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# Atypical Hemolytic Uremic Syndrome in Children Aged <2 Years

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**Keywords**
Atypical hemolytic uremic syndrome · Children · Plasma therapy · Eculizumab · Treatment · Outcome

**Abstract**
***Background:*** There are limited data on infants with atypical hemolytic uremic syndrome (aHUS). The aim of this study was to determine the clinical and laboratory features, and to evaluate treatment modalities and outcomes in infants with aHUS. ***Materials and Methods:*** Relevant data on patients with onset of aHUS at age <2 years were obtained from the Turkish Pediatric aHUS Registry. ***Results:*** Among the 146 patients included in the Registry, 53 (36%) (23 male and 30 female) were enrolled for the study. Age at disease onset was ≤1 year in 29 of the patients. In all, 21 (40%) of the patients developed neurological symptoms. Disease-causing mutations were noted in 14 (36%) of the 39 patients in which genetic analysis was performed. Plasma therapy was performed in 42 (79%) patients; eculizumab therapy was administered to treat the first episode of aHUS in 33 (62%) patients and in 5 patients as the first- line therapy. In total, 38 (72%) patients received renal replacement therapy (RRT), 3 (6%) died due to acute illness, and 4 (8%) were discharged from hospital with RRT. Follow-up visit data were available for 46 patients and the median duration was 23 months (range 3–129 months). End-stage renal disease developed only in 1 patient. Proteinuria and hypertension persisted in 17 (37%) and 20 patients (44%) respectively. Eculizumab treatment was continued in 25 of the 39 patients during the follow-up period. ***Conclusion:*** One-third of the aHUS patients had disease onset during infancy. The prognosis of this life-threatening disease seems to get better with improved treatment modalities.



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## Introduction

Hemolytic uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure [1]. HUS induced by Shigatoxin-producing Escherichia coli (STEC) is the most common type of the disease and generally has a good prognosis, but atypical HUS (aHUS) is a rare and heterogeneous disease, with a relapsing course and poor prognosis [2–4]. The frequency of aHUS in childhood is similar in boys and girls [5, 6], and it can occur at any time, from the perinatal period to adulthood. Upper respiratory tract infection, diarrhea, varicella, and H1N1 influenza trigger the development of the disease [2].

Atypical HUS was determined to be associated with complement alternative pathway dysregulation in the last two decades. In approximately 60–70% of aHUS patients mutations in the genes encoding complement regulatory proteins, components of the alternative pathway C3 convertase and anti-complement factor H autoantibodies have been reported [7, 8].

Although nearly 50% of aHUS patients have disease onset in childhood, especially during infancy, data are limited on the clinical features and treatment outcome in patients with infantile onset of aHUS [6]. The aim of the present study was to determine the demographic and clinical characteristics, and to evaluate treatment, clinical course, and outcome in patients with infantile onset aHUS.

## Materials and Methods

The Turkish Pediatric aHUS Registry was initiated in November 2013. The Registry includes retrospective and prospective patients with onset of aHUS at age <18 years that were diagnosed between January 2001 and December 2015, and were recorded in the database at www.ahusnet.org, a web-based national registry system. Data for patients with disease onset at age <2 years were obtained from the Registry and included in this study.

The complete triad of HUS is defined as hemolytic anemia characterized by a hemoglobin (Hb) level <10 g/dL, detection of schistocytes in peripheral blood smear with a negative Coombs test confirming microangiopathic hemolysis, thrombocytopenia with a platelet count $<150 \times 10^9$/L, and the presence of acute renal impairment. Patients with HUS secondary to drugs, an autoimmune disease, infection (caused by STEC, *Streptococcus pneumoniae*, or HIV), bone marrow or solid organ transplantation, or cobalamin deficiency were excluded. STEC-HUS was excluded with specific cultures of stool specimens and PCR was performed for the isolated EHEC serotypes.

All patients were screened for ADAMTS 13 deficiency. The activity of ADAMTS13 above 10% was considered normal. Serum creatinine was estimated based on Jaffe's reaction. The new Schwartz formula (K = 0.413) [9] was used to calculate the estimated glomerular filtration rate (eGFR). Chronic kidney disease staging was performed according to KDOQI Clinical Practice Guidelines for Chronic Kidney Disease [10]. Proteinuria was defined as ≥1+ via urine test strip analysis and/or a random urine protein creatinine ratio ≥0.5 mg/mg [11].

