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Genetics and Consequences of Atypical Hemolytic-uremic Syndrome in Turkish Children

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Original Paper

Introduction. Atypical hemolytic uremic syndrome (aHUS) is associated with mutations or antibodies that affect the regulation of the alternative complement pathway. Several studies were published recently, describing these mutations. We present the initial clinical findings, treatments, and long-term follow-up results of 19 patients hospitalized with the diagnosis of aHUS.
Methods. Nineteen patients who were diagnosed as aHUS were enrolled from January 2010 to March 2017. Initial clinical signs and clinical follow-up of patients with aHUS were evaluated. The reasons for complement factor H (CFH) mutations were investigated.
Results. CFH mutations were detected in 5 of the 19 aHUS cases. Of these, one was novel, while four were previously reported. We reported here the clinical course of aHUS patients with CFH previously defined mutations (p.Glu936Asp, Val 1197Ala) and a novel mutation (Glu927Lys), which caused previously defined aHUS. Two of the CFH mutation cases developed end-stage kidney disease that required hemodialysis, and one patient developed chronic kidney disease. Two cases were in remission; one of them under supportive therapy and the other one in remission with eculizumab treatment.
Conclusions. Morbidity rates are higher in children with aHUS. However, renal prognosis and morbidity rates are higher in children with CFH mutations than other children with aHUS. Poor prognosis in aHUS-children with CFH mutation depends on the genetic background.

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INTRODUCTION

Atypical hemolytic uremic syndrome (aHUS) is a serious disease related to the dysregulation of the complement system.[1] aHUS has a tendency to relapse, is associated with a positive family history, and has a poor outcome. Families with aHUS may have intrinsic abnormalities of the complement system, such as the mutations in complement factor H (CFH), membrane cofactor protein (MCP), complement factor I, deficiency in Von Willebrand factor-cleaving protease (VWF-CP) activity, presence of anti-factor H antibodies, or combinations of mutations for CFH and MCP.[2] Recently identified mutations have been identified in the gene encoding diacylglycerol kinase ε (DGKE).[3] Genetic mutations play a role in such activation, and above fifty percent of the patients with aHUS have causative mutations in complement genes[4]. Each day new information accumulates to unfold the genetic background of aHUS. Clinical features and patient outcomes are identified, and genotype and phenotype correlations continue to be established. Because of the low incidence of genetic aHUS, new patient records are reported to assess the prognosis

of the disease. The genetic abnormalities of patients from various ethnic backgrounds may be different and, thus, the characteristics of aHUS may vary between patient populations. CFH is a regulatory alternative pathway protein and locates in the cluster of complex activation regulators (CRA) in the CFH gene chromosome 1q32.[5] For the first time in 1998, Warwicker *et al.* identified mutations in three families and sporadic aHUS cases[6] and was the commonest genetic abnormality in many cohorts of aHUS patients. Richards *et al.* found 18, 19, and 20 exons FH mutations in 19 familial and 31 sporadic aHUS cases.[7] The prognosis of the disease correlates to some degree with the type of the genetic defect. It has been reported that the genotype-phenotype association in aHUS cases is imperative in predicting the course of the disease.[8] The recurrence rate is between 70% and 90%, and poor graft function is the worst graft function among all atypical HUS forms due to mutation. The CFH-mutated aHUS cases have the worst clinical course. It has been reported that 60%-70% of the cases with CFH-mutation develop end-stage kidney disease (ESKD) one year after the onset or even death.[9] We studied 19 sporadic cases with aHUS in a center in Izmir, Turkey; and investigated genetic variations seen in aHUS patients. This study provides an overview of the initial findings. We wanted to report a long-term prognosis in CHF mutated patients. We also reported the clinical features of these 19 Turkish patients. Demographic, clinical, laboratory and genetic characteristics of children with aHUS and their treatment were evaluated. Here we describe a series of 5 CHF-mutated patients with a clinical diagnosis of atypical HUS.

MATERIALS AND METHODS

Patients who were diagnosed as having aHUS were prospectively enrolled from January 2010 to May 2017. Patients with multiple episodes of microangiopathic hemolytic anemia and thrombocytopenia accompanied by acute renal failure were considered as aHUS.[10-12] To exclude other causes of secondary aHUS, the presence of bone marrow transplantation and drug use were questioned in all cases, tests for Streptococcus pneumonia, Influenza A, H1N1, HIV, and viral load polymerase chain reaction (PCR) were performed. Patients with ADAMTS13 activity lower than 5% or having anti-ADAMTS13 antibodies were excluded. Stool or rectal swab culture for STEC and real-time PCR for Stx genes performed in all patients at the time of the diagnosis. Antinuclear antibodies, lupus anticoagulants, and antiphospholipid antibodies were investigated in all patients to exclude HUS associated with other autoimmune diseases. All HUS cases that were due to secondary causes such as infections, systemic or autoimmune diseases and patients who had anti-factor H autoantibodies were excluded from the study. CFH mutation was studied in a total of 19 aHUS patients.

