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Exceptional Case

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Deficiency of complement factor H-related proteins and autoantibody-positive hemolytic uremic syndrome in an infant with combined partial deficiencies and autoantibodies to complement factor H and ADAMTS13

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Abstract

A 3-month-old male infant developed an extremely severe episode of atypical hemolytic uremic syndrome (aHUS) associated with partial deficiencies of full-length complement factor H (FH; ∼15% of infant normal) and a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) (39% of normal) and autoantibodies reactive with both proteins. His FH and ADAMTS13 genes were normal, indicating that the partial deficiencies were acquired, probably as the result of autoantibodies against full-length FH and ADAMTS13. The child also had a homozygous deletion of the complement factor H–related (CFHR)3–CFHR1 portion in the complement factor H (*CFH*) gene cluster. He therefore had deficiency of CFHR proteins and autoantibody-positive hemolytic uremic syndrome (DEAP-HUS) with an unusual early onset associated with a partial deficiency of ADAMTS13 and an anti-ADAMTS13 autoantibody. His clinical episode of aHUS responded to plasma infusion and subsequent treatment with mycophenolate and rituximab. We believe that this is the first report of DEAP-HUS in an infant with partial deficiencies in both ADAMTS13 and full-length FH acquired in association with autoantibodies to both proteins.

Key words: acute kidney injury, atypical HUS, autoantibody to ADAMTS13, factor H autoantibody, thrombotic microangiopathy

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is a thrombotic microangiopathy (TMA) characterized by thrombocytopenia, microangiopathic hemolytic anemia and renal failure. It is a complication of excessive alternative pathway (AP) activation that results in damage or perturbation of renal endothelial cells (ECs) and episodes of acute renal failure [1]. Prominent causes of aHUS are heterozygous loss-of-regulatory-function mutations in the gene for complement factor H [*CFH*; full-length factor H protein with 20 short consensus repeats (SCRs)]. Full-length FH is a negative AP regulator that prevents amplification of the C3 and C5 convertases and acts as a cofactor for factor I (FI)-mediated conversion of C3b to inactive C3b (iC3b) [2–4].

Approximately 10–16% of aHUS patients have homozygous deletions *CFHR1–CFHR3* (both with five SCR domains) within the *CFH* gene cluster, resulting in the absence of these plasma proteins [5]. When this latter entity is associated with an acquired autoantibody against FH, it is known as deficiency of CFHR proteins and autoantibody-positive HUS (DEAP-HUS) [5–7]. These FH autoantibodies frequently bind to the C-terminal portion of full-length FH that contains the binding recognition site [8].

Recent *in vitro* data show that AP initiation can occur on EC-secreted and anchored ultralarge von Willebrand factor (ULVWF) strings [9]. The attachment of C3b, Bb and C5b to EC-secreted/anchored ULVWF strings occurs in quantitative patterns consistent with the assembly of active complexes of C3 and C5 convertases. ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif 13) is the Ca^{2+}/Zn^{2+}-metalloprotease responsible for cleaving EC-secreted/anchored ULVWF multimeric strings.

It has been hypothesized [10] that even partial deficiencies of ADAMTS13 (e.g. ~50% of normal) may result in platelet adhesion/aggregation and AP activation in excess of normal on secreted ULVWF strings that are uncleaved by ADAMTS13 and remain anchored to ECs after secretion. In this report we describe an infant with a severe episode of TMA correlated with an initial partial deficiency of plasma ADAMTS13 and full-length FH associated with homozygous gene deletions of *CFHR1* and *CFHR3* and antibodies against both FH and ADAMTS13.

Case report

The patient, a 3-month-old African American male who was in foster care due to history of child abuse, presented with a 2-day history of diarrhea, lethargy, fever (101°F), hypertension [blood pressure (BP) 150/90 mmHg] and decreased oral intake. Initial evaluation (Day 1) showed acute renal failure (oliguria with creatinine 2.2 mg/dL and blood urea nitrogen (BUN) 47 mg/dL), microangiopathic hemolytic anemia (hemoglobin 6.5 g/dL, schistocytosis, lactate dehydrogenase (LDH) elevated at 5063 IU/L, decreased haptoglobin), thrombocytopenia (platelet count 102 000/μL) and leukocytosis (total white blood cell count 20 000/μL, neutrophils 10 010/μL). Stool and urine culture were negative for *Escherichia coli*.