Beyond the immediate neonatal period, oliguria was defined as urine output <0.5 mL/kg/h or 500 mL/24 h/1.73 $m^2$. The plasma complement C3 level was measured using the nephelometric technique; if the decrease in C3 level was more than 10% (>0.07 g/L) of the lower limit of normal (N: 0.70–1.2 g/L), it was defined low. Complete remission was defined as normalization of the blood count (Hb >10 g/dL, thrombocyte count $>150 \times 10^9$/L, and lactate dehydrogenase level <450 U/L) and renal function (eGFR >90 mL/min/1.73 $m^2$, and no proteinuria). Complete hematologic recovery, with renal insufficiency or failure was considered as partial remission. Renal remission was defined as a normal eGFR. Renal sequelae were defined as eGFR ≤89 mL/min/1.73 $m^2$ and/or proteinuria and/or hypertension during the chronic period (>3 months). Recurrence was considered a new episode of aHUS occurring >4 weeks after remission.

*Genetic Analysis*

Mutation analysis via Sanger sequencing of the coding regions of the *CFH*, *CFI*, *MCP*, *CFB, C3*, *DGKE*, and *CHFR5* genes was performed in 37 of the 53 study patients at the Hacettepe University Nephrogenetics Laboratory, Ankara, Turkey, and in 2 patients, genetic analysis was performed at a private genetic diagnosis laboratory in Turkey. *CFHR1–3* deletion was evaluated via multiplex ligation-dependent probe amplification (MLPA) analysis. MLPA analyses were performed using SALSA MLPA probemix P236-A3 ARMD mix-1kit (MRC-Holland™), according to the manufacturer's recommendations.

A variation was considered disease-causing mutation if it introduces a stop, frameshift, abrogation of start or stop, or leads to the disruption of obligatory splice site in a corresponding gene

- In case of missense variation in a corresponding gene if (a) it causes a change in a continuously conserved aminoacid at least up to *Daniorerio* (zebrafish) (b) loss of function in human allele is supported by functional data in the literature, and (c) it has been previously described in the literature as disease causing and exists in available databases such as HGMD
- Heterozygous allele frequency is <1% in available databases (ExAC, 1000G etc.)
- Inslico analyses using various software (i.e., PolyPhen2, MutationTaster, SIFT for missense variants and Human Splicing-Finder 3.0 forsplice site variations) predicts as pathogenic

Anti-complement Factor H autoantibody was searched using the CFH IgG ELISA Kit (Abnova™), according to the manufacturer's recommendations, with a detection limit of 0.6 AU/mL.

The study protocol was approved by the Hacettepe University Ethics Committee (FON10/03-22) and written informed consent was obtained from the parents of each patient.

*Statistical Analysis*

Data were analyzed using IBM SPSS Statistics for Windows version 21 (IBM Corp., Armonk, NY, USA). Descriptive statistical analysis was used to evaluate demographics and clinical data, and the results are shown as mean, median, SD, and range. Frequency tables were used to describe categorical data.

**Table 1.** Clinical and laboratory features of patients at first attack

| | *n* = 53, *n* (%) |
|---|---|
| Female/male | 30/23 |
| Age at diagnosis, month, mean ± SD | 11.64±6 |
| Age range, month | |
| ≤6 | 13 (24) |
| 7 to ≤12 | 16 (30) |
| 13 to ≤24 | 24 (46) |
| Triggering events | |
| Diarrhea | 32 (60) |
| Respiratory infections | 11 (21) |
| Varicella | 1 (2) |
| Extrarenal involvement | 33 (62) |
| Neurologic | 21 (40) |
| Gastrointestinal | 7 (13) |
| Respiratory system | 3 (5) |
| Cardiac | 2 (3.7) |
| Anuria | 16 (30.2) |
| Duration, day, median (range) | 5 (1–15) |
| Oliguria | 29 (54.7) |
| Duration, day, median (range) | 3 (1–30) |
| Hemoglobin, g/dL, mean ± SD | 7.67±1.81 |
| Hemoglobin <10 g/dL | 48 (90) |
| Platelet count, $\times 10^9$/L, mean ± SD | 73±41 |
| Creatinin, mg/dL, mean ± SD | 2.47±1.54 |
| eGFR, mL/min/1.73 $m^2$, mean ± SD | 23.09±24.67 |
| LDH, IU/L, mean ± SD | 2,110±1,158 |
| Complete triad | 45 (85) |
| Proteinuria | 44 (83) |
| Decreased C3[a] | 28 (57) |

[a] Data are available for 49 patients.

eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase.

