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Original Article

Official Journal of Erciyes University Faculty of Medicine

DOI: 10.14744/cpr.2026.91265
J Clin Pract Res 2026;48(0):0–0

Complement Genetic Findings and Clinical Phenotypes in Adult Atypical Hemolytic Uremic Syndrome

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ABSTRACT

Objective: To characterize genetic findings, clinical phenotypes, kidney outcomes, and eculizumab withdrawal in adults with atypical hemolytic uremic syndrome (aHUS).

Materials and Methods: This single-center, retrospective study reviewed 44 adults who underwent next-generation sequencing (NGS)-based aHUS/thrombotic microangiopathy (TMA) panel testing between 2010 and 2022. After excluding patients with secondary TMA, individuals undergoing asymptomatic donor evaluations, and those with insufficient follow-up, 24 patients were included in the primary cohort. Given the small and unequal genetic subgroups, analyses were descriptive, without formal between-group hypothesis testing.

Results: All 24 patients underwent therapeutic plasma exchange, 15 received eculizumab, and 18 required hemodialysis during the acute phase. Final patient-level genetic classifications were pathogenic/likely pathogenic in 2 patients (8.3%), variants of uncertain significance (VUS) in 6 (25.0%), and benign/likely benign variants or no significant complement variants in 16 (66.7%). During follow-up, 12 patients (50.0%) had an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2, and 7 (29.2%) progressed to end-stage kidney disease. Among the 15 eculizumab-treated patients, 2 had pathogenic/likely pathogenic variants, 5 had VUS, and 8 had benign/likely benign variants or no significant complement variants. Eculizumab was discontinued in all 15 patients. After excluding 5 patients who discontinued treatment because of irreversible kidney dysfunction or nonresponse, 2 of the remaining 10 monitored patients experienced relapse. One patient carried the likely pathogenic CD46 p.Tyr189Asp variant, whereas the other had no pathogenic complement variant but carried the benign/likely benign C3 p.Arg102Gly variant. Eculizumab was restarted in both patients.

Conclusion: Severe kidney outcomes and relapse following treatment withdrawal were not confined to a single genetic category. Genetic findings should inform, but not independently determine, surveillance strategies or decisions regarding complement inhibitor withdrawal. Longitudinal clinical phenotype, kidney recovery, prior disease severity, and the capacity for rapid retreatment remain essential considerations.

Keywords: Atypical hemolytic uremic syndrome, complement, eculizumab, genetic variants, kidney outcomes, thrombotic microangiopathy.

Cite this article as:
Ural Z, Güz G, Derici Ü. Complement Genetic Findings and Clinical Phenotypes in Adult Atypical Hemolytic Uremic Syndrome. J Clin Pract Res 2026;48(0):0–0.

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Submitted: 06.06.2026
Revised: 17.09.2026
Accepted: 05.10.2026
Available Online: 09.11.2026

Erciyes University Faculty of Medicine Publications - Available online at www.jcpres.com

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INTRODUCTION

Atypical hemolytic uremic syndrome (aHUS), increasingly referred to as complement-mediated hemolytic uremic syndrome (HUS), is a thrombotic microangiopathy (TMA) characterized by microangiopathic hemolytic anemia, thrombocytopenia, and organ injury, most notably acute kidney injury. Diagnosis requires the exclusion of other TMAs, particularly Shiga toxin-associated HUS and thrombotic thrombocytopenic purpura, as well as an assessment of secondary endothelial insults. Dysregulation of the alternative complement pathway may result from rare genetic variants, acquired abnormalities, or a combination of genetic susceptibility and environmental triggers.[1–4]

Genetic testing can support etiologic assessment and inform genetic counseling, transplantation planning, family evaluation, and discussions regarding relapse risk. However, the detection of a sequence variant does not necessarily establish causality. According to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) framework, a variant of uncertain significance (VUS) should not be used as a stand-alone molecular diagnosis or considered evidence of pathogenicity.[5] Conversely, the absence of a pathogenic variant does not exclude complement-mediated HUS, as disease expression is influenced by incomplete penetrance, genetic susceptibility, acquired complement abnormalities, and environmental or endothelial triggers. Furthermore, conventional genetic panels do not capture the full spectrum of complement dysregulation.[4,6] Functional complement studies, segregation data, population allele frequencies, and periodic variant reinterpretation can further refine genetic assessments. Terminal complement blockade with eculizumab has substantially improved clinical outcomes; however, the optimal duration of treatment and criteria for identifying patients in whom treatment can be safely discontinued remain unresolved.[7–13]

Adult cohorts integrating contemporary genetic variant interpretation with kidney outcomes and treatment withdrawal remain limited. Therefore, we aimed to characterize complement genetic findings, clinical phenotypes, kidney outcomes, and eculizumab discontinuation in a cohort of adults with aHUS.