Plasma complement C3 level was reduced on Day 1 [16 mg/dL (normal pediatric range 63–179)] and complement C4 level was within normal limits, suggesting overactivation of the AP. The patient initially received therapeutic plasma exchange (TPE) (1.5 × plasma volume) after blood samples were obtained for genetic testing of complement components and ADAMTS13.

His treatment was subsequently changed to plasma infusion (PI). Although TPE is superior to PI in most studies of TMA [11], TPE is technically difficult and clinically dangerous in infants. PI alone, nevertheless, has therapeutic effectiveness and has been used successfully as treatment in some TMA patients [12]. In our patient, the PI (containing both ADAMTS13 and FH) increased antigen levels of the two proteins and was probably capable of partially overcoming the negative effects of the autoantibodies.

Computed tomography and electroencephalography studies of the patient were normal and a T-antigen test for neuraminidase was negative. Pre-TPE ADAMTS13 activity showed a partial deficiency (39%). Daily PI was begun on Day 5 because of ongoing hemolysis and thrombocytopenia and continued until Day 12. At this time (2010), eculizumab was not US Food and Drug Administration (FDA)-approved for the treatment of aHUS.

Platelet count and C3 progressively increased and LDH progressively decreased (Figure 1). The patient initially received labetalol intravenous infusion for control of hypertension. Because of his excellent clinical response, PI was tapered to once weekly by Day 20. He received intermittent red cell transfusion and hemodialysis until creatinine was normalized by Day 30. Three months after the episode he was discharged on once a week PI. He had normal creatinine and BUN values (0.3 mg/dL and 15 mg/dL, respectively), hemoglobin of 9.7 g/dL and platelet count of 357 000/μL.

Total plasma FH levels were 56 μg/mL on Day 1 and 72 μg/mL on Day 7 (normal infant range 170–397). A plasma FI level obtained on Day 1 was normal. The enzyme-linked immunosorbent assay (ELISA) used for FH measures the total quantity of plasma proteins that cross-react with a polyclonal antibody

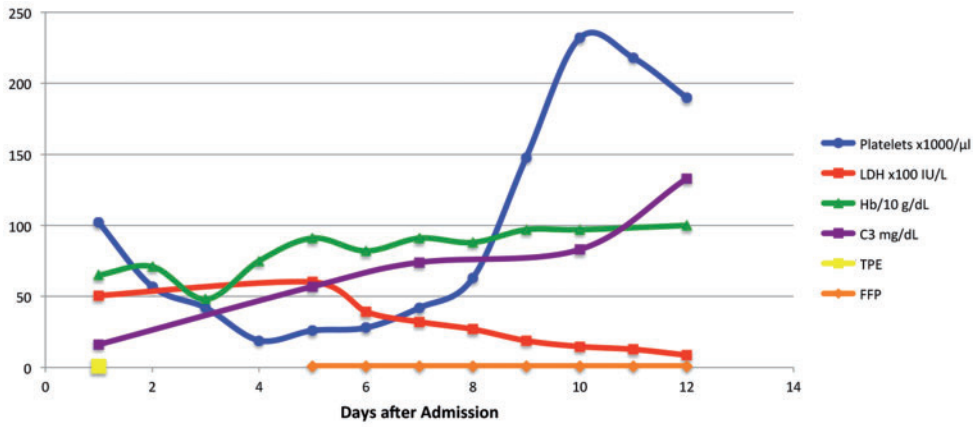

FIGURE 1: Patient response to treatment. The x-axis shows the number of days since initial presentation. Hemoglobin at presentation was 6.5 g/dL, platelet count was 100 000/μL, C3 was 16 mg/dL and LDH was 5000 IU/L. The patient improved in all parameters with plasma therapy.

that identifies all forms of FH, including FH-like protein and the five FH-related proteins. In a normal adult, FHR-1 protein levels are ~10–20% of FH levels [13] and FHR-3 levels are extremely low [14]. Complete deficiency of FHR-1 and FHR-3 proteins can therefore potentially reduce modestly the total plasma FH antigen levels measured by the anti-FH antibody [15].

An antibody against FH was detected in each of six patient samples collected on Day 7 through Day 45 and on Day 440 using a specific antibody detection ELISA (Figure 2A). The assay used immobilized, plasma purified, full-length FH to capture patient, normal adult and normal infant plasma antibodies. In Figure 2A, the gray dashed line shows the average fluorescent FH antibody intensity of three normal infant samples plus 2 standard deviations (SD) and the black dashed line indicates the average fluorescent antibody intensity from nine normal adult plasma samples plus 2 SD.

