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## A case of atypical hemolytic uremic syndrome associated with the *c.1273C>T* mutation in the complement *C3* gene

**TO THE EDITOR:** Hemolytic uremic syndrome (HUS) is characterized by non-immune hemolytic anemia, thrombocytopenia, and renal impairment [1]. The disease typically develops in children under the age of 5 years and is preceded by bloody diarrhea caused by infection associated with Shiga

toxin-producing *Escherichia coli* or Shiga-like toxin-producing bacteria (STEC-HUS) [2].

Atypical HUS (aHUS), which comprises 5–10% of HUS cases, is not associated with a prodrome of diarrhea and has a worse prognosis than that caused by STEC-HUS [3]. Uncontrolled complement activation, whether sporadic or familial, plays a major role in the pathogenesis of aHUS. Genetic abnormalities in the complement system that lead to uncontrolled complement activation have been demonstrated in 60% of aHUS cases [4]. The most common mutation in aHUS occurs in *complement factor H (CFH)*, followed by *membrane cofactor protein (MCP)*, *complement factor I (CFI)*, *thrombomodulin*, and *complement component 3 (C3)* [3]. So far, only 11 patients with aHUS associated with mutations in *CFH*, *CFI*, *MCP*, and *diacylglycerol kinase epsilon* have been reported in Korea, owing to the rarity of this syndrome and the lack of a suitable laboratory for its genetic diagnosis [5–7].

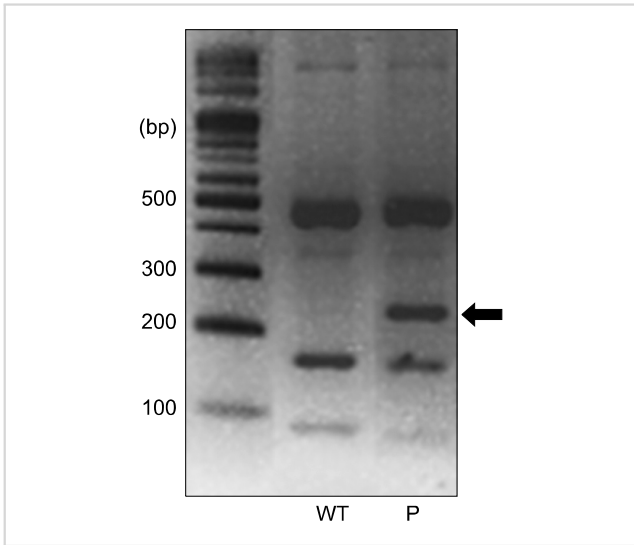
*C3* mutation occurs in about 4–10% of all aHUS cases [3]. This mutation results in a resistance to C3b inactivation caused either by decreased regulatory binding of MCP, CFH, and CFI to C3, or by increased binding of C3 to complement factor B (CFB) to produce a high amount of C3 convertase [8]. The prognosis of aHUS with a *C3* mutation is known to be poor, where about 50% of the cases recur after the initial treatment, and the rate of death or development of end-stage renal disease is about 60% [3].

Although *C3* mutations are a well-known etiology of aHUS, the *c.1273C>T* mutation has never been reported in Caucasians and Koreans. Herein, we report a case of aHUS with *C3* mutation in Korea and review of the literature.

### CASE

A 66-year-old woman was admitted to the hospital owing to altered mental status. She had previously been diagnosed with systemic lupus erythematosus (SLE) and diabetes mellitus and was on medications. She was treated with a 1,000 mg intravenous steroid pulse regimen for cerebral infarction and SLE. Five days after steroid treatment, she was discharged home with clinical improvement.

