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Glucose 6 Phosphate Dehydrogenase Deficiency Mimicking Atypical Hemolytic Uremic Syndrome

Patrick R Walsh MRCPCH¹, Sally Johnson MRCPCH, PhD², Vicky Brocklebank MRCP¹,
Jacobo Salvatore¹, Martin Christian MRCPCH³, David Kavanagh, MRCP, PhD^{1,2}

¹Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom; ²National Renal Complement Therapeutics Centre, Royal Victoria Infirmary, Newcastle, United Kingdom; ³Nottingham Children's Hospital, Queen Medical Centre, Nottingham, United Kingdom.

Correspondence to: David Kavanagh. National Renal Complement Therapeutics Centre, aHUS Service, Building 26, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP.

Telephone: +44 (0) 191 282 5094; Fax: +44 (0) 191 282 0798; Email:

david.kavanagh@ncl.ac.uk

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Abstract

A four-year-old boy presented with non-immune hemolysis, thrombocytopenia and acute kidney injury. Investigations for an underlying etiology failed to identify a definitive cause and a putative diagnosis of complement-mediated atypical hemolytic uremic syndrome (aHUS) was made. The patient was commenced initially on plasma exchange and subsequently eculizumab, after which his renal function rapidly improved. Whilst on eculizumab, despite adequate complement blockade, he represented twice with hemolytic anemia and thrombocytopenia, but without renal involvement. Genetic analysis did not uncover a mutation in any known aHUS gene (*CFH*, *CFI*, *CFB*, *C3*, *CD46*, *THBD*, *INF2* and *DGKE*) and anti-factor H antibodies were negative. Whole exome sequencing was undertaken to identify a cause for the eculizumab resistance, this revealed a pathogenic variant in *G6PD* (Glucose 6 Phosphate Dehydrogenase); which was confirmed by functional analysis demonstrating decreased erythrocyte G6PD activity levels. Eculizumab was withdrawn. Complement-mediated aHUS is a diagnosis of exclusion and this case highlights the diagnostic difficulty that remains without an immediately available biomarker for confirmation. This case of G6PD deficiency presented with a phenotype clinically indistinguishable from complement-mediated aHUS. We recommend G6PD deficiency is included in the differential diagnosis of patients presenting with aHUS and suggest measuring erythrocyte G6PD levels in these patients.

Keywords: atypical hemolytic uremic syndrome (aHUS), Glucose 6 Phosphate Dehydrogenase (G6PD), eculizumab, chronic non-spherocytic hemolytic anemia (CNSHA)

Introduction

Inherited or acquired dysregulation of the alternative complement pathway have been demonstrated to predispose to many cases of atypical hemolytic uremic syndrome (aHUS)(MIM 235400)¹. This understanding of the pathogenesis of aHUS ultimately resulted in the successful introduction of the complement inhibitor eculizumab into the clinic².

The term primary aHUS has been used when an underlying defect in the complement system is suspected (mutations in *CFH*, *CFI*, *CFB*, *C3*, *CD46*, or anti factor H antibodies) and secondary aHUS where there is another cause (e.g. infection associated, bone marrow transplant associated, drug-induced, cobalamin C disease etc.)³. Complicating such a classification is the finding that, in many cases a trigger (e.g. infection) is required for disease to manifest in those with a complement abnormality¹. No biomarker exists that will confirm the diagnosis of a primary complement mediated aHUS in the acute setting and the diagnosis is therefore one of exclusion. Early initiation of eculizumab has been shown to lead to better outcomes². Thus, treatment with eculizumab is often commenced in patients with suspected primary complement mediated aHUS, and discontinued if an alternative etiology is subsequently identified.

With the increasing use of eculizumab in clinical practice, it has become apparent that there are subgroups of non-complement mediated aHUS that do not respond to eculizumab (e.g. *DGKE*⁴ and *INF2*⁵). Additionally, a rare polymorphism in C5 has been demonstrated to impair eculizumab efficacy⁶.

Here we describe a child presenting with non-immune hemolysis, thrombocytopenia and acute kidney injury (AKI) who relapsed whilst on eculizumab and did not carry genetic risk factors for eculizumab non-response. Using whole exome sequencing we identified a

pathogenic variant in *G6PD* (c.1160G>A, pR387H) that caused a phenotype indistinguishable from aHUS.