Citrated plasma obtained pre-TPE showed a subnormal level of functional ADAMTS13 (39% by fluorescence resonance energy transfer assay). Patient plasma ADAMTS13 antigen level (by ELISA) on Day 7 was ~55% of normal and ~65% on Day 10 [normal infant level 713 ng/mL ($n = 3$)]. The slightly higher antigenic values on Days 7 and 10 were obtained during daily PI. An antibody detection ELISA showed that the patient had autoantibodies to ADAMTS13 (Figure 2B). In Figure 2B, the gray dashed line shows the average fluorescent ADAMTS13 antibody intensity of three normal infant samples plus 2 SD and the black dashed

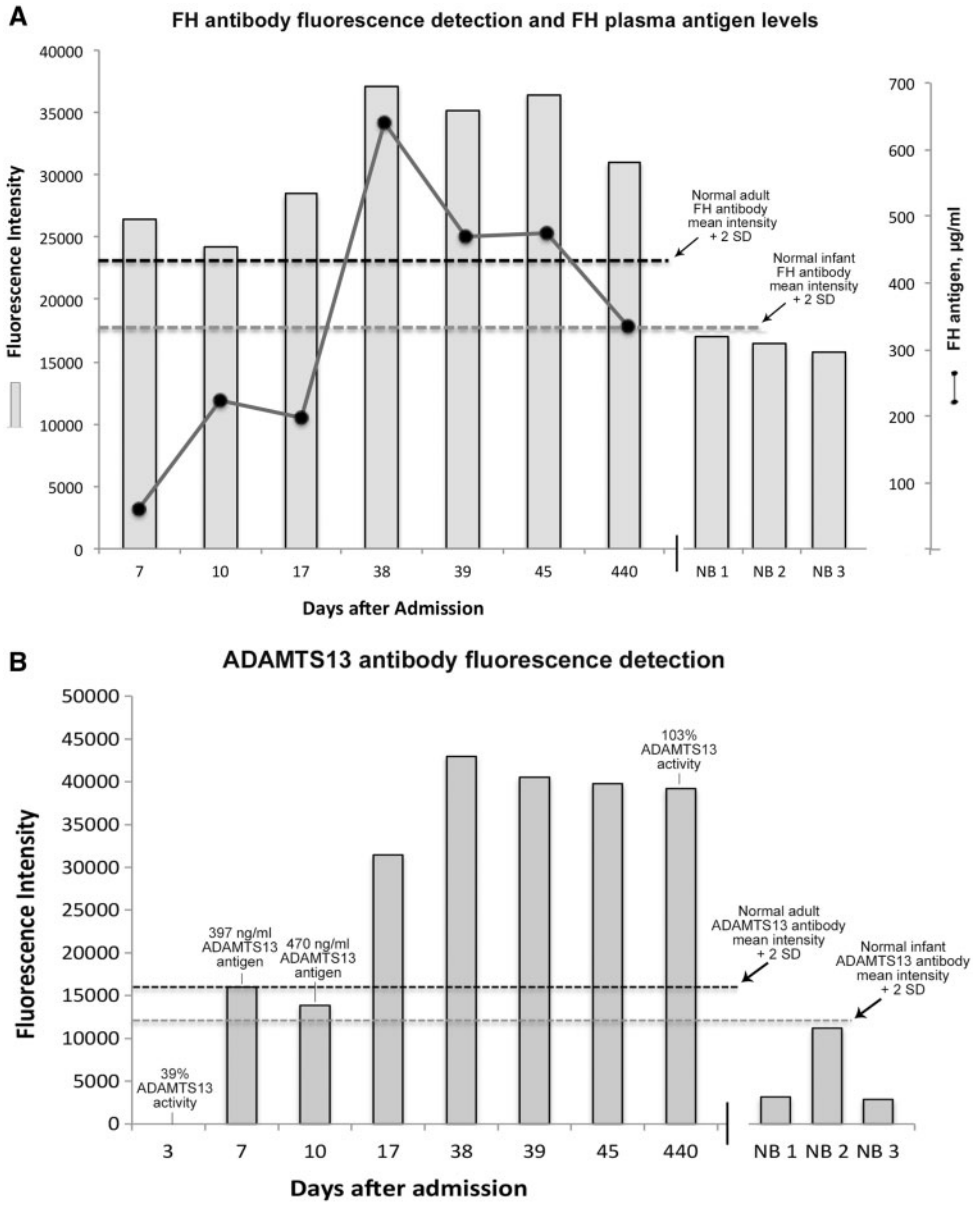

FIGURE 2: Detection of autoantibodies against full-length FH and ADAMTS13 in patient plasma. Autoantibodies against (**A**) immobilized human full-length FH and (**B**) immobilized EC-released ADAMTS13 were detected in patient plasma samples using an ELISA. The relative antibody levels were detected with goat antihuman-IgG-horseradish peroxidase plus a fluorescent substrate. Control plasma samples from three normal newborns are shown for comparison. The gray dashed line shows the average fluorescent antibody intensity of the newborn samples plus 2 SD and the black dashed line indicates the average fluorescent antibody intensity from nine (**A**) and five (**B**) normal adult plasma samples plus 2 SD. In (**A**), the black circles show patient full-length FH antigen levels (μg/mL, right side y-axis). Newborn FH antigens were 290, 293 and 192 μg/mL, respectively. In (**B**), ADAMTS13 antigen levels (ng/mL) are shown for Days 7 and 10 and patient ADAMTS13 activity levels (% normal by fluorescence resonance energy transfer) are shown for Days 3 and 440.

line indicates the average fluorescent antibody intensity from five normal adult plasma samples plus 2 SD. We could not determine conclusively if the antibodies caused a modest reduction in ADAMTS13 activity or in ADAMTS13 survival time. VWF multimer patterns that were analyzed in patient samples collected on Days 7, 10, 17, 38, 39 and 45 after hospital admission were similar to normal newborns and adult plasma VWF multimers analyzed in parallel.

Genetic testing showed that *CFH*, *thrombomodulin (THBD)*, *complement factor B (CFB)*, *membrane cofactor protein (CD46)*, *complement factor H-related protein 5 (CFHR5)*, *complement factor I (CFI)*, *component 3 (C3)* and *ADAMTS13* were normal [7]. There was a homozygous deletion of *CFHR3–CFHR1* within the *CFH* gene cluster on chromosome 1 [5]. It was not until 2 years later that a recessive mutation of the gene encoding diacylglycerol kinase ε (*DGKE*) was found to be associated with aHUS in children < 1 year of age. Although testing for a *DGKE* mutation has not been performed in our patient, it should be noted that most of *DGKE* aHUS patients do not have complement abnormalities [16].