KEY MESSAGES

- Genetic findings in atypical hemolytic uremic syndrome should be interpreted in the clinical context; variant classification alone neither confirms pathogenicity nor excludes clinically significant complement-mediated disease.
- Surveillance after treatment withdrawal should be individualized based on the longitudinal clinical phenotype, kidney recovery, relapse history, triggering factors, and genetic findings rather than genotype alone.
- Because relapse risk remains difficult to predict, shared decision-making, close monitoring, and prompt access to retreatment are essential following the discontinuation of complement inhibition.

MATERIALS AND METHODS

Study Setting and Design

This single-center, retrospective observational study was conducted at the Gazi University, Division of Nephrology. The study was conducted in accordance with the Declaration of Helsinki and was approved by Gazi University Faculty of Medicine Clinical Research Ethics Committee (Approval Number: 2022-914, Date: 26.07.2022). Adult patients who underwent next-generation sequencing (NGS)-based aHUS panel testing between January 2010 and December 2022 were retrospectively reviewed.

Patients and Data Collection

Electronic medical records, laboratory information systems, pathology reports, treatment records, and nephrology follow-up files were reviewed. A total of 44 adults with available aHUS genetic panel results were identified. Twenty individuals were excluded from the primary analysis of symptomatic patients for the following reasons: malignancy-associated TMA (n=5), transplantation-related immunosuppressive drug-associated TMA (n=2), insufficient clinical data or ongoing follow-up at an external center (n=7), and asymptomatic individuals who underwent testing during kidney donor evaluation (n=6). Thus, the primary cohort comprised 24 symptomatic adults (Fig. 1).

Diagnostic Criteria

The diagnosis of aHUS was based on a compatible TMA phenotype and the exclusion of alternative primary and secondary causes of TMA. Clinical and laboratory findings supporting TMA included microangiopathic hemolytic anemia, thrombocytopenia, acute kidney injury, and, when available, kidney biopsy findings consistent with TMA. Evidence of microangiopathic hemolysis was assessed using lactate dehydrogenase (LDH) levels, haptoglobin levels, and the presence of schistocytes on peripheral blood smears. Kidney biopsy findings consistent with TMA were considered supportive but were not required for diagnosis. Acute kidney injury was assessed according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria. Severe