Case Presentation

A four-year-old boy presented to his local hospital with coryzal symptoms and macroscopic hematuria, having been treated with trimethoprim for a presumed UTI one week previously. On presentation, he was profoundly anemic (hemoglobin 40g/L), with evidence of non-immune intravascular hemolysis (direct coombs test negative, lactate dehydrogenase (LDH) 1590U/L, undetectable haptoglobins) and acute kidney injury (creatinine 3.3mg/dL) (figure 1), urinalysis demonstrated dipstick positive hemoglobin and numerous red blood cells on microscopy. He required transfusion with packed red blood cells and was transferred to the regional nephrology center. There was continued evidence of hemolysis (maximum LDH 9000U/L), further deterioration of renal function (maximum creatinine 5.4mg/dL with oliguria and fluid overload, necessitating one session of hemodialysis) and thrombocytopenia (platelets $94 \times 10^9/L$). His initial investigations ruled out shiga toxin HUS (negative *E.coli* culture and serology), thrombotic thrombocytopenic purpura (ADAMSTS13, 113%) and other causes of aHUS (normal homocysteine levels, negative anti-nuclear antibodies, negative HIV serology, negative T-antigen, and negative cold agglutinins). Complement levels were normal (C3 1.22mg/L, C4 0.24mg/L)

A presumptive diagnosis of primary complement-mediated aHUS was made and he was commenced on plasma exchange (PEX). Following initiation of PEX his renal function, hemoglobin, LDH, and platelets improved (figure 1). Six weeks following presentation he was commenced on eculizumab (600mg day 1 and 7, 300mg day 9, 300mg every fortnight thereafter) and PEX was discontinued.

Shortly after starting eculizumab, he contracted metapneumovirus and presented with acute deterioration of his hematological parameters (hemoglobin 77g/L, LDH 2248U/L and platelets of $121 \times 10^9/L$) with stable creatinine; his third loading dose of eculizumab was brought forward and this episode settled.

He was maintained on eculizumab for 6 months, with adequate suppression of the alternative pathway (AH50 absent), although there was evidence of ongoing hemolysis (LDH above reference range and persistent anemia).

Screening for complement abnormalities did not reveal any pathogenic variants in aHUS associated genes (*CFH*, *CFI*, *CFB*, *C3*, *CD46*, *THBD*, *INF2* and *DGKE*) or factor H autoantibodies⁷.

Six months after commencing eculizumab, he presented with a lower respiratory tract infection. Despite evidence of effective complement blockade (AH50 absent), he was found to have acute hemolysis (hemoglobin 51g/L and LDH 4890U/L) and thrombocytopenia (platelet $123 \times 10^9/L$), without a change in serum creatinine. This settled spontaneously after treatment with intravenous antibiotics and a single blood transfusion.

Due to eculizumab resistance with chronic hemolysis, we undertook whole exome sequencing (as previously described⁸) and identified a pathogenic variant in *G6PD* (c.1160G>A, pR387H). Functional erythrocyte G6PD activity levels confirmed severely depressed G6PD levels 0.4IU/ 10^{12} erythrocytes (4.5 – 13.5).

Following the decision to discontinue eculizumab, at 3 months the patient had one relapse, again associated with metapneumovirus infection, which required a blood transfusion, but without a change in his renal function or complement levels (C3 0.96mg/L, C4 0.32mg/L).

At last follow-up, 3 years after discontinuation of eculizumab he has not suffered any further acute relapses, although he has persistent chronic hemolysis (raised LDH and decreased hemoglobin) requiring folate supplementation.

Discussion

G6PD deficiency typically presents with hemolysis provoked by infection or drugs without AKI or thrombocytopenia. The presence of these features in this case lead to a delay in the eventual diagnosis.

Glucose 6 Phosphate dehydrogenase deficiency (MIM 134700) is an X-linked recessive condition, which occurs as a result of mutations in *G6PD*⁹. G6PD is ubiquitously expressed¹⁰ and catalyzes the conversion of glucose-6-phosphate to 6-phosphoglucono- δ -lactone generating NADPH, in the first step of the pentose phosphate pathway⁹. This is the sole NADPH source within erythrocytes and is required to maintain the redox state intracellularly and prevent damage from free radical and reactive oxygen species produced during cellular metabolism¹¹.