## Results

*Patient Characteristics*

Among the 146 aHUS patients in Turkish Pediatric aHUS Registry, 53 (36%) who had disease onset at age <2 years (range 7 days – <2 years) were included in the study. Clinical and laboratory features of the patients at disease onset are shown in Table 1. There were no familial cases. In total, 60% of the patients had diarrhea (4 of the patients had bloody diarrhea and 1of them also had invagination). Gastrointestinal involvement was seen as pancreatitis (*n* = 3), gallstones (*n* = 2), and ischemic hepatitis (*n* = 2). Cranial MRI was performed in 20 of the patients with neurologic manifestations and the findings were abnormal in 11, including ischemic changes or/infarct (*n* = 7), signal changes secondary to hypertension (*n* = 1), hemorrhage (*n* = 1), and non-specific changes (*n* = 2). Among the patients with neurologic involvement, 1 had ischemic hepatitis, 1 had cardiomyopathy, and 1 had pancreatitis. Acute respiratory distress syndrome requiring mechanical ventilation occurred in 3 patients, 2 of whom faced complications caused by cardiomyopathy during the disease course.

Eight patients did not have complete triad at onset. The Hb level was >10 g/dL in 4 patients who had uremia and thrombocytopenia, and the thrombocyte count was $150 \times 10^9$/L-$190 \times 10^9$/L in 3 patients who had microangiopathic hemolytic anemia and uremia. In 1 patient with homozygous *MCP* mutation, the creatinine level was normal, but anemia and thrombocytopenia were present. All patients had an elevated lactate dehydrogenase level.

*Genetics*

Genetic analysis was completed for all corresponding genes in 39 (74%) of the patients who had an available DNA sample, and mutations were noted in 14 (36%), as follows: *CFH*: (*n* = 3); *C3*: (*n* = 2); *MCP*: (*n* = 1); *CFB*: (*n* = 1); *CFB/MCP*: (*n* = 1); *CFB/CFR 1–3 del*: (*n* = 1); *CFI*: (*n* = 1); *DGKE*: (*n* = 4). Among the patients, 8 had 2 mutations; homozygous or compound heterozygous. Anti-CFH antibody was not detected in any of the patients.

The genetic variations and patient characteristics are summarized in Table 2.

*Treatment*

Treatment and outcome data of the patients during the first attack are shown in Table 3. The majority of the patients (79%) were treated with plasma therapy. The daily dose of plasma infusion was 10–20 mL/kg, and plasma exchanged volume for each session was 40–60 mL/kg. Plasma therapy was combined with dialysis in 34 patients. Fourteen patients were treated with plasma only, 8 (57%) of them achieved complete hematologic and renal remission, 2 had hematologic remission but no renal remission, and 4 had neither hematologic nor renal remission. Five patients received eculizumab as a first-line treatment at the first attack of aHUS. The median age of these patients was 10 months (range 2–15 months). Three of them underwent peritoneal dialysis, and 4 (80%) had complete remission. The remaining patient was discharged from hospital with a low GFR, but the patient was not on dialysis. No statistically significant difference was found according to disease outcome between patients treated with plasma and eculizumab (*p* = 0.603).Twenty-eight patients were treated with plasma and eculizumab; complete remission was achieved in 22 (78%) of them. In the remain-

**Table 2.** Detected genetic variations and patients' characteristics

| Patient (No. 14) | Disease-causing mutations (references of known mutations) | Zygosity | Age at onset, month | Follow-up time, month | Diarhea | Extrarenal involment | C3 mg/dL N >70 mg/dL | Relaps | Treatment at first episode | Eculizumab during follow-up | eGFR at last visit mL/min/1.73 $m^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *CFH*:p.Tyr1177Cys [23] | Hom | 1 week | 54 | – | – | 61 | – | PD, PI | Started 2 years later after first attack Continued | >90 |
| 2 | *CFH*: p.Tyr711del [28] | Het | 8 | 57 | – | Neurologic Ischemichepatitis | 76 | – | HD, PI, PE | Started 1 year later after first attack Continued | >90 |
| 3 | *CFH*: c.245-18insTT [29] | Het | 23 | 14 | – | – | 105 | – | PD, PI ECU | Continued | >90 |
| 4 | *DGKE*: p.Arg337Stop [in dbSNP) [28] | Hom | 11 | 12 | + | Neurologic | 77 | 1 | HD, PE | Started at second attack | Exitus |
| 5 | *DGKE*:p.Leu316Pro [28] | Hom | 8 | 111 | – | – | 130 | 4 | PD, PI | Started at 8 years after first attack Continued | >90 |
| 6 | *DGKE*:p.Gln143Stop; p.Pro378Arg [28] | C-Het | 2.5 | 10 | + | – | 97 | 1 | PD, PI | Started at second attack Continued | >90 |
| 7 | *DGKE*:p.Ser41Metfs*2 [28] | Hom | 5 | 11 | – | ARDS | 69 | – | PD, PI ECU | Discontinued | >90 |
| 8 | *MCP*: c.286 + 2T>G [30] | Hom | 7 | 27 | – | – | 107 | – | – | – | >90 |
| 9 | *CFB*: p.Val42Ala [28] | Het | 7 | 130 | – | – | 60 | – | – | – | >90 |
| 10 | *CFB*: p.Glu566Ala [31] MCP: c.286 + 18_27delT [28] | Het Het | 15 | 22 | + | – | 70 | – | ECU | Continued | >90 |
| 11 | *CFB*: p.Glu566Ala [31]; p.Lys350Asn; [32] *CFHR1-3Del* | C-Het Het | 6 | 120 | – | – | 35 | – | PD, PI | Started at prophylactically before Tx Continued | 80 |
| 12 | *C3*: p.Arg1042Leu [33] | Het | 7.5 | 38 | – | Neurologic ARDS | 49 | – | PI, ECU | Continued | >90 |
| 13 | *C3*: c.1-5delG [28] | Het | 14.5 | 42 | – | Neurologicinvolvement | 65 | – | PD, ECU | Continued | 55 |
| 14 | *CFI*: p.Asp403Asn [13] | Het | 10 | 46 | – | – | 53 | – | ECU | Discontinued | >90 |