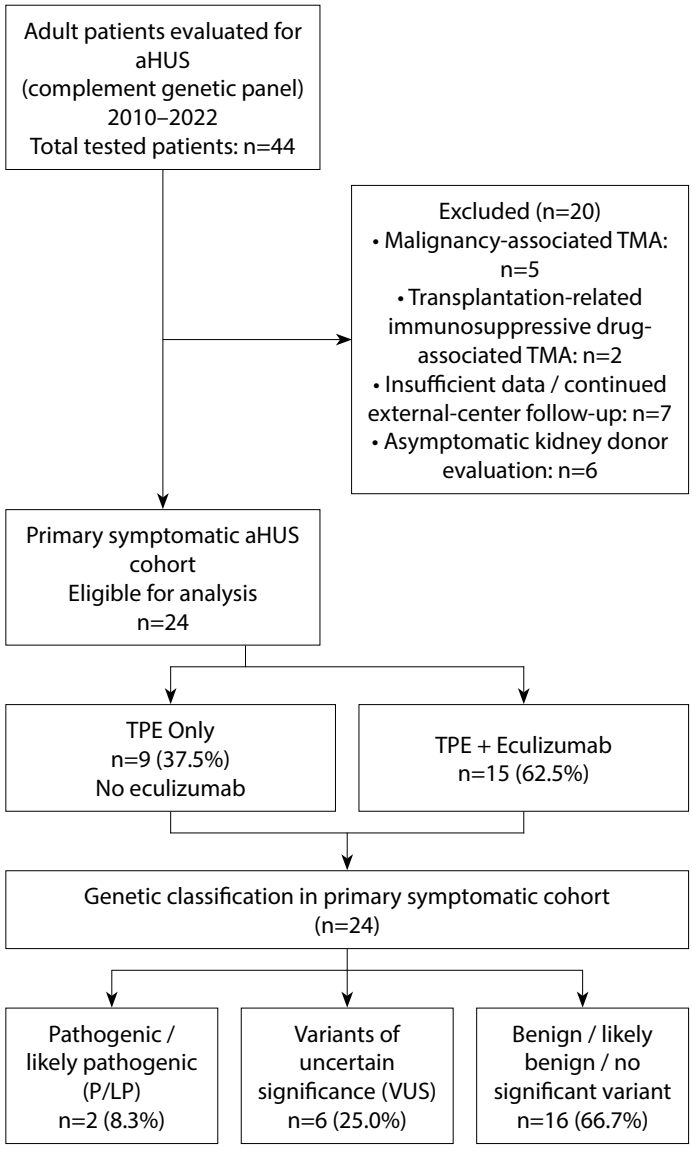


Figure 1. Study flow and genetic classification of the primary adult aHUS cohort included in the analysis. The flow diagram illustrates patient selection, reasons for exclusion, treatment groups, and final patient-level genetic classification within the primary cohort of 24 patients (P/LP, n=2; VUS, n=6; benign/likely benign variants or no significant complement variants, n=16).

aHUS: Atypical hemolytic uremic syndrome; P/LP: Pathogenic/likely pathogenic; TMA: Thrombotic microangiopathy; TPE: Therapeutic plasma exchange; VUS: Variant of uncertain significance.

ADAMTS13 deficiency (activity <10%), consistent with thrombotic thrombocytopenic purpura, and Shiga toxin-associated HUS were excluded when clinically suspected and appropriate testing was available. Potential disease triggers, including pregnancy, infection, severe hypertension, autoimmune disease, transplantation, malignancy, and drug exposure, were systematically reviewed.

Definitions

End-stage kidney disease (ESKD) was defined as dialysis dependence persisting for >90 days or receipt of a kidney transplant. An estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 during follow-up was evaluated as an additional kidney outcome. A schistocyte proportion >1% was considered supportive of TMA.[14] Relapse was defined as the recurrence of clinical and laboratory features of TMA following hematologic and/or kidney remission.

Inclusion Criteria

The primary clinical cohort included adults (≥18 years) with a clinically established aHUS phenotype, available NGS-based aHUS/TMA panel testing results, and sufficient clinical, treatment, and follow-up data to assess outcomes.

Exclusion Criteria

Patients were excluded from the primary cohort if an alternative cause of TMA was established, including Shiga toxin-associated HUS, severe ADAMTS13 deficiency (activity <10%) consistent with thrombotic thrombocytopenic purpura, malignancy-associated TMA, or transplantation-related immunosuppressive drug-associated TMA. Other clearly attributable secondary TMA syndromes were also excluded when considered responsible for the clinical presentation. Patients with insufficient clinical follow-up or incomplete outcome data, as well as asymptomatic individuals tested during kidney donor evaluation, were excluded from the primary clinical outcome cohort.

Clinical, Laboratory, and Genetic Investigations

Baseline data included age, sex, potential triggering conditions, serum creatinine, hemoglobin, platelet count, LDH, haptoglobin, peripheral blood smear findings, dialysis requirements, extrarenal involvement, treatment exposure, relapse, and kidney outcomes. ADAMTS13 activity was assessed in patients considered for eculizumab treatment to exclude severe ADAMTS13 deficiency consistent with thrombotic thrombocytopenic purpura. Stool culture and/or Shiga toxin testing was performed when enteric HUS was clinically suspected.