A month after the discharge, her mental status became aggravated again and was re-admitted to the department of nephrology under the impression of thrombotic thrombocytopenic purpura. Her complete blood count showed a hemoglobin level of 11.2 g/dL, reticulocyte count of 0.2%, platelet count of 60,000 /mm<sup>3</sup>, and white blood cell count of 13,450 /mm<sup>3</sup>. The prothrombin time was 12 s, and the activated partial thromboplastin time was 42 s. The peripheral blood smear revealed many schistocytes. In the blood chemistry, her blood urea nitrogen and creatinine levels were increased to 46.3 mg/dL and 1.25 mg/dL, respectively. Her total and direct bilirubin levels were 0.58 mm/dL and 0.19 mm/dL, respectively. Her lactated dehydrogenase (LDH) level was elevated to 1,192 U/L. The complement levels (reference ranges in parentheses) were as follows:

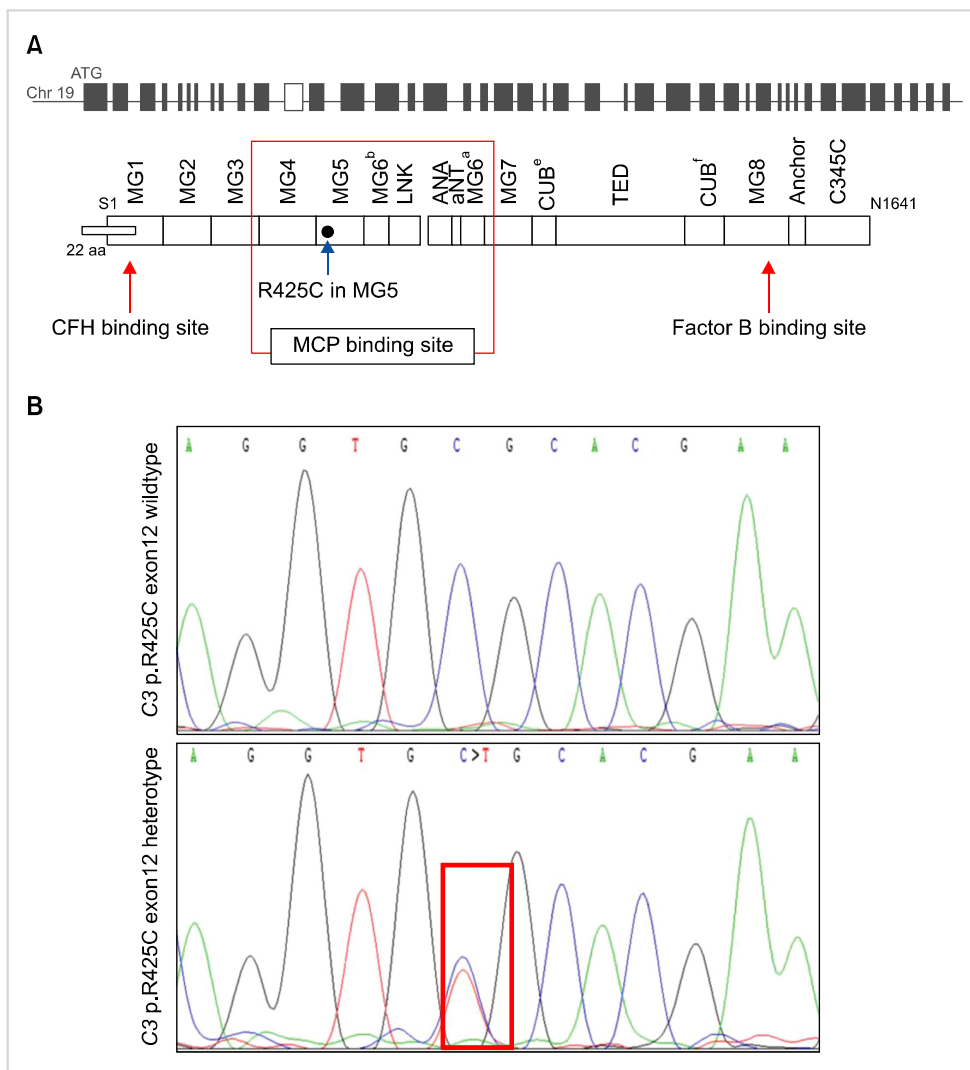


**Fig. 1.** Restriction fragment length polymorphism analysis of our patient with heterozygote *c.1273C>T* mutation in *C3* gene showing 250 bp product (solid arrow). Abbreviations: P, patient; WT, wild type.