Laboratory data were obtained before performing plasma exchange in all cases. Acute kidney injury was defined as an increase in baseline serum creatinine levels (sCr) according to the pediatric RIFLE criteria (risk, serum creatinine × 1.5; damage, serum creatinine × 2; insufficiency, sCr × 3).[13] eGFR was calculated using the modified Schwartz formula.[14] All cases were followed up regularly in our clinic until May 2017. eGFR < 15 mL/min/1.73m^2 was defined as ESKD during follow-up.[15] Hematological remission was defined as having a platelet count of ≥ 150.000/mm^3 and low lactate dehydrogenase at two consecutive measurements in the absence of hemolytic findings.[16] A predicted glomerular filtration rate (eGFR) > 90 mL/min/1.73m^2 and the absence of proteinuria were defined as renal remission. Age, sex, laboratory data, plasma treatments, renal replacement therapy, and clinical follow-up were recorded in patients with aHUS. Physical examination findings, laboratory data, and genetic results, therapies applied during the acute phase (e.g., plasma treatments, plasma infusion and/or plasma exchange, eculizumab, antihypertensive, hemodialysis, peritoneal dialysis, and continuous renal replacement therapies), renal/hematological status, proteinuria, hypertension, serum creatinine and hemoglobin levels and platelet count were recorded. The plasma volume for plasma exchange was calculated as 20 mL/kg per session. The plasma exchange was performed once a day and continued for two days after a complete recovery.

All cases were regularly followed-up at the outpatient clinics of the Ege university children hospital in İzmir, Turkey from January 2010 to March 2017. During the follow-up, patients requiring chronic renal replacement were considered as ESKD. The study was approved by the ethical committee of Ege university hospital. Written

informed consent was obtained from the parents of all patients.

According to the algorithm we had established in our clinic, we primarily studied the CFH gene mutation in aHUS cases. In patients with a negative CFH gene mutation, we investigated the other aHUS-causing complement genes. In this study, we aimed to establish genotype and phenotype relationships by performing CFH gene mutation analysis in patients with aHUS, as well as to evaluate the results of treatment and long-term prognosis in patients with negative or positive mutations.

Genetic Studies

The genomic DNA was obtained from 200 µL sample of 1 mL peripheral blood in EDTA-anticoagulated tube. DNA was extracted by using an automatic DNA isolation method with magnetic beads (Invitrogen Co. Paisley UK). DNA measurements were done under 260/280 nm wavelength by a NanoDrop Spectrophotometry instrument. PCR amplification for all coding exons of the CFH gene was performed using oligonucleotide primers specific to each exon and a Platinum Taq polymerase with enhancer buffer. PCR amplification was performed using the Veriti thermal cycler. The PCR products were purified by enzymatic methods using Exo SAP. Big Dye chemistry sequencing was done after purification of the PCR samples. Purified samples were put on the ABI 3130XL genetic analyzer automated DNA sequencing instrument, and nucleotide sequences were read according to the pikes. Nucleotide changes after DNA sequencing were compared with the gene bank and protein database reference sequences in the web pages of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/).

Statistical Analysis

Descriptive statistical methods were used to present demographic and clinical data. Mean, median, standard deviation, and interquartile range (IQR) was calculated for numeric variables. Tables were used to define categorical data in frequencies. Data were analyzed by the Statistical Package for Social Sciences v. 21 (SPSS Inc., Chicago, IL, USA).

RESULTS

From January 2010 to May 30, 2017, 19 patients (9 male, 10 female) were admitted to the pediatric nephrology department with the diagnosis of aHUS. All 22 CFH exons were sequenced and analyzed for all patients. CFH mutations were detected in five of the 19 aHUS cases. We presented the clinical progression of five atypical HUS patients with Factor H mutations. All study participants were from Turkey. None of the patients had a family history of aHUS or kidney disease. The mean age of diagnosis was 5.9 ± 2.3 years (range = 1-16 years). The first presenting symptoms of the patients were diarrhea (8 patients, 42.1%), dark urine (6 patients, 31.6%), pale color of the skin, edema, and jaundice (5 patients, 26.3%). Gastrointestinal symptoms such as vomiting were the most common extra renal symptoms (16 patients, 84.2%). Three (15.8%) cases were accompanied by neurological findings such as loss of consciousness, headache, and encephalopathy. Five patients (26.3%) were under two years of age, six patients (31.6%) were between two and five years of age, and eight patients (42.1%) were older than five years. All patients had thrombocytopenia, hemolytic anemia, and acute renal failure. Ten patients (52.6%) were accompanied by hypertension. Demographic, clinical, and laboratory features of the patients are presented in Table 1.

