atypical haemolytic-uraemic syndrome (aHUS) is characterised by microangiopathic haemolytic anaemia, thrombocytopenia and renal failure in the absence of shigatoxin[1]. aHUS is rare, accounting for approximately 10% of all cases of HUS, and has a poor prognosis[2]. aHUS can occur at any age and may be sporadic or familial. It is now known that aHUS is due to the loss of complement regulation resulting from mutations in the complement regulatory proteins complement factor H (CFH), factor I, complement factor H related (CFHR)1-3, CFHR-5, CD46 membrane cofactor protein (MCP), thrombomodulin (THBD) or complement activators such as factor B, and complement 3 (C3)[2,3]. The presence of a mutation, or variant (single nucleotide polymorphisms and haplotype blocks), and a triggering event increasing complement activation may be necessary for manifestation of the disease[4,5]. In this paper we report the onset of aHUS in association with heterozygous deletion of CFHR1 and a mutation in *CFH*, c.497G>T (p.Arg166Leu) that has not previously been reported with aHUS. We also report this patient's response to complement inhibition with eculizumab and the effect of the patient's subsequent pregnancy.

Case report

The patient is a 20-year old Caucasian woman with an unremarkable past medical history except for a spontaneous foetal loss 3 months prior to presentation. She presented with complaints of abdominal pain and bloody diarrhoea 3 days after eating raw fish. Within 4 days of hospitalisation, the patient developed overt microangiopathic haemolytic anaemia, thrombocytopenia and renal failure requiring dialysis. The ADAMTS13 activity level was 40% and she was negative for shigatoxin. A diagnosis of thrombotic thrombocytopenic purpura/aHUS was made. The patient was treated with dialysis for several weeks and daily, 1.5 plasma volume plasma exchange. Because of persistent thrombocytopenia, microangiopathic haemolytic anaemia, and renal insufficiency, the frequency of the plasma exchange was increased to twice daily. The patient developed seizures, lethargy, and fever and was treated with high-dose corticosteroids. After 6 weeks of plasma exchange, she was transferred to the University of Southern California (USC) - Los Angeles County Medical Center. Plasma exchange was discontinued and therapy was initiated with eculizumab, on a compassionate use protocol approved by the USC Investigational Review Board. She received eculizumab 900 mg weekly for 4 weeks followed by 1,200 mg every 2 weeks. The patient developed a small intracranial haemorrhage 4 days after starting eculizumab, when her platelet count dropped to 24×10^9/L. The patient was given a transfusion of platelets, fresh-frozen plasma and an additional 600 mg of eculizumab, with improvement of her symptoms. The patient responded slowly to treatment. Eculizumab trough values were sub-therapeutic (52 mg/mL, therapeutic level >99 mg/mL) at week 4. In week 5 she was switched to a maintenance dose of 1,200 mg (given every 2 weeks), which resulted in a rapid improvement of her platelet count, lactate dehydrogenase level, mental status and renal function (Table I). Genetic tests evaluating factor H, factor I, membrane cofactor protein (MCP), thrombomodulin, and factor B failed to identify any mutations previously associated with aHUS and no antibodies to factor H were found. However, the patient was found to be hemizygous for CFHR1-3, as well as having a point mutation in short consensus repeat (SCR) 3 of CFH, 497 c.497G>T. The patient continued eculizumab for 9 months then decided to discontinue treatment. She became pregnant shortly afterwards. The pregnancy was complicated by severe hypertension and proteinuria of 3 g at week 34. Delivery was induced shortly thereafter. The patient's platelet count was 148×10^9/L, haemoglobin concentration 12 g/dL, lactate dehydrogenase level 190 IU, and creatinine concentration 0.8 mg/dL. A healthy baby, weighing 2,583 g, was delivered. Following delivery, the proteinuria and hypertension improved, although the patient remains on one antihypertensive medication at this time. Both mother and child are doing well.