Eleven months after initial presentation, the frequency of PI was reduced to once every 2 weeks. Transient attempts to reduce PI to once every 3 weeks were unsuccessful (LDH increased to 1150 U/L), possibly because the replenishment of FH and ADAMTS13 using an every 3-week regime was inadequate to counteract the effect of autoantibody-mediated decreases in FH (and ADAMTS13) survival. At 14 months of age he remained dependent on biweekly PI and autoantibodies were still detectable. At that time, our therapeutic goal was to induce a decrease in FH (and ADAMTS13) autoantibodies to levels that would allow a reduction in the frequency of PIs. Although eculizumab had been FDA-approved by this time, the continued presence of the two types of autoantibodies led to our decision to treat him with 2 weekly doses of rituximab (375 mg/m^2), 3 months of oral prednisone (1 mg/kg daily × 4 weeks, then weaned over 2 months), 6 additional weeks of PI and mycophenolate (200 mg twice daily) for 24 months. At this point his FH level was normal without detectable FH autoantibody and mycophenolate was discontinued. Four months later the FH autoantibody was again positive and he was restarted on mycophenolate. This has continued to the present time without adverse reaction. He is now 6.5 years old with height 121 cm (74%) and weight 25.1 kg (84%), with normal renal function [serum creatinine 0.51 mg/dL and estimated glomerular filtration rate (eGFR) 97 mL/min/1.73 m^2], normal BP (108/53 mmHg), negative urine protein, hemoglobin 12 g/dL, LDH 214 IU/L, haptoglobin 106 mg/dL and platelet count 320 000/μL.

Discussion

The 3-month-old infant described in this report had a severe episode of acute renal failure in association with both reduced FH antigen levels (~25% of normal infant levels) and reduced functional ADAMTS13 activity (39%) [17] ADAMTS13 activity levels in aHUS studies are typically >10%. Patients with <10% plasma ADAMTS13 usually have severe thrombotic thrombocytopenic purpura (TTP), however, ADAMTS13 levels of 11–25% [18] and 11–70% [19] have been reported in two large TMA patient studies.

Genetic studies showed normal full-length *CFH* and *ADAMTS13* genes combined with a homozygous deletion of the *CFHR1* and *CFHR3* genes of the *CFH* gene cluster. These deletions and the consequent absence of FHR-1 and FHR-3 proteins from the patient's plasma did not explain the ~75% reduction in total plasma FH antigen [15]. The additional reduction was likely to be associated with the autoantibody that was detected in full-length FH.