Treatment options and long-term clinical outcomes were evaluated. The mean follow-up duration was 4.47 ± 2.1 years (range, 2-7 years). Seven patients (36.8%) achieved remission with supportive treatment. Plasma exchange was performed in eleven patients (57.8%). During the initial diagnosis, 15 patients (78.9%) required acute renal replacement therapy, 5 cases hemodialysis, and 10 cases peritoneal dialysis. Ten patients (52.6%) received eculizumab treatment after the approval of the ministry of health of Turkey according to the regulations and parental consent. Eculizumab treatment was still continuing in only one case. The other 9 instances received eculizumab treatment for three months, and they did not need further doses. Oral penicillin prophylaxis was given before eculizumab treatment to all patients. Meningococcal vaccination was performed during the therapy with eculizumab. None of the patients treated with plasma exchange and eculizumab had any relapses or worsened renal function.

In the long-term clinical follow-up, two patients (10.5%) progressed to ESKD. One of the patients

Table 1. Demographic Features and Clinical Profiles of Patients with aHUS

Characteristic	aHUS
Patients	19
Male, n (%)	9 (47.3%)
Mean Age at Onset Year, ± SD (min-max)	8.6 ± 3.8 (4-19)
Family History, n (%)	0
Mean Age at Diagnosis, ± SD (min-max)	5.9 ± 2.3 (1-16)
< 2 years	5 (26.3%)
2 - 5 years	6 (31.5%)
> 5 years	8 (42.1%)
Duration of the Follow-up Time, ± SD (min-max)	7.3 ± 3.2 years (8-3)
Clinical Findings	
Anemia, n (%)	19 (100%)
Acute Kidney Injury, n (%)	19 (100%)
Thrombocytopenia, n (%)	19 (100%)
Hypertension, n (%)	10 (52.6%)
Dialysis Required, n (%)	15 (78.9%)
Presenting Symptom	
Diarrhea	8 (42.12%)
Dark Urine	6 (31.57%)
Other Symptoms (Pale Color of the Skin, Edema, Jaundice)	5 (26.31%)
Extra Renal Involvement	
Gastrointestinal System, n (%)	16 (84.22%)
Central Nervous System, n (%)	3 (15.78%)
Low C3 Level, n (%)	2 (10.5%)

(case 5) developed ESKD after the first episode and was followed up in the chronic hemodialysis program. Eculizumab treatment could not be given to this patient because this drug was not in use during the first episode. Eculizumab treatment was applied when the patient was in the chronic hemodialysis program, but kidney damage did not show any improvement. She died after six years of follow-up due to complications of hemodialysis (case 5). The other patient (case 9) developed ESKD after the third episode and was in the chronic dialysis program. Eculizumab treatment could not be performed during the first episode of this patient because this therapy was not available in Turkey in 2010. Therefore, in this patient, eculizumab administered after the third episode. Eculizumab treatment did not improve renal function. The patient had a living donor kidney transplantation. There was no complication in the patient after renal transplantation. Eculizumab treatment was resumed after the patient had a recurrence at the 6th month of transplantation. In the end, this patient has been followed up for three years with remission and has good graft function (Table 2). Characteristics and outcomes of aHUS patients in the study are shown in Table 2. All 22 CFH exons were sequenced and analyzed in all patients. CFH mutation was present in 5 (26.3%) of the patients. Of these mutations, 4 were already

Table 2. Characteristics and Outcomes of Atypical HUS Patients Included in this Study

Case no	Gender (F/M)	Duration of the Disease, years	PE	RRT	Low C3	Tx	Eculizumab	ESRD	Current State	Follow-up, years
1	F	11	+	-	-	-	+	-	Remission	2
2	M	1	+	-	-	-	+	-	Remission	2
3	M	2	-	-	-	-	-	-	Remission	4
4	F	4	+	+	-	-	+	-	Remission	3
5	F	4	+	+	+	-	+	+	ESKD Died	6
6	F	1	-	-	-	-	-	-	Remission	4
7	M	4	-	+	-	-	+	-	Remission	4
8	M	1	-	-	-	-	+	-	CKD on Eculizumab	3
9	M	8	+	+	+	+	-	+	ESKD Remission (with tx) on Eculizumab	7
10	M	5	-	+	-	-	-	-	Remission	6
11	M	12	+	+	-	-	-	-	Remission	6
12	F	16	+	+	-	-	-	-	Remission	4
13	M	7	+	+	-	-	-	-	Remission	5
14	F	1	-	-	-	-	+	-	Remission	4
15	F	11	+	-	-	-	-	-	Remission	8
16	F	2	+	-	-	-	+	-	Remission	3
17	F	4	-	-	-	-	+	-	Remission	4
18	F	15	+	-	-	-	-	-	Remission	5
19	M	1	-	+	-	-	+	-	CKD	5