Discussion

The patient presented with an acute onset of non-shigatoxin related abdominal pain and diarrhoea and rapidly developed fulminant aHUS. The patient failed to respond to 6 weeks of intense plasma exchange.

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Table I - Summary of interventions and response.

	7/4	7/9	7/16	8/1	8/15	8/22	8/29	9/5	9/12	9/19	9/26	10/10
Intervention	Fluid	PE 1.5 PV D Tx	PE 1.5 PV bid Tx	PE + HD Solu-medol	DC PE Ecu 900 mg weekly	Ecu 900 mg Tx plts/BC	Ecu 900 mg	Ecu 900 mg	Ecu 1,200 mg		Ecu 1,200 mg	Ecu 1,200 mg
Creatinine (mg/dL)	0.75	4.0			2.1		1.5		1.0		1.2	1.0
Hb (mg/dL)	14.4	8.8	6.0		7.5	6.9	7.9	8.0	8.8	8.8	9.0	9.4
Platelets ($\times 10^9$/L)	267	44	50	50-70	70	24-50	60	80	84	134	284	214
Retic (%)		4%			3%							
Smear f/HPF		5-6	5-6		5-6	3	2-3	2-3	2-3		<1	<1
LDH		2,330	800	700	700	595	700	600	600	434	239	178

PE: plasma exchange; PV: plasma volume; D: dialysis; HD: high-dose; DC: discontinued; Ecu: eculizumab; Tx: transfusion; plts: platelets; RBC: red blood cells; f/HPF: fragments/high power field; Retic: reticulocytes; LDH: lactate dehydrogenase.

Mutation analysis revealed that she was a heterozygous for CFHR1-3. CFHR1 has binding sites for C3b, C3d, C5 and C5b6 and competes with factor H for binding to C3b inhibiting C5 convertase activity, and interferes with C5b surface deposition and formation of the terminal complement complex (TCC)[6]. CFHR3 inhibits C3 convertase binding C3b and C3d[6,7]. Homozygous deficiency is frequently associated with factor H auto-antibodies, which our patient did not have[8]. It occurs with a frequency of 3% in the normal population but 16% in the aHUS population[8]. Previously reported registry studies have shown that CFHR1-3 is highly responsive to plasma exchange in 70-80% of patients[9]. The fact that our patient had such a poor response to plasma exchange suggested that she might have additional mutations.

Further studies revealed a novel point mutation in the N-terminus of CFH, 497 c.497G>T. This variant is not reported in the NHLBI Exome Sequencing Project or the 1000 Genomes Project. The majority of the aHUS-associated factor H mutations cluster within the carboxy-terminal SCR domains of the protein[10-13], are frequently associated with normal C3 and CFH levels, and result in defective binding of CFH to heparin, C3b, and endothelium[13]. The N-terminal of CFH, where the patient's mutation is located, acts as a cofactor for factor I-mediated proteolytic inactivation of C3b, and competes with factor B for C3b binding, therefore increasing the rate of dissociation of the alternative pathway convertase C3bBb[12,13]. We postulate that the onset of the disorder in our patient and the poor response to plasma exchange were the results of the compounding effects of the two mutations, with a reduction in effective complement H levels as well as reduced C3b binding. However, if a non-functional factor H and poor C3b binding were the sole mechanisms in our patient, we might have expected an improvement with plasma exchange. Our patient's poor response to plasma exchange suggests that she may have additional mutations which we have not yet been able to identify. Further analysis to identify additional mutations is ongoing.