Hom, homozygous; Het, heterozygous; C-Het, compound heterozygous; CFH, complement factor H; CFHR, CFH-related protein; CFI, complement factor I; CFB, complement factor B; MCP, membrane cofactor protein; DGKE, diacylglycerolkinase-ε; ARDS, acute respiratory distress syndrome; PD, peritoneal dialysis; HD, hemodialysis, PI, plasma infusion; PE, plasma exchange; ECU, eculizumab; eGFR, estimated glomerular filtration rate; Tx, renal transplantation.

ing 6 patients; 3 had renal, 1 hematologic, and 2 had hematologic and renal impairment. The overall outcome of the patients at the first episode of aHUS is also shown in Table 3. Renal replacement therapy (RRT) was performed in 38 (72%) patients. The causes of death were pulmonary hemorrhage, resistant seizures, and sepsis.

*Long-Term Follow-Up*

Follow-up data are shown in Table 4. During the follow-up, only 1 patient received plasma infusion therapy for 8 years, and then was switched to eculizumab after the drug was available. In addition to 33 patients who received eculizumab for the first episode of aHUS, 6 addi-

tional patients were treated with eculizumab during the follow-up period (Tables 3, 4). Three of these 6 patients had complement and 3 had *DGKE* mutations (Table 2). One of the patients (No. 4) with *DGKE* mutation died at the second attack of aHUS despite plasma and eculizumab treatment (Table 2). There were no relapses in the follow-up period with eculizumab treatment in the remaining 5 patients who survived, they were in full remission.

Eculizumab treatment was withdrawn in 14 of 39 patients for various reasons, including family choice, genetic testing results, drug availability, and physician decision. Two of these patients (No. 7, 14) had disease-causing mutations (Table 2). At the last follow-up visit, the median creatinine level was 0.44 mg/dL (range 0.26–0.8 mg/dL) and the median eGFR was 102 mL/min/1.73 $m^2$ (range 67–198 mL/min/1.73 $m^2$) in these 14 patients and relapses did not occur. The remaining 25 (64%) patients, including 5 additional patients in whom eculizumab was started during the follow-up period, were maintained on eculizumab treatment. Among these patients, only 1 relapse occurred when eculizumab was retarded for a few days due to an inter-current infection. At the follow-up last visit, the median creatinine level was 0.52 mg/dL (range 0.2–1.3 mg/dL) and the median eGFR was 106 mL/min/1.73 $m^2$ (range 43–225 mL/min/1.73 $m^2$) in these 25 patients.

In total, 7 relapses of aHUS occurred in 4 patients, including 1 patient who had just 1 hematologic relapse. The time from the onset of aHUS and relapse of disease ranged from 4 months to 2 years. Six relapses occurred in 3 patients (No. 4, 5, and 6) with *DGKE* mutation (Table 2). One of the patients who relapsed had no mutation.

In all, 6 (11%) patients were not treated with plasma or eculizumab during the first attack of aHUS and did not receive any treatment during the follow-up period. At their last follow-up visit, 5 of the 6 patients were in full remission, whereas the remaining 1 had a renal sequela, an eGFR of 77 mL/min/L/1.73 $m^2$, and mild proteinuria.

End-stage renal disease developed in 1 patient (No. 11) before eculizumab was available and was treated only with plasma. This patient carried both a *CFB* mutation and *CFHR1–3* deletion (Table 2).

