Anti-complement factor H (CFH) antibodies and a novel CFH gene mutation in an atypical hemolytic uremic syndrome patient with complement activation of the classical pathway

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ABSTRACT
Atypical hemolytic uremic syndrome (aHUS) is a rare disease caused by overactivation of the complement alternative pathway. aHUS involves the presence of antibodies against complement factor H and its mutations in the complement genes. A 2-month-old boy presented with discoid rash, hemolytic anemia, thrombocytopenia, multiple antibodies, and hypocomplementemia with a very low level of C4 (< 3 mg/dL), indicating activation of the complement pathway, together fulfilling the systemic lupus erythematosus (SLE) criteria of the American College of Rheumatology at 5 months of age. However, most of these findings normalized spontaneously without any intervention. Further investigations revealed a high level of anti-complement factor H antibodies and a novel heterozygous missense mutation (p.Glu1172Ala, located in exon 22) in a complement gene, CFH. At 2 years of age, his SLE-like symptoms have not recurred, but hematuria and schistocytes were persistent. Eventually, aHUS was diagnosed rather than SLE. Our findings suggest that multiple antibody complex, including anti-complement factor H antibody, may temporarily activate the classical pathway, resulting in SLE-like findings.

1. Introduction
Atypical hemolytic uremic syndrome (aHUS) is a type of thrombotic microangiopathy (TMA) caused by overactivation of the complement alternative pathway. aHUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal dysfunction [1]. Approximately 10% of aHUS cases had antibodies against complement factor H (CFH) that regulates the alternative pathway [2]. Recent studies have shown that mutations of complement genes (such as CFHR1 and CFHR3) are strongly associated with the generation of anti-CFH antibodies [3,4]. However, the mechanism of action between complement gene mutation and the generation of anti-CFH antibodies has been poorly understood. Here we describe a patient with symptoms of aHUS, anti-CFH antibodies, and a novel missense CFH mutation.

2. Case report
The male patient was born after an uneventful pregnancy of 38 weeks, weighing 2,578 g. The family history was unremarkable and the parents were non-consanguineous. He was born in Japan. His mother was Thai, and his father was half Chinese and half Filipino. He had an aneurysmal malformation in the vein of Galen and received therapeutic embolization at 18 days of age. Symptomatic epilepsy after embolization was treated with carbamazepine for 2 months.

At 2 months of age, he was admitted to our intensive care unit because of microangiopathic hemolytic anemia (hemoglobin 7.9 g/dL with schistocytes (Figure 1(a)), reticulocytes, 133,000/μL; lactate dehydrogenase, 685 U/mL; total bilirubin, 0.30 mg/dL; direct and indirect Coombs test, negative) with thrombocytopenia (platelets 15,000/μL) during a respiratory syncytial virus infection. The patient had discoid rash over his entire body, which almost vanished on the following day after admission. Serum creatinine level was normal (0.23 mg/dL; reference range 0.14–0.26 mg/dL). An abdominal ultrasound examination showed increased renal parenchymal density. ADAMTS13 activity was low (16.1%; reference range, 60–123%) with an
ADAMTS13 inhibitor (1.3 BU/mL) that did not meet the definition of TTP (ADAMTS13 activity < 10%). Bone marrow examination showed normocellularity, without leukemic blasts or decreased megakaryocytes. Thrombocytopenia normalized in 2 days. Although hemoglobin level gradually increased, schistocytes remained.