Homozygous *CFHR1* and *CFHR3* deletions have been found in 2–4% of clinically normal individuals [13]. This indicates that a complete absence of circulating FHR-1 and FHR-3 does not cause clinical disease in the absence of an autoantibody to full-length FH, additional complement mutations or other autoantibodies against complement proteins [20]. It has been reported that ~87% of aHUS patients with autoantibodies against full-length FH have homozygous *CFHR1* and *CFHR3* gene deletions [21]. It is not known, however, how these observations might explain the appearance of these autoantibodies in association with *CFHR1/CFHR3* gene deletions. One possibility is that one (or both) of these molecules normally impairs the antibody production by B-lymphocytes [22]. Therefore, in the absence of CFHR-1 and/or CFHR-3 proteins, autoantibody production by B-lymphocytes may be inadequately controlled. There are several case series [23, 24] reporting successful treatment of FH autoantibody-associated HUS with plasma exchange, prednisone and immunosuppression with cyclophosphamide or rituximab, with or without maintenance immunosuppression. We therefore chose to use the immunosuppressive treatment detailed above for our patient.

The association of *DGKE* mutations and aHUS was not known 6 years ago when our patient initially presented [16]. Genetic testing demonstrated homozygous deletion of *CFHR1–CFHR3* and associated autoantibodies to FH and ADAMTS13. Because of his clinical response to plasma therapy, and later immunosuppression, testing for *DGKE* mutations was not considered clinically important during later follow-up (after the association between *DGKE* mutations and aHUS became known).

In our patient, deletion of the *CFHR1* and *CFHR3* genes was also associated with autoantibodies to ADAMTS13, as well as to full-length FH and initial ADAMTS13 activity level that was 39% of normal. This lower ADAMTS13 activity would likely have compromised his capacity to cleave EC-anchored ULVWF strings. Increased numbers of ULVWF multimeric strings are secreted and anchored to ECs in the presence of inflammatory cytokines (TNF and interleukin (IL)-8) [25] or during trauma and/or hypoxia [26]. Increased secreted/anchored hyperadhesive ULVWF strings are sites of platelet adhesion/aggregation in the microvasculature. Binding of C3 (as C3b) to secreted/anchored ULVWF strings that remain uncleaved when ADAMTS13 is subnormal would be expected to increase AP activation, especially in the presence of reduced levels of full-length FH. In a previous study, Strauss *et al.* [27] showed that neonates with stressful conditions such as intrauterine growth restriction, retinopathy of prematurity and necrotizing enterocolitis had lower levels of ADAMTS13. Our patient was born full term but did have a history of child abuse (a serious stressful event) that may have contributed to his lower level of ADAMTS13.

Normal infant and adult plasmas were tested routinely for autoantibodies against ADAMTS13 and FH along with the patient plasma samples. As shown in Figure 2, the autoantibody levels against both antigens in the patient samples were higher than levels obtained in normal plasma samples (and more than double the values for autoantibodies against ADAMTS13). These results indicate that the autoantibodies detected in the patient plasma are unlikely to be false positives. Inverse correlations between ADAMTS13 and FH autoantibodies and corresponding antigen levels are not possible to determine because of the frequent PIs received by the patient. We did not determine the specific subset of the autoantibodies.

Autoantibodies against ADAMTS13 have been previously evaluated almost exclusively in TTP patients with <10% activity

levels. The findings in our patient of combined acquired deficiencies of both ADAMTS13 and FH, in association with autoantibodies against both, have rarely (if ever) been subjected to laboratory testing. The autoantibodies against ADAMTS13 in the patient's plasma are likely to be of pathophysiological importance because his ADAMTS13 genes are normal. It is probable that these antibodies against ADAMTS13 were binding to the patient's plasma ADAMTS13 and subsequently removed from circulation. The autoantibody reactive with ADAMTS13 may have decreased survival of the enzyme in the blood of the patient (rather than directly inhibiting ADAMTS13 activity using *in vitro* testing). Noninhibitory autoantibodies reactive with ADAMTS13 have been described in several studies [28–30].

In summary, the infant described had a severe episode of TMA resulting from acquired partial deficiencies of both FH and ADAMTS13 associated with autoantibodies to both proteins and with a homozygous deletion of the *CFHR1* and *CFHR3* genes.

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Conflict of interest statement

None declared.

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