## Discussion

The present findings show that 36% of the patients from the Turkish Pediatric aHUS Registry had disease onset at age <2 and that 20% had onset at age <1. Our study and earlier studies clearly show that the period from birth till 2 years is a critical time period for aHUS patients

**Table 3.** Treatment and outcome of patients at first attack

| | *n* = 53, *n* (%) |
|---|---|
| Plasma therapy | 42 (79) |
| PI | 23 (43) |
| PE | 9 (17) |
| PI + PE | 10 (18) |
| Eculizumab therapy | 33 (62) |
| First line | 5 (15) |
| Second line | 28 (85) |
| Renal replacement therapy | 38 (72) |
| PD | 23 (60) |
| HD | 6 (16) |
| PD + HD | 4 (10) |
| CRRT | 5 (13) |
| Supportive treatment only[a] | 6 (11) |
| Complete remission | 39 (74) |
| Renal remission | 42 (79) |
| Hematologic remission | 49 (92) |
| RRT at the time of discharge from hospital | 4 (8) |
| Mortality (during the acute episode) | 3 (6) |

[a] No PI/PE, no eculizumab.

PI, plasma infusion; PE, plasma exchange; PD, peritoneal dialysis; HD, hemodialysis; CRRT, continuous renal replacement therapy; RRT, renal replacement therapy.

**Table 4.** Treatment and outcome of the patients during follow-up

| | *n* = 46, *n* (%) |
|---|---|
| Follow-up time, months | |
| Mean ± SD | 31.44±30 |
| Median (range) | 22.68 (3–129) |
| End stage renal disease | 1 (2) |
| Kidney transplantation | 1 (2) |
| eGFR, mL/min/1.73 $m^2$ | |
| >90 | 38 (83) |
| 60–89 | 7 (15) |
| 30–59 | 1 (2) |
| Proteinuria | |
| No proteinuria | 29 (63) |
| Non nephrotic range | 15 (33) |
| Nephrotic range | 2 (4) |
| Hypertension | 20 (44) |
| Eculizumab treatment | 39 (74) |
| Continued | 25 (64) |
| Duration of follow-up with eculizumab, month, median (range) | 23 (3–50) |
| Discontinued | 14 (36) |
| Eculizumab free follow-up, month, median (range) | 15 (2–30) |
| Overall relaps | 7 (15) |

eGFR, estimated glomerular filtration rate.

and that special attention is required for its diagnosis and treatment [5, 6, 12, 13].

Gastroenteritis (most frequently non-bloody diarrhea) and upper respiratory tract infection were the most common precipitating factors for aHUS in the present study, as reported earlier [6, 9, 12]. Although, aHUS is characterized by renal manifestations, additional involvement can occur, including cardiovascular, pulmonary, and gastrointestinal system [14]. Neurological symptoms, including visual disturbance, seizures, reduced consciousness, agitation, and coma occur in 8–30% of patients with HUS [7, 15]. Among the patients in the present study, nearly half of the patients developed neurological symptoms, more than 10% had gastrointestinal involvement, 6% had acute respiratory distress syndrome, and 3% cardiomyopathy that increase morbidity and mortality.

More than 70% of our patients required dialysis during the first episode of aHUS. However the rate of progression to end-stage renal disease was 2% and overall mortality rate was 6%. Similarly, previous studies reported that most children with aHUS have acute renal failure and >50% require dialysis during the first episode of aHUS [5, 6, 16]. However, detailed analysis about the prognosis of infantile aHUS was not extensively evaluated in the previous studies; thus, it is hard to arrive at firm conclusions about the prognosis of infantile aHUS [5, 6, 12, 13, 16].

Disease-causing mutations, mostly heterozygous, were noted in 44–60% of patients with aHUS, of which approximately 30% had *CFH* mutation [6, 12, 13]. In the present study, disease-causing mutations were observed in 14 (36%) patients in whom genetic analysis was performed, of which 11 had disease onset at age <1. Ten of these mutations were complement mutations, and *CFH* and *CFB* mutations were the most common. Interestingly, *MCP* mutations were observed in 2 patients in the present study; such mutations are very rarely reported in patients of this age [6]. In our study, 4 of the 14 patients had *DGKE* mutations and all were <1 year of age. Three of them had disease relapses and 1 died due to septicemia. These findings are compatible with the findings of earlier reports of frequent relapses and poor prognosis in patients with *DGKE* mutations [17].

Before the availability of eculizumab, the European Pediatric Study Group for HUS 2009 Guidelines [18] primarily recommended plasma therapy as the first-line treatment in patients with aHUS. Plasma treatment may provide partial or complete recovery, but more than half of the patients experienced aHUS relapses [6, 12, 16]. More recent data show an improvement in the prognosis of aHUS in pediatric patients, primarily due to early diagnosis and intensive plasma therapy [7]; however, the intensity and duration of plasma therapy remains unknown because of high variability in the severity of aHUS. Moreover, low total blood volume and technical difficulties associated with vascular access pose significant challenges to successful plasma therapy, especially in young children. In 2014, it was reported the effects of early and intensive plasma therapy for aHUS showed that 31% of children experienced catheter-related complications [14]. Our present findings show that during the first episode of aHUS, 14 infants were treated with plasma therapy only and relapse occurred in 4 of the patients.