At 4 months of age, he was hospitalized for additional therapeutic embolization. His general condition was good and he had no history of diarrhea, fever, or cough. However, laboratory data exhibited an elevation of serum creatinine (0.30 mg/dL, eGFR 66.2 mL/min/1.73m²), microangiopathic hemolytic anemia (hemoglobin, 9.3 g/dL with schistocytes), and slightly increased platelet count (564,000/µL). Ultrasonography still showed increased renal parenchymal density. A urinalysis showed hematuria without proteinuria. Immunological examinations revealed hypocomplementemia (CH50, < 12 U/mL; C3, 63 mg/dL; C4, < 3 mg/dL [reference ranges: CH50, 25–48 U/mL; C3, 65–135 mg/dL; C4, 13–31 mg/dL]). Additionally, he had multiple autoantibodies (antineuclear, anti-single-stranded DNA, anti-double-stranded DNA, anti-Smith, and anti-β2-glycoprotein I) (Table 1), suggesting activation of the classical pathway (Figure 1(b)). His mother did not have any antibodies leading to his neonatal lupus (antinuclear antibodies < x40, anti-single-stranded DNA antibodies < 10 AU/mL, anti-double-stranded DNA antibodies < 10 IU/mL, anti-Smith antibodies < 1.0 U/mL, anti-SS-A antibodies 4.6 U/mL, or anti-SS-B antibodies 1.0 U/mL). He fulfilled the SLE criteria of the American College of Rheumatology. However, without any intervention, his blood cell counts and levels of complement normalized within 3 months, and multiple antibody titers decreased. He had a normal coagulation profile except around the time of therapeutic embolization. His coagulation test results at 5 months when anti-CLβ2GPI was detected were as followed; PT 104%, APTT 33.3s, D-dimer 1.6 µg/mL. Anti-CLβ2GPI antibody titer was very low at 5 months and turned negative later. Thus, we did not suspect that the antiphospholipid antibody syndrome caused the reduced complements. Besides, there was no evidence of infections, malignancies, or metabolic disorders. Further investigations of the complement system indicated an exceedingly high level of anti-CFH IgG at 5 months of age (37480.9 AU/mL; reference range, 393.9–1069.0 AU/mL). Levels of CFH (436.1 µg/mL; reference range 285.9–710.7 µg/mL) and sC5b-9 (1158 ng/mL; reference range 148.0–1243.6 ng/mL) were normal. At 11 months of age, both C3 and C4 levels normalized (Figure 1(b)). Anti-CFH IgG level remained high (3838 AU/mL), CFH level was slightly low (272.5 µg/mL), and sC5b-9 level was normal (627.0 ng/mL).

Complement genes of genomic DNA were analyzed by the Miseq Sequencing System (Illumina, San Diego, CA) at The Japanese Association for Complement Research. Genetic variants with rare allele frequency (< 0.005) were identified using the Exome Aggregation Consortium. The sequencing revealed a novel heterozygous missense mutation (p.Glu1172Ala in exon 22) in the CFH gene. Although this mutation was not listed in HGVD, p.Glu1172Ala was predicted by in silico analysis using PolyPhen-2, PROVEAN, and SIFT analyses as ‘probably damaging’, ‘deleterious’, and ‘damaging’.

Table 1. Autoantibodies and complement levels.

| Reference range | Age (months) | 5 | 6 | 8 | 11 | 19 |
|-----------------|--------------|---|---|---|----|----|
| anti-CFH-IgG    | 393.9–1069.0 (AU/mL) | 37480.9 | 3838.3 |
| CFH             | 285.9–710.7 (µg/mL) | 436.1 | 272.5 |
| C3a             | 0.50–32.33 (ng/mL) | 16.79 | 13.36 |
| sC5b-9          | 148.0–1243.6 (ng/mL) | 1158 | 627 |
| Ba              | 419.6–1714.0 (ng/mL) | 1254 | 923 |
| CFI             | 28.8–55.6 (µg/mL) | 33.9 | 25.6 |
| ANA             | < x40         | x40 | x40 | x40 |
| anti-ss DNA     | 25 (AU/mL) ND | 27 | 18 |
| anti-ds DNA     | 12 (IU/mL) | 30 | 21 | 17 |
| anti-Smith      | < 10 (U/mL) | 15.9 | 6 | 5.1 |
| anti-CLβ2GPI    | < 3.0 (U/mL) | 3.7 | < 1.2 | < 1.2 |

Abbreviations: CFH: complement factor H; CFI: complement factor I; ANA: antinuclear antibodies; ss: single strand; ND: not done; ds: double strand; anti-CLβ2GPI: beta 2-glycoprotein I dependent anti-cardiolipin antibody.

Figure 1. (a) Peripheral blood smear with schistocytes. (b) Changes in C3 and C4 levels, showing complement activation.
respectively. Complement C4 gene copy number variation was measured using QuantStudio 3D Digital PCR System (Thermo Fisher Scientific). He had five copies of C4 (reference range 2–8), two copies of C4A, and three copies of C4B, suggesting that his genetic status of the C4 gene did not reduce the C4 level.

Based on the combination of microangiopathic hemolytic anemia, thrombocytopenia, acute kidney injury, high level of anti-CFH IgG, and mutation in the CFH gene, his condition was diagnosed as aHUS, rather than SLE. Thereafter, symptoms of aHUS or SLE have not recurred for 2 years, except for persistent hematuria and schistocytes.