G6PD deficiency is classified (I to V) depending on enzyme activity¹², with class I variants representing the most severe deficiency (activity <10%). Most individuals with G6PD deficiency remain asymptomatic but are susceptible to acute hemolytic crisis, due to oxidative damage, precipitated by infections, drugs or eating fava beans⁹. As with G6PD, primary complement mediated aHUS is frequently triggered by environmental stimuli. In addition to susceptibility to acute hemolytic crisis, individuals with class I variants suffer chronic non-spherocytic hemolytic anemia (CNSHA).

Within erythrocytes, oxidative damage leads to the formation of neoepitopes on band 3 protein on the cell membrane. This results in the binding of naturally occurring antibodies

and ultimately C3b deposition and opsonization¹³. Clearance of these cells is thought to be, at least in part, complement dependent, mediated by CR1¹⁴. That hemolysis continued in this case, despite eculizumab indicates that the hemolysis and clearance of erythrocytes in G6PD is independent of the terminal pathway of complement.

In vivo G6PD functions as a homodimer or tetramer¹⁵. The variant identified in this child, R387H, lies at this interface immediately adjacent to the β sheet strand “ β L” (figure 2). This acts as a scaffold for both the dimer interface and NADP⁺ binding. This variant has been described in patients with CNSHA and in keeping with this, functional analysis has demonstrated severely reduced G6PD (class I) activity¹⁶.

In this case, the patient presented with a clinical picture indistinguishable from aHUS, including AKI. AKI is a rare complication of acute hemolytic crisis in G6PD and is also seen in other hemolytic conditions such as paroxysmal nocturnal hemoglobinuria and sickle cell disease. Free hemoglobin released during hemolysis exceeds the binding capacity of haptoglobin, resulting in filtration of free hemoglobin and heme at the glomerulus. This leads to oxidative damage and apoptosis of the tubular cells with subsequent obstruction and acute tubular necrosis (ATN)¹⁷. Renal biopsies from patients with G6PD deficiency induced AKI, frequently demonstrate ATN¹⁸. Our patient has had one episode of AKI requiring dialysis; renal biopsy was not performed due to thrombocytopenia. His renal function has returned to baseline, without any evidence of chronic damage in keeping with a presumed diagnosis of ATN.

Intriguingly, our patient developed thrombocytopenia during three of four acute hemolytic episodes, returning to normal when he was healthy. Mild thrombocytopenia has only rarely been reported during episodes of acute hemolytic crisis^{19, 20}. Previous analysis has shown the activity of G6PD in platelets of patients with G6PD deficiency is severely reduced without an

appreciable effect on their function although increased platelet microparticles have been reported, especially in severe deficiency²¹. R387H is one of the most severe variants in G6PD¹⁶; and the thrombocytopenia in this case may be due to overwhelming oxidative stress.

In summary, we report a child presenting with non-immune hemolysis, thrombocytopenia and AKI who did not respond to treatment with eculizumab. Using whole exome sequencing, we identified a pathological variant in *G6PD* that likely caused the phenotype that is clinically indistinguishable from aHUS. This demonstrates the utility of next generation sequencing techniques in cases where there may be diagnostic uncertainty, allowing personalized management of these clinical syndromes.

G6PD is a rare but important differential diagnosis in patients presenting with an aHUS phenotype. The diagnosis of G6PD deficiency is most easily made by measuring reduced G6PD activity in erythrocytes. This test must be done whilst in remission, as there is a refractory period after acute hemolytic crisis where measured levels will be falsely elevated, as older erythrocytes with lower enzyme levels will be destroyed and only younger erythrocytes with higher residual G6PD levels are measured. We recommend testing for G6PD deficiency in aHUS patients, particularly in those who fail to respond to complement blockade, thus avoiding the risk and cost of long-term eculizumab treatment.

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Figure Legends**Figure 1 Clinical course**

Creatinine, LDH and platelet count plotted over the first 400 days following the initial presentation. Clinical interventions with plasma exchange (PEX) and eculizumab are shown above. Arrows demonstrate infectious triggers (HMPV- human metapneumovirus, LRTI- lower respiratory tract infection, PLT – Platelets, LDH – Lactate dehydrogenase).

(Conversion factors: serum creatinine in $\mu\text{mol/L}$ to mg/dL , $\div 88.4$)

Figure 2. The location of the p.R387H mutation displayed on the G6PD homodimer structure.

An x-ray-derived crystal structure of G6PD (1QKI)¹⁵ was used to model the p.R387H mutation and displayed with Pymol (Delano Scientific). Individual monomers are shown in grey and green. The position of the bound structural NADP molecule is shown (black). The location of the p.R387H mutation is highlighted as a red sphere. R387H lies immediately adjacent to the β sheet strand “ βL ” and the main chain atom is at the dimer surface.