NGS-based testing included ADAMTS13, C3, CD46, CFB, CFH, CFHR1–CFHR5, CFI, DGKE, MASP2, PLG, and THBD; the complete panel is presented in Appendix 1. Variants were described using the ACMG/AMP terminology. For patient-level analyses, complement genetic findings were classified into three mutually exclusive categories: pathogenic/likely

Table 1. Baseline clinical and laboratory characteristics of the primary aHUS cohort (n=24)

Variables	Overall cohort
Demographic characteristics	
Age, years, median (minimum–maximum)	30 (22–61)
Female sex, n (%)	13 (54.2)
Potential triggers	
No identified trigger, n (%)	9 (37.5)
Pregnancy-associated trigger, n (%)	4 (16.7)
Infection-associated trigger, n (%)	7 (29.2)
Drug-associated trigger, n (%)	4 (16.7)
Kidney involvement	
Serum creatinine at diagnosis, mg/dL, mean±SD	6.3±4.2
Hemodialysis during the acute episode, n (%)	18 (75.0)
Hematologic findings	
Hemoglobin at diagnosis, g/dL, mean±SD	9.0±2.2
Platelet count, $\times10^3$/µL, median (minimum–maximum)	84 (20–370)
Schistocytes >1%, n (%)	22 (91.7)
LDH, U/L, median (minimum–maximum)	872 (340–4724)
Haptoglobin, mg/dL, median (minimum–maximum)	2 (1–30)
Extrarenal involvement	
CNS involvement, n (%)	5 (20.8)
Hypertension requiring IV treatment, n (%)	9 (37.5)
Cardiovascular involvement, n (%)	1 (4.2)
Follow-up	
Follow-up duration, months, median (minimum–maximum)	22 (4–108)

Values are presented as n (%) unless otherwise indicated. aHUS: Atypical hemolytic uremic syndrome; CNS: Central nervous system; IV: Intravenous; LDH: Lactate dehydrogenase; SD: standard deviation.

pathogenic (P/LP), VUS, and benign/likely benign or no significant complement variant. When multiple variants were identified in the same patient, a hierarchical classification approach was applied, with a P/LP finding taking precedence over a co-occurring VUS. ADAMTS13 findings were evaluated separately as part of the differential diagnosis of TMA. Variant-level findings are presented in Table 2.

Treatment and Follow-up

Treatment decisions were individualized based on disease severity, hematologic activity, kidney involvement, and response to initial therapy. Therapeutic plasma exchange (TPE) was administered to all 24 patients during the acute phase. Eculizumab was initiated in patients with persistent or recurrent TMA activity and/or inadequate kidney recovery despite TPE, including persistent thrombocytopenia, ongoing dialysis requirements, or insufficient improvement in kidney function.

Eculizumab was administered according to the standard adult dosing regimen of 900 mg intravenously once weekly for four weeks, followed by 1200 mg in week 5 and every two weeks thereafter. Vaccination against Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae, along with antimicrobial prophylaxis, was provided in accordance with institutional practices and prevailing recommendations for terminal complement inhibition.

Eculizumab exposure, treatment duration, discontinuation, and follow-up after discontinuation were retrospectively determined from medical records. Eculizumab treatment duration was defined as the interval between the first administered dose and the final dose before discontinuation. Off-treatment follow-up was analyzed separately and was not included in the treatment duration. No prespecified tapering protocol was used. Following eculizumab discontinuation, longitudinal follow-up data were reviewed to assess TMA recurrence, hematologic activity, kidney function, dialysis requirements, and reinitiation of complement inhibition.

Statistical Analysis

Given the rarity of aHUS, the small cohort size, and the P/LP subgroup comprising only two patients, the revised analysis was intentionally descriptive. Continuous variables were summarized as median (minimum–maximum) or mean±standard deviation, as appropriate, and categorical variables were expressed as numbers and percentages. No formal between-group hypothesis testing, p values, multiple-comparison procedures, or time-to-event or survival analyses were performed. Statistical summaries were generated using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Cohort Selection and Baseline Characteristics

Of the 44 adults who underwent aHUS/TMA genetic panel testing, 20 were excluded from the primary clinical cohort for the reasons described above, leaving 24 patients for outcome analysis. The median age was 30 years (range, 22–61 years), and 13 patients (54.2%) were female. All 24 patients underwent therapeutic plasma exchange during the acute episode, and 18 (75.0%) required hemodialysis. Nine patients were managed without complement inhibition, whereas 15 received eculizumab. Baseline demographic, clinical, and laboratory characteristics are summarized in Table 1.

