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【 CASE REPORT 】

Atypical Hemolytic Uremic Syndrome Triggered by Acute Pancreatitis in a Patient with a Membrane Cofactor Protein (CD46) Genetic Variant

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Abstract:

Atypical hemolytic uremic syndrome (aHUS) is a type of HUS. We herein report a case of aHUS triggered by pancreatitis in a patient with a heterozygous variant of membrane cofactor protein (*MCP*; P165S), a complement-related gene. Plasma exchange therapy and hemodialysis improved thrombocytopenia and anemia without leading to end-stage kidney disease. This *MCP* heterozygous variant was insufficient to cause aHUS on its own. Pancreatitis, in addition to a genetic background with a *MCP* heterozygous variant, led to the manifestation of aHUS. This case supports the "multiple hit theory" that several factors are required for the manifestation of aHUS.

Key words: atypical HUS, *MCP* variant, multiple hit theory, pancreatitis

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Introduction

Atypical hemolytic uremic syndrome (aHUS) is a type of thrombotic microangiopathy (TMA) characterized by acute kidney injury, thrombocytopenia, and microangiopathic hemolytic anemia. However, internationally unified diagnostic criteria for aHUS have not yet been established.

In the Kidney Disease: Improving Global Outcomes (KDIGO) classification, TMA is classified into four categories: STEC-HUS induced by Shiga toxin-producing *Escherichia coli* (STEC) infection, thrombotic thrombocytopenic purpura (TTP), "primary aHUS," and "secondary aHUS." TTP is caused by a marked deficiency in a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13 (ADAMTS-13) activity, either because of genetic abnormalities or acquired autoantibodies. Conversely, more than 90% of HUS cases are STEC-HUS, and the remaining 10% are aHUS, which does not involve STEC infection (1, 2). The term "primary aHUS" is used when an underlying abnormality of the alternative pathway of complement is strongly suspected, and other causes of "secondary aHUS" have been excluded.

Pathogenic genetic variants in a patient with aHUS include complement factor H (*CFH*), membrane cofactor protein (*MCP*; *CD46*), complement factor I (*CFI*), complement 3 (*C3*), complement factor B (*CFB*), thrombomodulin (*THBD*), complement factor H related protein 1 (*CFHR1*), complement factor H related protein 5 (*CFHR5*), and diacylglycerol kinase epsilon (*DGKE*) (3-6). Fujisawa et al. reported that, in an analysis of 118 aHUS patients in Japan, the frequencies of *C3*, *CFH, MCP* and *DGKE* genetic abnormalities were 32 (27%), 10 (8%), 5 (4%), and 1 (0.8%), respectively, and the frequency of anti-CFH antibodies was 20 (17%). Unidentified genetic abnormalities were reported in 36 patients (30%) (7). However, even in some patients, a complement abnormality could not be identified.

In such cases, several triggers are considered to be related to the manifestation of aHUS. The triggers include autoimmune conditions, transplants, pregnancy, infections, drugs, and metabolic conditions, which we have collectively classified as "secondary aHUS."

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Table 1. Progress of Lab-date in This Case.

Variable	DAY0	DAY2	DAY3	DAY4	DAY7	DAY11	DAY14
WBC ($\times 10^3/\mu L$)	54	112	105	107	210	171	77
Haemoglobin (g/dL)	15.5	12.4	10.5	8.8	7.2	7.9	8.3
Platelets ($\times 10^4/\mu L$)	31.3	0.5	2.1	1.6	19.6	63.8	83
Cr (mg/dL)	0.75	1.33	1.51	1.67	1.34	1	0.98
LDH (U/L)	151	2,469	2,513	2,024	550	447	362
T-Bil (mg/dL)	0.77	4.35	4.57	4.49	1.98	1	0.82
P-Amy (U/L)	34	1,632	1,080	302	196	134	173
CRP (mg/dL)	0.47	16.76	25.95	15.21	21.22	5.52	1.43
Haptoglobin (mg/dL)	23			<10			88