Although soon after the discovery of *DGKE* mutations early aggressive and continuous plasma infusion was recommended to prevent podocyte damage in aHUS patients carrying *DGKE* mutation [19], treatment of these patients has not yet been resolved. In our study, 4 patients with *DGKE* mutation were treated with eculizumab. One died during the second attack, Eculizumab was continued in 2, discontinued in 1 patient without relapses during the follow-up. One of the patients (No. 5) with *DGKE* mutation experienced 4 relapses during regular plasma therapy (every 2 weeks) for 8 years (Table 2). The patient was subsequently followed by treatment with eculizumab and no relapses were observed over four years. These results suggest that some patients with *DGKE* mutation also seem to respond well to eculizumab.

Eculizumab, a humanized monoclonal antibody that blocks the terminal complement pathway by targeting C5, was approved by the US Food and Drug Administration for the treatment of aHUS in September 2011. This new drug was reported to be safe and effective in children in several case reports and clinical trials [20–23]. In a prospective study reported by Greenbaum et al. [24], 64% of 22 pediatric patients achieved complete remission after 26 weeks of treatment. A recent international consensus on the management of aHUS in children also proposed eculizumab as a first-line treatment, when possible [25]. In the present study, 33 of the 45 patients who were diagnosed as aHUS after 2011 received eculizumab treatment during the first episode; 5 of these patients used the drug as first-line treatment.

Discontinuation of eculizumab maintenance therapy was reported in 10 patients with a genetic defect in complement-regulating genes and/or anti-CFH antibodies, and 3 of these patients had disease relapsed within 6 weeks of discontinuation of eculizumab therapy [26]. It was recently reported that relapses occurred after a mean follow-up of 22 months in 12 of 38 aHUS patients in which eculizumab was discontinued. All 12 of the relapsed patients

had a *CFH* or *MCP* mutation, and 9 of them were children [27]. In the present study, all patients with *CFH* mutations received uninterrupted eculizumab treatment. In 1 patient (No. 14) with *CFI* mutation, eculizumab treatment was discontinued after an acute episode of aHUS and 1 patient (No. 8) with a homozygous *MCP* mutation did not receive eculizumab treatment (Table 2). Furthermore, no relapses occurred during the follow-up period in any of the patients with complement mutations. Although most of the patients in the present study received eculizumab following the onset of aHUS, it was discontinued in 14 patients who were monitored closely during follow-up for the signs of recurrence. End-stage renal disease developed in 1 patient (No. 11) with both a *CFB* mutation and *CFHR1–3* deletion for whom eculizumab therapy was not available at onset of aHUS (Table 2). Subsequently, cadaveric transplantation was performed in this patient, followed with continuous prophylactic eculizumab treatment and satisfactory kidney function for 4 years.

The present study has some limitations, including few patients with genetic mutations for effective clinical comparison and interpretation of prognosis. Shortcomings of the Turkish Pediatric aHUS Registry system may have resulted in incomplete data concerning the family history of aHUS. Lastly, other limitations include lack of standardized patient treatment and care.

In conclusion, the present study is the first study including the clinical findings and prognosis in patients with pure infantile onset aHUS. The prognosis of infantile aHUS has improved in recent years following the advent of plasma and eculizumab therapy. Considering the fact that onset of aHUS was <2 years in a substantial number of patients, clinicians should be well informed about infantile aHUS, and pediatric nephrology centers should be sufficiently equipped for the proper management of such patients.


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## Ethics Statement

The study protocol was approved by the Hacettepe University Ethics Committee (FON10/03-22) and written informed consent was obtained from the parents of each patient.

## Disclosure Statement

The authors declare that they have no conflict of interest.

## Author Contributors

N.Ç. designed, wrote the manuscript, and analysed of data, Z.B.O. and F.Y. designed, wrote the manuscript, F.O. evaluated and wrote genetic results and revised the text. E.K. carried out the molecular genetic studies. O.S. revised the text. M.K., B.C.A., E.B., B.G., A.Y., and S.Y. participated in collection of data. All authors have read and approved the final manuscript.

## References


1 Ruggenenti P, Noris M, Remuzzi G: Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Kidney Int 2001;60:831–846.

2 Noris M, Remuzzi G: Atypical hemolytic-uremic syndrome. N Eng J Med 2009;361:1676–1687.

3 Loirat C, Frémeaux-Bacchi V: Atypical hemolytic uremic syndrome. Orphanet J Rare Dis 2011;6:60.

4 Besbas N, Karpman D, Landau D, Loirat C, Proesmans W, Remuzzi G, Rizzoni G, Taylor CM, Van de Kar N, Zimmerhackl LB; European Paediatric Research Group for HUS: A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int 2006;70:423–431.