3. Discussion

It is often difficult to clarify the etiology of TMA. This case presented aHUS symptoms with anti-CFH antibodies and a novel CFH mutation. Typically, the alternative pathway is activated in aHUS patients. In this case, multiple autoantibodies may have been involved in the activation of the classical pathway and the SLE-like symptoms.

In this case, symptoms of TMA were speculated to occur secondarily to SLE at first. However, his condition recovered without any intervention. Complement levels that were low at 5 months old normalized at 11 months old, accompanying a decrease in multiple autoantibody levels. In contrast, hematuria and schistocytes persisted. This clinical course was atypical for SLE. Further studies revealed an exceedingly high level of anti-CFH antibodies and a heterozygous mutation in the CFH gene. CFH is a major regulatory protein of the complement alternative pathway and is composed of 20 short consensus repeats (SCRs). Two major functional regions are located at the N-terminal SCRs 1–4 and C-terminal SCRs 19–20. N-terminal SCRs mediate regulatory activities of decay of the C3 convertase and cofactor. C-terminal SCRs includes binding sites for ligands such as C3b, C3d, and glycosaminoglycan, and mediates surface recognition leading inhibition of complement activation. Acquired autoantibodies against CFH have been described in about 10% of aHUS cases [4–6]. Anti-CFH antibodies induce functional CFH deficiency by binding to its C-terminal region and thereby reducing its regulatory function [2–4].

In a different pattern from typical aHUS cases, this case showed the activation of the classical pathway and SLE-like symptoms, which may be caused by immune complexes of multiple autoantibodies that bind the C1q, leading to type 3 hypersensitivity, and consequently SLE-like symptoms, similar to serum disease or type 3 allergy. In fact, the levels of anti-CFH antibody in this patient were higher than those in the previous cases [7,8]. Although we did not measure C1q level and immune complexes binding to C1q, his C4 and C3 levels decreased together and he had multiple autoantibodies, suggesting the activation of the classical pathway rather than the alternative or lectin pathways. The cause of antibodies generated in this patient was unclear. Although, to our knowledge, no studies have examined the association of specific pathogen and anti-CFH antibody, respiratory syncytial virus infection might have induced to generate multiple autoantibodies, and consequently activated the classical pathway. There is also a possibility that therapeutic embolization causes the autoimmune-like phenomenon. However, to our knowledge, there is no report of this phenomenon in patients with aneurysmal malformation in the vein of Galen. Besides, the same phenomenon did not occur again even though he underwent therapeutic embolization seven times.

Several causative genetic variants for aHUS have been identified. Approximately 50% of aHUS patients have loss-of-function variants in complement regulatory genes (CFH, MCP, CFI) or gain-of-function variants of complement factors (C3, CFB). In our patient, a single novel missense mutation, p.Glu1172Ala in the CFH gene, was found in a heterozygous state. No other mutations were found in complement genes. The majority of the reported CFH mutations are heterozygous likely pathogenic variants [9]. Previous reports have shown that only 9.2% of aHUS patients with CFH mutation carried abnormalities in other complement genes, suggesting that a mutation in the CFH gene alone may be sufficient to cause aHUS [9]. In addition, in silico analysis algorithms suggested the pathogenicity of the mutation. This mutation (exon 22 of CFH gene) is located in the major functional region C-terminal SCR 20, which is the recognition region and binding sites for the surface of endothelial cells. The exon 20–22 of the CFH gene is known as a hotspot for mutations in aHUS [10]. Therefore, the heterozygous CFH mutation in our case may be a cause of aHUS. Further studies are needed to elucidate this association.

4. Conclusions

We found anti-CFH antibodies and a novel CFH mutation (p.Glu1172Ala) in an aHUS patient with temporary activation of the classical complement pathway. Our findings suggest that high levels of anti-CFH antibodies may activate the classical pathway, resulting in SLE-like symptoms.
Acknowledgment

We thank The Japanese Association for Complement Research for their contribution to complement testing and gene analysis, which were financially supported by Alexion GK as company-sponsored research.

Ethical approval

This study was approved by the Ethics Committees of the National Center for Child Health and Development and the Japan Pediatric Society in May, 2018 (# 2020-163). Written informed consent was obtained from the patient’s parents for all procedures, tests, and publications.

Author contributions

SM, HI, and AI contributed to the conception and design of this study; YH performed gene analyses; SM and HI drafted the manuscript; HN, KN, MK, and AI critically reviewed the manuscript. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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