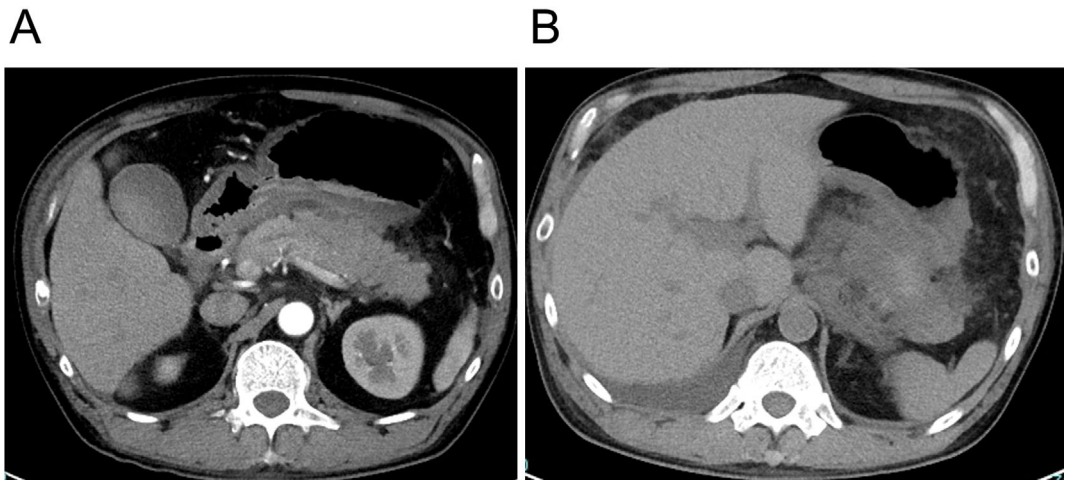

Figure. Abdominal computed tomography (CT) findings in this patient. (A) Day X+2 after performing endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). The pancreatic body tail has a mildly reduced contrast effect in the arterial phase, consistent with the findings of acute pancreatitis. (B) Day X+7. CT indicates a low-absorption area suspected of being walled-off necrosis (WON) around the peripancreatic to the gastric body and gastric antrum.

We herein report a patient with an underlying genetic variant of *MCP* who developed aHUS triggered by the onset of acute pancreatitis.

Case Report

A 49-year-old man with no medical or family history of TMA presented with pancreatic swelling and an elevated IgG4 level of 139 mg/dL. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was performed for suspected autoimmune pancreatitis (AIP) on day X. The pathology results of the pancreas submitted after EUS-FNA were negative for AIP, and the pancreas was diagnosed as normal.

The laboratory data are shown in Table 1. After EUS-FNA, the patient complained of left-sided abdominal pain, and the pancreatic amylase level was found to have increased to 1,547 U/L. The patient's vital signs were as follows: body temperature, 37.5°C; and blood pressure, 136/74 mmHg. Contrast-enhanced computed tomography (CT) revealed a contrast-impaired area of the pancreatic tail and increased peripancreatic fatty tissue density (Figure A). The patient was diagnosed with acute pancreatitis after EUS-FNA. Treatment was started with fasting, analgesia with acetaminophen, treatment with nafamostat-mesylate, and large extracellular fluid replacement of 4,000 mL/day. Serum creatinine increased from 0.75 to 1.33 mg/dL, and the estimated glomerular filtration rate (eGFR) decreased from 87 to 46.5, indicating acute kidney injury. The urinary protein excretion was 6.15 g/gCr, and hematuria (3+) was observed. Blood samples showed progression of anemia from Hb 15.5 to 12.4 mg/dL, and platelets decreased from 313,000 to 5,000/μL. Schistocytes were detected at a rate of 50-70/2,000 red blood cells (RBCs), lactate dehydrogenase (LDH) levels were elevated to 2,469 U/L, and haptoglobin was 23 mg/dL.

Based on these findings, plasma exchange therapy (PE) was initiated on day X+4. Hemodialysis (HD) was initiated because of oliguria and a tendency toward weight gain. Methylprednisolone (500 mg) was administered. An analysis to evaluate the course of TMA revealed serum levels of C3, C4, and CH50 at 91, 25, and 37.8/mL, respectively. The patient had no diarrhea, and Shiga toxin levels were negative. The ADAMTS-13 activity was 44%, and ADAMTS-13 inhibitor was negative. The Coombs test results were negative.

Table 2. Profiles of the Patients with *MCP* P165S Variant.

patient	MCP mutation	Other gene variant	sex	Age at onset (yr)	Triggers	onset of aHUS	outcome at first episode	reference
This case	P165S		M	49	pancreatitis	+	Remission	
HUS68	P165S	CFI(T538X) *MCP* ggaac	F	57	n.a.	+	Remission	(9)
HUS84	P165S	CFI(T538X) *MCP* ggaac	F	41	No trigger	+	Remission	(9)
III-10	P165S		M	52		-		(9)
III-11	P165S	CFI(T538X)	M	54		-		(9)
IV-1	P165S	CFI(T538X)	F	37		-		(9)
IV-2	P165S	CFI(T538X)	M	35		-		(9)
IV-3	P165S		F	30		-		(9)

*MCP*ggaac : one type of *MCP* risk haplotype of aHUS that has been reported to increase the penetrance of aHUS

Based on these findings, we diagnosed the patient with secondary TMA secondary to pancreatitis. PE and HD were terminated on day X+7 because the TMA activity had settled and the fluid volume status improved.