5 Licht C, Ardissino G, Ariceta G, Cohen D, Cole JA, Gasteyger C, Greenbaum LA, Johnson S, Ogawa M, Schaefer F, Vande Walle J, Frémeaux-Bacchi V: The global aHUS registry: methodology and initial patient characteristics. BMC Nephrol 2015;16:207.

6 Sellier-Leclerc AL, Fremeaux-Bacchi V, Dragon-Durey MA, Macher MA, Niaudet P, Guest G, Boudailliez B, Bouissou F, Deschenes G, Gie S, Tsimaratos M, Fischbach M, Morin D, Niveet H, Alberti C, Loirat C: Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. J Am Soc Nephrol 2007;18:2392–2400.

7 Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, Daina E, Fenili C, Castelletti F, Sorosina A, Piras R, Donadelli R, Maranta R, van der Meer I, Conway EM, Zipfel PF, Goodship TH, Remuzzi G: Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol 2010;5:1844–1859.

8 Bu F, Maga T, Meyer NC, Wang K, Thomas CP, Nester CM, Smith RJ: Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome. J Am Soc Nephrol 2014;25:55–64.
9 Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL: New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009;20:629–637.
10 Kidney Disease Outcomes Quality Initiative: KDOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. IV. Definition and Classification of Stages of Chronic Kidney Disease, 2002. http:/www2.kidney.org/professionals/kdoqi/guidelines_ ckd/p4_class.htm.
11 Hogg RJ, Portman RJ, Milliner D, Lemley KV, Eddy A, Ingelfinger J: Evaluation and management of proteinuria and nephrotic syndrome in children: recommendations from a pediatric nephrology panel established at the national kidney foundation conference on proteinuria, albuminuria, risk, assessment, detection, and elimination (PARADE). Pediatrics 2000;105:1242–1249.
12 Geerdink LM, Westra D, van Wijk JA, Dorresteijn EM, Lilien MR, Davin JC, Kömhoff M, Van Hoeck K, van der Vlugt A, van den Heuvel LP, van de Kar NC: Atypical hemolytic uremic syndrome in children: complement mutations and clinical characteristics. Pediatr Nephrol 2012;27:1283–1291.
13 Fremeaux-Bacchi V, Fakhouri F, Garnier A, Bienaime F, Dragon-DureyMA, Ngo S, Moulin B, Servais A, Provot F, Rostaing L, Burtey S, Niaudet P, Deschênes G, Lebranchu Y, Zuber J, Loirat C: Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. Clin J Am Soc Nephrol 2013;8:554–562.
14 Johnson S, Stojanovic J, Ariceta G, Bitzan M, Besbas N, Frieling M, Karpman D, Landau D, Langman C, Licht C, Pecoraro C, Riedl M, Siomou E, van de Kar N, Walle JV, Loirat C, Taylor CM: An audit analysis of a guideline for the investigation and initial therapy of diarrhea negative (atypical) hemolytic uremic syndrome. Pediatr Nephrol 2014;29:1967–1978.
15 Eriksson KJ, Boyd SG, Tasker RC: Acute neurology and neurophysiology of haemolytic-uraemic syndrome. Arch Dis Child 2001;84:434–435.
16 Taylor CM, Chua C, Howie AJ, Risdon RA; British Association for Paediatric Nephrology: Clinico-pathological findings in diarrhoea-negative haemolytic uraemic syndrome. Pediatr Nephrol 2004;19:419–425.
17 Lemaire M, Frémeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, Fakhouri F, Taque S, Nobili F, Martinez F, Ji W, Overton JD, Mane SM, Nürnberg G, Altmüller J, Thiele H, Morin D, Deschenes G, Baudouin V, Llanas B, Collard L, Majid MA, Simkova E, Nürnberg P, Rioux-Leclerc N, Moeckel GW, Gubler MC, Hwa J, Loirat C, Lifton RP: Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet 2013;45:531–536.
18 Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, Loirat C, Pecoraro C, Taylor CM, Van de Kar N, Vandewalle J, Zimmerhackl LB; European Paediatric Study Group for HUS: Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatr Nephrol 2009;24:687–696.
19 Westland R, Bodria M, Carrea A, Lata S, Scolari F, Fremeaux-Bacchi V, D'Agati VD, Lifton RP, Gharavi AG, Ghiggeri GM, Sanna-Cherchi S: Phenotypic expansion of DGKE-associated diseases. J Am Soc Nephrol 2014;25:1408–1414.