Table 2. Genetic findings across the aHUS/TMA Gene Panel in the primary cohort (n=24)

A. Final patient-level genetic classification		
Patient-level category	**Patients, n (%)**	**Genes represented**
Pathogenic/likely pathogenic	2 (8.3)	CFH (pathogenic); CD46 (likely pathogenic)
Variants of uncertain significance (VUS)	6 (25.0)	CFHR2, CFHR4, THBD, PLG, C3, CFHR5
Benign/likely benign or no significant complement variant	16 (66.7)	CFB, MASP2, CFH, C3, THBD; or no significant complement variant
ADAMTS13 findings are presented separately in Panel B as part of the TMA differential diagnostic workup and do not determine the complement genetic classification of aHUS.		
B. Genetic variants identified in the cohort, grouped by study classification		
Gene	**Variant (HGVS)**	**Zygosity**
Pathogenic/likely pathogenic variants		
CFH	NM_000186.4:c.3644G>A (p.Arg1215Gln) — Pathogenic	Heterozygous
CD46	NM_002389.4:c.565T>G (p.Tyr189Asp) — Likely pathogenic	Homozygous
Variants of uncertain significance (VUS)		
CFHR2	NM_005666.4:c.602C>T (p.Pro201Leu)	Heterozygous
CFHR4	NM_001201551.1:c.909C>A (p.Asp303Glu)	Heterozygous
THBD	NM_000361.2:c.127G>A (p.Ala43Thr)	Heterozygous
PLG	NM_000301.3:c.266G>A (p.Arg89Lys)	Heterozygous
C3	NM_000064.4:c.2338G>A (p.Glu780Lys)	Heterozygous
CFHR5	NM_030787.4:c.1067G>A (p.Arg356His)	Heterozygous
Additional VUS detected in a P/LP variant carrier		
CFHR3	NM_021023.5:c.522A>T (p.Lys174Asn)	Homozygous
CFHR3	NM_021023.5:c.613+434G>A	Homozygous
Benign/likely benign variants and common polymorphisms		
CFB	NM_001710.5:c.95G>A (p.Arg32Gln)	Heterozygous
CFB	NM_001710.5:c.672C>T (p.Tyr224=)	Heterozygous
MASP2	NM_006610.3:c.1111G>T (p.Asp371Tyr)	Heterozygous
CFH	NM_000186.4:c.2808G>T (p.Glu936Asp)	Heterozygous
C3	NM_000064.4:c.304C>G (p.Arg102Gly)	Heterozygous
CFB	NM_001710.5:c.94C>T (p.Arg32Trp)	Heterozygous
C3	NM_000064.4:c.941C>T (p.Pro314Leu)	Heterozygous
THBD	NM_000361.3:c.1456G>T (p.Asp486Tyr)	Heterozygous
ADAMTS13 findings: TMA differential diagnostic component		
ADAMTS13	NM_139025.4:c.2936G>A (p.Arg979Gln)	Heterozygous
ADAMTS13	NM_139025.4:c.19C>T (p.Arg7Trp)	Heterozygous
ADAMTS13	NM_139025.4:c.1342C>G (p.Gln448Glu)	Heterozygous
ADAMTS13	NM_139025.4:c.1852C>G (p.Pro618Ala)	Heterozygous
ADAMTS13	NM_139025.4:c.3097G>A (p.Ala1033Thr)	Heterozygous

Patient-level categories are mutually exclusive and hierarchical. CFH p.Arg1215Gln is classified as pathogenic, and CD46 p.Tyr189Asp is classified as likely pathogenic. Additional CFHR3 VUS in the CD46 variant carrier do not alter the patient-level classification. THBD p.Ala43Thr, PLG p.Arg89Lys, and CFHR5 p.Arg356His are retained as VUS for the purposes of this study. C3 p.Arg102Gly is classified as benign/likely benign and is presented only as genetic context for relapse. ADAMTS13 findings are included as part of the differential diagnosis and do not determine the complement genetic classification of aHUS. aHUS: Atypical hemolytic uremic syndrome; HGVS: Human Genome Variation Society; P/LP: Pathogenic/likely pathogenic; TMA: Thrombotic microangiopathy; VUS: Variant of uncertain significance.