Additionally although the mutation is over 8 Å from the structural NADP⁺ the R387H function can be restored by addition of NADP⁺, suggesting that there is also interference with the structural NADP⁺ site resulting in destabilization of the G6PD homodimer¹².

Figure 1.

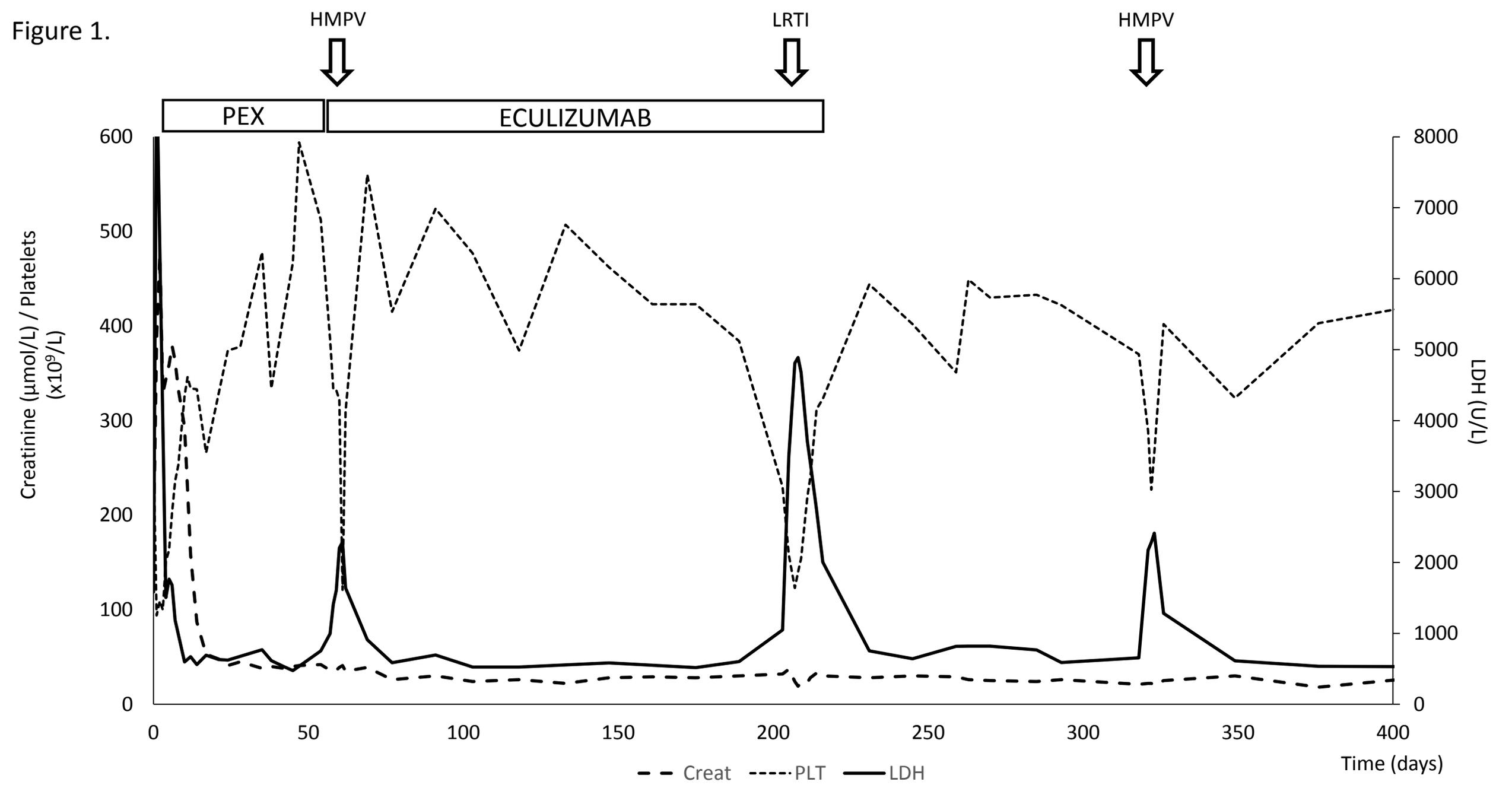


Figure 2.