The role of complement dysregulation in the development of aHUS has led to the use of the complement inhibitor, eculizumab, in this disease. Two completed trials have demonstrated significant, rapid reduction of thrombotic microangiopathy, recovery of platelet counts, elimination of the need for plasma exchange/plasma infusion and sustained overall improvement in patients with aHUS both in the acute setting and in those who have been on long term plasma exchange/plasma infusion[13,14]. Our patient's slow response to eculizumab may have been due to third spacing of the drug secondary to hypoalbuminaemia with anasarca, documented by sub-therapeutic eculizumab levels in week 4. When the dose was increased to 1,200 mg (every other week), the patient rapidly improved, with resolution of all the aHUS signs and symptoms. It may be important to measure trough levels of eculizumab in patients not responding appropriately to treatment with this antibody, since values below the therapeutic level would suggest the need to increase the dose. Although we recommended that the patient continued eculizumab treatment, she decided to stop eculizumab after 9 months of therapy. Shortly afterwards, she became pregnant. She did well until the third trimester when she developed significant hypertension and proteinuria. Pregnancy has been associated with increased complement activation[15]. Mutations in complement regulatory proteins have been linked to pre-eclampsia/eclampsia, and the HELLP syndrome[15]. While she did not have overt thrombotic microangiopathy at the time she presented, the emergent delivery may have reduced the risk of developing the full blown syndrome. Given the previous episode of aHUS, the development of pre-eclampsia suggests that this patient's mutations are clinically significant.

Keywords: aHUS, complement factor H, plasma exchange, eculizumab, pre-eclampsia.

Contributions

Ilene C. Weitz: data collection, manuscript revisions and manuscript review; Preeti Chaudhary: data collection, first draft of the manuscript and manuscript review; Mehmet Hepgur: data collection and manuscript review; Sarmen Sarkissian: data collection and manuscript review; Richard J.H. Smith: mutation analysis, manuscript revision and manuscript review.

Conflict of interest disclosure

Ilene C. Weitz, Speakers' Bureau and Consultant for Alexion Pharmaceuticals; Preeti Chaudhary, Mehmet Hepgur, Sarmen Sarkissian and Richard J.H. Smith have no conflicts of interest to declare.

References

1) Taylor CM, Chua C, Howie AJ, Risdon RA. Clinico-pathological findings in diarrhea-negative haemolytic uraemic syndrome. Pedriatr Nephrol 2004; **19**: 419-25.
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**: 1844-59.
3) Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatr Nephrol 2010; **25**: 2431-42.
4) Warwicker P, Goodship TH, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int 1998; **53**: 836-44.
5) Rodríguez de Cordoba S. aHUS: a disorder with many risk factors. Blood 2010; **115**: 158-60.
6) Heinen S, Hartmann A, Lauer N, et al. Factor H related protein 1 (CFHR-1) inhibits complement C5 convertase activity and terminal complex formation. Blood 2009; **114**: 2439-47.
7) Zipfel PF, Edey M, Heinen S, et al. Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. PLoS Genet 2007; **3**: e41.
8) Jozsi M, Licht C, Strobel S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood 2008; **111**: 1512-4.
9) Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med 2009; **361**: 1676-87.
10) Manuelian T, Hellwage J, Meri S, et al. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. J Clin Invest 2003; **111**: 1181-90.
11) Pechtl IC, Kavanagh D, Mcintosh N, et al. Disease-associated N-terminal complement factor H mutations perturb cofactor and decay-accelerating activities. J Biol Chem 2011; **286**: 11082-90.
12) Hocking HG, Herbert AP, Kavanagh D, et al. Structure of the N-terminal region of complement factor H and conformational implications of disease-linked sequence variations. J Biol Chem 2008; **283**: 9475-87.
13) Licht C, Muus P, Legrende C, et al. Eculizumab is an effective long term treatment in patients with atypical hemolytic uremic syndrome previously receiving plasma exchange/plasma infusion (PE/PI): extension study results. Blood 2011; **118**: abstr 3303.
14) Greenbaum L, Babu S, Furman RK, et al. Eculizumab is an effective, long term treatment in patients with atypical hemolytic uremic syndrome resistant to plasma exchange/plasma infusion (PE/PI): results of an extension study. Blood 2011; **118**: abstr 193.
15) Fakhouri F, Roumenia L, Provot F, et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. J Am Soc Nephrol 2010; **21**: 857-67.

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