Table 3. Kidney outcomes according to final genetic classification

Final genetic category	Patients, n (%)	Genes represented	eGFR <30 mL/min/1.73 m² during follow-up, n/N (%)	Progression to ESKD, n/N (%)	Relapse following eculizumab withdrawal, n
Pathogenic/likely pathogenic	2 (8.3)	CFH, CD46	2/2 (100)	1/2 (50.0)	1
VUS	6 (25.0)	CFHR2, CFHR4, THBD, PLG, C3, CFHR5	4/6 (66.7)	2/6 (33.3)	0
Benign/likely benign or no significant complement variant	16 (66.7)	CFB, MASP2, CFH, C3, THBD; or no significant complement variant	6/16 (37.5)	4/16 (25.0)	1
Overall cohort	24 (100)	—	12/24 (50.0)	7/24 (29.2)	2/10 (20.0)*

The eGFR <30 mL/min/1.73 m² outcome and ESKD are not mutually exclusive; ESKD represents the more severe kidney endpoint. *Relapse denominator. eGFR: Estimated glomerular filtration rate; ESKD: End-stage kidney disease; VUS: Variant of uncertain significance.

Genetic Findings

Final patient-level genetic classification identified P/LP complement variants in 2 of 24 patients (8.3%), variants of uncertain significance (VUS) in 6 (25.0%), and benign/likely benign variants or no significant complement variants in 16 (66.7%). The P/LP group included one patient with CFH p.Arg1215Gln, classified as pathogenic, and one with homozygous CD46 p.Tyr189Asp, classified as likely pathogenic. The six patients in the VUS group carried variants involving CFHR2, CFHR4, THBD, PLG, C3, or CFHR5. Two additional CFHR3 VUS were identified in the patient carrying the CD46 variant and were retained in the variant-level analysis without assigning the patient to an additional VUS category. ADAMTS13 findings were reported separately as part of the differential diagnosis of TMA. Variant-level findings are summarized in Table 2.

Kidney Outcomes

During follow-up, 12 of 24 patients (50.0%) had an eGFR <30 mL/min/1.73 m², and 7 (29.2%) progressed to ESKD. An eGFR <30 mL/min/1.73 m² was observed in 2/2 patients in the P/LP group, 4/6 in the VUS group, and 6/16 in the benign/likely benign or no significant complement variant group. Progression to ESKD occurred in 1/2, 2/6, and 4/16 patients, respectively.

Given the small and unequal genetic subgroups, these kidney outcomes are presented descriptively, without formal between-group hypothesis testing. Kidney outcomes stratified by final genetic classification are summarized in Table 3.

Eculizumab Exposure, Discontinuation, and Relapse

Among the 15 patients who received eculizumab, 2 were classified in the P/LP group, 5 in the VUS group, and 8 in the benign/likely benign or no significant complement variant group. Eculizumab was discontinued in all 15 patients during follow-up. In five patients, treatment was discontinued because of irreversible kidney dysfunction or a lack of meaningful kidney recovery; therefore, these patients were not considered evaluable for relapse following treatment withdrawal.

Of the remaining 10 patients monitored after treatment withdrawal, 2 (20.0%) experienced relapse. One patient carried homozygous CD46 p.Tyr189Asp, classified as likely pathogenic. The other had no identified pathogenic complement variant but carried the benign/likely benign C3 p.Arg102Gly polymorphism. Eculizumab was promptly reinitiated in both patients and continued thereafter. Treatment exposure and outcomes following treatment withdrawal are summarized in Table 4.

DISCUSSION

In this adult cohort with aHUS, we observed substantial heterogeneity in both complement genetic findings and clinical outcomes. P/LP variants were identified in only 2 of 24 patients, whereas severe kidney outcomes occurred across all three genetic categories. Relapse following eculizumab withdrawal was also observed in two genetically distinct patients, including one without an identified pathogenic complement variant. Although previous studies have demonstrated genotype-specific differences in kidney prognosis, particularly poorer outcomes associated with certain CFH and CFI abnormalities compared with CD46-associated disease, the small and unequal subgroups in the present cohort preclude meaningful comparative conclusions.[7,15] Therefore, our findings should not be interpreted as evidence that genetic categories confer equivalent kidney risk. Rather, they demonstrate that severe kidney involvement may occur in the absence of a currently identifiable pathogenic variant and that clinical severity alone cannot establish variant pathogenicity.