C3, 40.1 mg/dL (90–180 mg/dL) and C4, 17.9 mg/dL (16–49 mg/dL). Brain MRI showed a subtle diffusion-restricted lesion in the right corpus callosum and left cerebellum, suggestive of cerebral infarction. The ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity was within the normal range. She had no history of bloody diarrhea. The Shiga-toxin assay on stool samples showed negative results. Immediately after admission, the patient received plasma exchange (daily for 2 days), followed by hemodialysis and steroids, and her consciousness improved to the level that she could recognize her family. The hemoglobin, platelet, and creatinine levels recovered to the normal range. Her LDH level also decreased to 456 U/L. The patient completely recovered and was discharged 56 days after admission. She is currently doing well without recurrence of disease under outpatient management on day 340 after the initial diagnosis.

**Detection of complement *C3* gene mutation**

Genomic DNA was extracted from the patient’s peripheral



**Fig. 2.** Localization of the R425C *C3* mutation. (A) Position within the *C3* gene and the protein primary structure. (B) Representative genome sequencing histogram for our patient (red square).

blood leukocytes using a G-DEX II Genomic DNA Extraction Kit (iNtRON Biotechnology, Korea). For mutation detection, the coding exons and intron flanking regions of *C3* (NM\_000064.2) were amplified by the polymerase chain reaction (PCR), using a forward primer (5'-CAA TTC CCA GGT CTC AGG GA-3') and a reverse primer (5'-GAG AGA AAA GGA GAA AGG G-3'). The 743 bp PCR product was digested with 5 U *Ban*II for 16 h at 37°C for restriction fragment length polymorphism analysis. In contrast to the wild type (171 bp only), an additional 273-bp band was detected in our patient (Fig. 1). Direct sequencing of the *C3* gene revealed that she had heterozygous mutation *1273C>T* in exon 12, that affects codon 425 resulting in substitution of arginine with cysteine (R425C) in the  $\beta$ -chain of C3 (Fig. 2). The family study could not be done due to proband's refusal.

## DISCUSSION

To the best of our knowledge, this is the first case report of a Korean patient with aHUS associated with the *c.1273C>T* mutation in the complement *C3* gene. Although direct evidence to define this polymorphism as a causative mutation for aHUS onset is lacking, *in silico* analyses assumed it as the potentially causative mutation [9].

The mutation was first demonstrated in an 8-month-old Japanese male infant, who had developed aHUS after cardiac surgery [10]. The infant died because of progressive renal and neurologic damage on postoperative day 50. Plasma infusions and hemodialysis were not effective. The response rate to short-term plasma therapy is known to be 40–50% [3]. In contrast, our patient experienced less severe renal damage than the infant and was responsive to plasma exchange. It suggests that the clinical response to plasma therapy in patients with a *C3* mutation is variable, depending on the stage of renal or neurologic damage. The father and aunt of the Japanese patient had the same heterozygous mutation but they had no history of aHUS. Our patient also has no family members who experienced symptomatic aHUS. It suggests that the penetrance of aHUS associated with the *c.1273C>T* mutation in the complement *C3* gene is low. This is consistent with a previous finding of only a 10% penetrance of aHUS in individuals with a mutation in *C3* as compared with higher penetrance rates in patients with mutations in the *CFH*, *CFI*, *MCP*, and *CFB* genes [11]. This means that a heterozygous carrier with the *c.1273C>T* mutation of *C3* itself may not develop aHUS without an additional environmental trigger or genetic modifier as a second hit, as suggested by Fan *et al.* [9].

The *c.1273C>T* mutation is located within the *C3* gene domain that binds with MCP. Therefore, we suspect that the pathogenesis of aHUS caused by *c.1273C>T* is likely to be associated with dysregulation of the alternative pathway, caused by prevention of proper binding of the MCP regulator to C3b.

Recently, eculizumab, a humanized monoclonal antibody that blocks complement C5 activation and the formation

of the terminal complement component, has been widely used for the treatment of aHUS in the USA, Europe, and Japan. Eculizumab has been reported to induce long-term remission in patients with aHUS associated with *C3* gene mutations [12, 13]. In conclusion, we describe the first Korean case of aHUS associated with the *c.1273C>T* mutation in the complement *C3* gene, where the patient was responsive to plasma exchange. Given the poor prognosis of aHUS and its low penetrance of development, identifying carriers in the family of this proband and properly educating them on how to avoid risk factors that trigger aHUS would be required to manage the patient and her carrier family members.