PE: Plasma Exchange, RRT: Renal Replacement Therapy, Tx: Transplantation, ESKD: End Stage Kidney Disease, CKD: Chronic Kidney Disease

Table 3. Mutations in Patients with atypical HUS

Case number	Mutation type	Nucleotide substitution	Genotype	Exon	Renal outcome
1	p.Glu936Asp	2808G>T	Heterozygous	18	Remission
5	p.Glu936Asp	2808G>T	Homozygous	18	ESKD
6	Val 1197Ala	3590T>C	Heterozygous	21	Remission
8	Glu936Asp	2808G>T	Heterozygous	18	CKD
9	Glu927Lys	1204G>T	Homozygous	9	ESKD

ESKD: End Stage Kidney Disease, CKD: Chronic Kidney Disease

reported in aHUS cases.[13-16] However, we found one new mutation in our patients (Glu927Lys). This mutation has not previously been published in the Human Gene Mutation Database in aHUS patients. Clinical course of the instances that had CFH mutation were as follow: two patients (case numbers 5 and 9) had ESKD (40%), one case (case number 8) developed chronic kidney disease (20%), one case was in remission with eculizumab (case 6), and one case (case 1) was in remission after short-term eculizumab treatment (Table 3). Table 3 shows detailed information about cases that had CHF mutations.

DISCUSSION

The pathogenic mechanisms behind the endothelial damage and microvesicular thrombosis caused by aHUS were linked to deteriorations in complement regulation. The genetics of aHUS is complex. Complement gene mutations may have different results from pathogenic mutations associated with the disease and other mutations and/or variants that result in CFH and MCP together with aHUS.[8] Mutations were found in sporadic patients, clustered on the exons 18-20. Familial HUS is a heterogeneous condition.[7] Mutations are commonly found in FH and membrane cofactor protein, whereas mutations within C3 and complement factor I occur less frequently. The alternative pathway of complement activation is regulated with the contribution of the plasma protein Factor H. A common area bounded by D1S212 markers, and D1S306 is a distance of 26 cm in 1q32 separated by disease (Zmax 3.94). It has been shown that the H factor gene is present in the region.[6] Approximately, 60% of the aHUS cases have genetic defects causing mutations in the components of the alternative pathway or FH autoantibodies. Complement factor H (CFH) mutation is the most common type of mutation in aHUS cases. It accounts for 23% to 27% of the mutations in the aHUS cases in the United States and Europe.[4,15,16] In a study in Hungary, CHF mutations were detected in 48.1% of (13 out of 27) aHUS cases.[15] In our study, 26.3% of the aHUS cases were found to have CFH mutations. This proportion is similar to those found in Europe and in the United States. We showed that CFH mutations in our Turkish cohort were about 26.3%. Novel mutations that are associated with aHUS are discovered as genetic investigations of aHUS cases continue. The ratio of CFH mutations among aHUS cases increases with new mutation discoveries. The clinical prognosis of patients with aHUS depends on the type of mutations. Frequent FH mutations usually have a poor prognosis, with combined mortality or ESKD rates of 50% to 70% and recurrence rates of 50%. Three out of four patients with CFH mutations die or develop ESKD.[16] The prognosis of our patients with CFH mutation was not favorable. Throughout the follow-up period, patients with CFH mutations had mortality and ESKD development rates of 20% and 40%, respectively. Two patients (40%) with CFH mutations had recurrent episodes. Two of the 5 patients with CFH mutations developed ESKD. Two cases with ESKD additionally showed low serum levels of C3. The mutations of these two patients were homozygous p.Glu936Asp and homozygous Glu927Lys. The complement profile of these cases illustrates the functional effect of the mutations. Low levels of C3 indicate an increase in the complement consumption and an increase in the complement consumption by the alternative path activity. Among our patients, two mutations (CFH Glu927Lys, p.Glu936Asp) showed alternative path activity and decreased C3 levels. These data can be considered as a sign of alternative path activity and an alternative path disorder of low C3 levels. In summary, out of the 19 patients, 5 had

potentially identified disease-causing mutations in the candidate genes pre-defined. One new mutation (Glu927Lys) and the two previously analyzed (p.Glu936Asp, Val 1197Ala) CFH mutations could be demonstrated.

CONCLUSIONS

In conclusion, this study endorses the significance of new mutations and reflects the contribution of these mutations in the pathogenesis of aHUS. In this study, CFH mutated clinical course of HUS patients was presented. We also detected a new mutation (Glu927Lys) associated with aHUS.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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