Variant classification and biological effects should not be considered synonymous. A VUS should not be interpreted as causative or used as the sole basis for clinical decision-making without supporting population, segregation, functional, or other disease-specific evidence.[5,7,16,17] Conversely, classification

Table 4. Genetic distribution of eculizumab-treated patients and outcomes following treatment withdrawal

A. Genetic distribution among patients treated with eculizumab			
Final genetic category	**Eculizumab-treated patients, n/15 (%)**	**Genes represented among treated patients**	
Pathogenic/likely pathogenic	2/15 (13.3)	CFH, CD46	
VUS	5/15 (33.3)	CFHR4, THBD, PLG, C3, CFHR5	
Benign/likely benign or no significant complement variant	8/15 (53.3)	Benign/likely benign variants or no significant complement variants; includes C3 p.Arg102Gly as genetic context for relapse	
Total	15/15 (100)	—	
B. Eculizumab discontinuation and relapse outcomes			
Variables	**Result**		
Eculizumab exposure in the primary cohort		15/24 (62.5%)	
Eculizumab discontinued during follow-up		15/15 (100%)	
Discontinuation due to irreversible kidney dysfunction or nonresponse		5/15 (33.3%)	
Patients monitored following treatment withdrawal		10	
Relapse following treatment withdrawal		2/10 (20.0%)	
Eculizumab restarted following relapse		2/2 (100%)	
Eculizumab continued following reinitiation		2/2 (100%)	
C. Genetic context of the two relapse cases			
Case	**Gene/variant**	**Final classification**	**Management following relapse**
1	CD46 NM_002389.4:c.565T>G (p.Tyr189Asp), homozygous	Likely pathogenic	Eculizumab restarted and continued
2	C3 NM_000064.4:c.304C>G (p.Arg102Gly), heterozygous	Benign/likely benign common polymorphism; no pathogenic complement variant identified	Eculizumab restarted and continued

The genetic distribution of the 15 eculizumab-treated patients was reconciled with the final patient-level classification presented in Table 2. The CFHR2 VUS carrier did not receive eculizumab; the five treated patients with VUS carried variants in CFHR4, THBD, PLG, C3, or CFHR5. The C3 p.Arg102Gly variant in relapse case 2 is reported solely to describe the genetic context and should not be interpreted as a causative aHUS variant. These treatment and relapse data are descriptive and should not be used to infer genotype-specific treatment efficacy. aHUS: Atypical hemolytic uremic syndrome; VUS: Variant of uncertain significance

as benign or likely benign argues against a monogenic disease-causing role, although common complement polymorphisms may still modulate alternative pathway activity as part of an individual's genetic susceptibility. C3 p.Arg102Gly illustrates this distinction: functional studies have demonstrated that the 102G allele can enhance alternative pathway amplification, but this does not establish the variant as a causative mutation for aHUS.[18] Accordingly, in our second relapse case, C3 p.Arg102Gly is reported solely as part of the patient's genetic background and should not be interpreted as the cause of relapse.

A negative or nonpathogenic result on a conventional sequencing panel does not exclude susceptibility to complement-mediated disease. The CFH-CFHR genomic region is particularly susceptible to copy-number changes, complex rearrangements, and hybrid genes that may not be detected by standard sequencing approaches. In a large cohort of patients with primary aHUS, uncommon CFH-CFHR structural variants were identified in approximately 8% of cases, highlighting the potential contribution of genomic abnormalities beyond single-nucleotide variants.[19] Therefore, additional evaluation in genetically unresolved patients with a convincing complement-mediated phenotype may include dedicated copy-number or structural variant analysis, segregation studies, broader genomic approaches, and functional complement assessment. More broadly, contemporary precision-oriented frameworks emphasize that aHUS may result from interactions among inherited susceptibility, acquired complement abnormalities, and environmental or endothelial triggers rather than a single identifiable monogenic defect in every patient.[6] Regulatory, noncoding, polygenic, and epigenomic mechanisms remain areas of active investigation and should be regarded as research hypotheses rather than established explanations in the present cohort.