20 Michaux K, Bacchetta J, Javouhey E, Cochat P, Frémaux-Bacchi V, Sellier-Leclerc AL: Eculizumab in neonatal hemolytic uremic syndrome with homozygous factor H deficiency. Pediatr Nephrol 2014;29:2415–2419.
21 Szarvas N, Szilágyi Á, Tasic V, Nushi-Stavileci V, Sofijanova A, Gucev Z, Szabó M, Szabó A, Szeifert L, Reusz G, Rusai K, Arbeiter K, Müller T, Prohászka Z: First-line therapy in atypical hemolytic uremic syndrome: consideration on infants with a poor prognosis. Ital J Pediatr 2014;40:101.
22 Vilalta R, Lara E, Madrid A, Chocron S, Muñoz M, Casquero A, Nieto J: Long-term eculizumab improves clinical outcomes in atypical hemolytic uremic syndrome. Pediatr Nephrol 2012;27:2323–2326.
23 Besbas N, Gulhan B, Karpman D, Topaloglu R, Duzova A, Korkmaz E, Ozaltin F: Neonatal onset atypical hemolytic uremic syndrome successfully treated with eculizumab. Pediatr Nephrol 2013;28:155–158.
24 Greenbaum LA, Fila M, Ardissino G, Al-Akash SI, Evans J, Henning P, Lieberman KV, Maringhini S, Pape L, Rees L, van de Kar NC, Vande Walle J, Ogawa M, Bedrosian CL, Licht C: Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. Kidney Int 2016;89:701–711.
25 Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A, Coppo R, Emma F, Johnson S, Karpman D, Landau D, Langman CB, Lapeyraque AL, Licht C, Nester C, Pecoraro C, Riedl M, van de Kar NC, Van de Walle J, Vivarelli M, Frémeaux-Bacchi V; HUS International: An international consensus approach to the management of atypical hemolytic uremic syndrome in children. Pediatr Nephrol 2016;31:15–39.
26 Ardissino G, Testa S, Possenti I, Tel F, Paglialonga F, Salardi S, Tedeschi S, Belingheri M, Cugno M: Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome: a report of 10 cases. Am J Kidney Dis 2014;64:633–637.
27 Fakhouri F, Fila M, Provôt F, Delmas Y, Barbet C, Châtelet V, Rafat C, Cailliez M, Hogan J, Servais A, Karras A, Makdassi R, Louillet F, Coindre JP, Rondeau E, Loirat C, Frémeaux-Bacchi V: Pathogenic variants in complement genes and risk of atypical hemolytic uremic syndrome relapse after Eculizumab discontinuation. Clin J Am Soc Nephrol 2017;12:50–59.
28 Besbas N, Gulhan B, Soylemezoglu O, Ozcakar ZB, Korkmaz E, Hayran M, Ozaltin F: Turkish pediatric atypical hemolytic uremic syndrome registry: initial analysis of 146 patients. BMC Nephrology 2017;18:6.
29 Abrera-Abeleda MA, Nishimura C, Smith JL, Sethi S, McRae JL, Murphy BF, Silvestri G, Skerka C, Józsi M, Zipfel PF, Hageman GS, Smith RJ: Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). J Med Genet 2006;43:582–589.
30 Fremeaux-Bacchi V, Moulton EA, Kavanagh D, Dragon-Durey MA, Blouin J, Caudy A, Arzouk N, Cleper R, Francois M, Guest G, Pourrat J, Seligman R, Fridman WH, Loirat C, Atkinson JP: Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. J Am Soc Nephrol 2006;17:2017–2025.
31 Marinozzi MC, Vergoz L, Rybkine T, Ngo S, Bettoni S, Pashov A, Cayla M, Tabarin F, Jablonski M, Hue C, Smith RJ, Noris M, Halbwachs-Mecarelli L, Donadelli R, Fremeaux-Bacchi V, Roumenina LT: Complement factor B mutations in atypical hemolytic uremic syndrome-disease-relevant or benign? J Am Soc Nephrol 2014;25:2053–2065.
32 Roumenina LT, Jablonski M, Hue C, Blouin J, Dimitrov JD, Dragon-Durey MA, Cayla M, Fridman WH, Macher MA, Ribes D, Moulonguet L, Rostaing L, Satchell SC, Mathieson PW, Sautes-Fridman C, Loirat C, Regnier CH, Halbwachs-Mecarelli L, Fremeaux-Bacchi V: Hyperfunctional C3 convertase leads to complement deposition on endothelial cells and contributes to atypical hemolytic uremic syndrome. Blood 2009;114:2837–2845.
33 Maga TK, Nishimura CJ, Weaver AE, Frees KL, Smith RJ: Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. Hum Mutat 2010;31:E1445–E1460.