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## Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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## Delta beta thalassemia: a rare hemoglobin variant

**TO THE EDITOR:** Delta beta ( $\delta\beta$ )-thalassemia results from a deletion in both the delta and beta genes on chromosome 11. The gamma genes on the affected chromosome increase their production of gamma globin, thereby increasing the amount of hemoglobin F (HbF).  $\delta\beta$ -Thalassemia heterozygotes clinically show characteristics of thalassemia minor. However, homozygous  $\delta\beta$ -thalassemia may give a clinical picture of thalassemia intermedia with a mild anemia.

A 12-month-old boy presented to the hematology outpatient department for evaluation of pallor and jaundice that had been for the past 2 months. He had no history of a blood transfusion. His family history was insignificant for congenital anemia. His parents had a consanguineous marriage. Physical examination revealed pallor and palpable spleen 2 cm below the left costal margin. Other examination findings were unremarkable. A complete blood count (CBC) revealed an Hb level of 8.0 g/dL, WBC count of  $8.9 \times 10^9/L$ , and platelet count of  $341 \times 10^9/L$  (Table 1). A peripheral blood smear revealed anisopoikilocytosis with hypochromic microcytic red cells, target cells, and basophilic stippling (Fig. 1A). The corrected reticulocyte count was 1.6%. Liver function tests showed raised levels of serum total bilirubin (3.5 mg/dL) and indirect bilirubin (3.0 mg/dL). High-perform-

ance liquid chromatography (HPLC) showed 100% HbF and an absence of HbA and HbA2 (Fig. 1B). The Kleihauer-Betke test revealed a pancellular pattern (Fig. 1C). Consequently, a CBC followed by HPLC was performed for both parents who were apparently healthy and had no history of blood transfusions (Table 1). Kleihauer-Betke tests of both parents showed a heterocellular distribution of HbF.

Hence, the patient was diagnosed with homozygous  $\delta\beta$ -thalassemia, whereas the parents with heterozygous  $\delta\beta$ -thalassemia. Unfortunately, mutational analysis could not be performed because the patient was lost to follow-up.

$\delta\beta$ -Thalassemia results from the deletion of both  $\delta$  and  $\beta$  genes. Homozygotes for  $\delta\beta$ -thalassemia have 100% HbF and, because of the increased synthesis of HbF, may have thalassemia intermedia rather than thalassemia major [1].

However, the phenotype of heterozygotes resembles that of the  $\beta$ -thalassemia trait, but the HbA2 percentage is not increased and is often normal. HbF in such individuals is consistently elevated, varying from 5% to 20%. Peripheral blood film findings are similar to those for the  $\beta$ -thalassemia trait, and the distribution of HbF is heterocellular, which is best observed via flow cytometry. It is necessary to distinguish it from hereditary persistence of fetal hemoglobin (HPFH). The two groups of disorders are distinguished by the phenotype of heterozygous individuals. Heterozygotes of  $\delta\beta$ -thalassemia mutations have 5% to 20% HbF, which is heterocellularly distributed in red cells, whereas heterozygotes of HPFH mutations have 17% to 30% HbF, with a pancellular distribution. In addition, homozygotes of HPFH are asymptomatic, whereas  $\delta\beta$ -thalassemic homozygotes have thalassemia intermedia-like features [2].

At least nine mutations can result in  $\delta\beta$ -thalassemia. This type of thalassemia is observed in many ethnic groups, including some Mediterranean populations (Italians, Greeks, and Turks). Although the exact diagnosis of  $\delta\beta$ -thalassemia requires genetic analysis for mutations, Hb electrophoresis or HPLC findings of markedly elevated HbF may be suggestive. An extensive PubMed search was done to determine the incidence of  $\delta\beta$ -thalassemia in different parts of the world, but owing to the rarity of this Hb variant,

**Table 1.** Laboratory parameters of the case and parents.

	Case	Mother	Father
CBC			
Hb (g/dL)	8.0	12.6	13.8
Mean corpuscular volume (fL)	76.4	76.0	73.0
Mean corpuscular Hb (pg)	23.4	25.4	23.5
HPLC			
HbA (%)	0	79.8	81.7
HbA2 (%)	0	2.2	2.3
HbF (%)	100	18	16

Abbreviations: CBC, complete blood count; Hb, hemoglobin; HPLC, high-performance liquid chromatography.