The patient was found to have suffered a relapse of inflammation and showed elevated pancreatic enzymes on day X+7. CT showed pancreatic swelling and hypo-absorptive areas in the peripancreatic, gastric fold, and transverse colonic mesentery, suggesting the formation of walled-off necrosis (WON) (Figure B). The WON improved with antibiotic treatment. After confirming no recurrence of postprandial abdominal pain, the patient was discharged on day X+26.

We outsourced next-generation sequencing of complement-related genetic abnormalities to the KAZUSA DNA Research Institute. In the laboratory, *CFH*, *CFI*, *MCP*, *C3*, *CFB*, *THBD*, *DGKE*, and *CFHR5* were analyzed using next-generation sequencing hybridization capture methods for rare single nucleotide substitutions and deletions with an allele frequency of <0.5%. In addition, complement function tests (sheep erythrocyte hemolysis test) and anti-CFH antibody tests were performed. The sheep erythrocyte hemolysis test was performed because it is useful for detecting genetic mutations in CFH and anti-CFH antibodies. The results showed the absence of anti-CFH antibodies (8). Genetic testing revealed a 493-base cytosine (C) to thymine (T) mutation in *MCP* (*MCP* P165S), a complement-related gene responsible for atypical HUS, resulting in a missense variant of proline-to-serine substitution and a heterozygous mutation in *MCP*. Based on the *MCP* findings, we considered this case to potentially be primary aHUS triggered by acute pancreatitis.

Discussion

We herein report a patient with a genetic mutation in *MCP*, a gene associated with the complement-related pathway, who developed aHUS following pancreatitis after EUS-FNA.

In the present case, the patient had a missense variant of *MCP* (*MCP* P165S). A previous report indicated a 50% reduction in MCP expression in leukocytes in patients with the *MCP* P165S variant (9). Another study reported that a 50% reduction in MCP expression leads to a decrease in the binding ability of C3b to <50% compared to normal MCP expression (10). The *MCP* P165S variant is not registered in Clinvar but is categorized as "likely pathogenic" according to the The American College of Medical Genetics and Genomics guidelines (ACMG guidelines) (11). Esparaza-Gordillio et al. reported families with the same genetic variant as this case and found that patients with only the *MCP* variant did not manifest aHUS, whereas those with coexisting the *CFI* mutation and *MCP*ggaac single-nucleotide variant haplotype block, another variant of *MCP*, did manifest aHUS (9, 12, 13). The clinical characteristics of the patients with the *MCP* P165S variant are summarized in Table 2. In the present case, only the protein-coding region exons of *CFH, CFI, CD46, C3, CFB, THBD*, and *DGKE* and their intron boundaries were searched using the next-generation sequencer, and the multiple ligation-dependent probe amplification (MLPA) method was not used to detect structural variants. The lack of a genetic analysis using MLPA is considered a limitation of this case (14).

Several triggers are thought to be involved in the manifestation of aHUS, in addition to genetic mutations, including infection, pregnancy, transplantation, metabolic diseases, vasculitis, and pancreatitis, called the "multiple hit hypothesis" (13, 15-17). Among the reported triggers, cases of aHUS triggered by pancreatitis are rare, accounting for just 3% (4/110) of secondary aHUS cases (18-21). Previously reported cases of aHUS triggered by pancreatitis were not specifically investigated for complement-related gene mutations, so whether or not complement-related gene mutations other than pancreatitis have any effects on the manifestation of aHUS is unclear.

In the present case, aHUS developed in a patient with a genetic variant (*MCP* P165S) that originally did not meet the threshold for aHUS development due to pancreatitis after EUS-FNA. This shows that several triggers, not just genetic mutations, are involved in the development of aHUS, supporting the "multiple hit hypothesis." Patients with *MCP*

variants have a better prognosis and lower probability of developing end-stage kidney disease (ESKD) and death than patients with other genetic mutations, such as *CFH*, *CFI*, *C 3*, and *THBD* (13, 17, 22).

The patient in this case responded well to plasma exchange therapy and PE. He recovered his kidney function, supporting the relatively good prognosis of patients with *MCP* mutations compared to other aHUS-related gene mutations. Although the efficacy of eculizumab as a treatment for aHUS has been described in recent years, eculizumab was not used in this case, as the patient had a good clinical course with plasma exchange therapy and HD (17, 19, 21). In addition, infection with WON after pancreatitis was suspected on day X+7; therefore, it was difficult to use eculizumab, which produces immunosuppressive effects. However, the use of eculizumab should be considered in the future if aHUS relapse is triggered by some event.

In conclusion, we encountered a case of aHUS triggered by acute pancreatitis after EUS-FNA in a patient with a heterozygous variant of *MCP* (*MCP* P165S), which is considered to not manifest aHUS. We speculate that the heterozygous variant of *MCP* in this case did not induce aHUS alone, instead representing a second hit by pancreatitis-induced aHUS.

The authors state that they have no Conflict of Interest (COI).

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