The two relapses observed following eculizumab withdrawal are clinically relevant in the context of current evidence on treatment discontinuation. One relapse occurred in a patient carrying homozygous CD46 p.Tyr189Asp, classified as likely pathogenic, whereas the other occurred in a patient without an identified pathogenic complement variant. Prospective and observational studies have demonstrated that relapse risk following complement inhibitor withdrawal is heterogeneous and higher among patients carrying rare or pathogenic complement variants, although recurrence remains difficult to predict at the individual level.[11,20] In the prospective multicenter study by Fakhouri et al.,[11] rare complement gene variants were associated with an increased risk of relapse following eculizumab discontinuation, while observational data similarly demonstrated that recurrence occurred predominantly among patients with rare complement variants.[20] A recent systematic review and meta-analysis further confirmed a measurable risk of recurrence following eculizumab discontinuation and highlighted the persistent uncertainty in identifying individual patients who can safely discontinue therapy.[13] In our cohort, eculizumab was reinitiated in both patients following relapse, emphasizing the importance of close surveillance and rapid access to retreatment after withdrawal.

Genetic testing should therefore inform, but not independently determine, follow-up strategies or decisions regarding C5 inhibitor withdrawal. Following withdrawal, structured longitudinal surveillance remains important across all genetic categories, including patients with benign/likely benign findings or no significant complement variants. Follow-up intensity may reasonably be determined by the severity of the initial episode, degree of kidney recovery, residual kidney dysfunction, previous relapses, triggering conditions, transplant status, and genetic findings, together with patient preferences and the feasibility of prompt reassessment. Importantly, a benign/likely benign or negative genetic result alone should not justify less intensive follow-up in a patient with a convincing complement-mediated phenotype. When treatment withdrawal is considered, shared decision-making is essential to balance the potential risk of relapse against treatment burden and the risks associated with prolonged complement inhibition.[11,13,21]

For patients requiring continued complement inhibition, long-acting C5 blockade with ravulizumab offers an alternative to eculizumab with less frequent dosing. Two-year phase 3 data have demonstrated sustained hematologic and kidney responses with ravulizumab, while data from the Global aHUS Registry support maintained effectiveness and safety following a switch from eculizumab.[22,23] Although these findings do not establish which patients can safely discontinue complement inhibition, the reduced treatment burden associated with extended dosing intervals may be relevant when considering long-term therapy and shared decision-making. Larger longitudinal aHUS registries integrating detailed genetic, phenotypic, treatment, relapse, kidney outcome, and transplant data may help refine individualized risk assessments and improve the interpretation of uncommon or incompletely characterized complement variants. Such genotype–phenotype resources may also facilitate the development of more evidence-based surveillance and treatment withdrawal strategies across genetically heterogeneous patient populations.[6,23]

Several limitations should be acknowledged. First, the retrospective, single-center design and small cohort size, particularly the P/LP subgroup comprising only two patients, precluded robust between-group comparisons, multivariable modeling, and time-to-event analyses. Second, genetic testing was performed over an extended period during

which sequencing platforms, panel composition, and variant annotation practices evolved. Dedicated CFH-CFHR copy-number and structural variant analyses, functional complement studies, segregation analyses, and broader genomic or epigenomic investigations were not systematically available, potentially limiting the characterization of genetically unresolved cases and VUS. Therefore, variant interpretation should be considered in the context of the available evidence and may change as additional functional and population data emerge. Furthermore, eculizumab treatment duration and withdrawal decisions were determined in routine clinical practice rather than according to a prospectively standardized discontinuation protocol. Despite these limitations, this study provides longitudinal data from an adult aHUS cohort, integrating detailed genetic findings with kidney outcomes, complement inhibitor exposure, and relapse following treatment withdrawal, while transparently reporting variant-level findings and clinically relevant follow-up data.

CONCLUSION

In this adult cohort with aHUS, genetic findings and clinical outcomes were heterogeneous. Severe kidney impairment occurred across genetic categories, whereas relapse following eculizumab withdrawal was observed in two genetically distinct patients, including one without an identified pathogenic complement variant. Genetically unresolved cases or those involving relapse may warrant expanded structural, functional, or genomic evaluation, while larger prospective, multicenter studies integrating advanced genetic analyses with longitudinal phenotyping are needed to clarify the clinical relevance of these findings and refine individualized risk stratification and management.

Ethics Committee Approval: Ethics committee approval was obtained from Gazi University Faculty of Medicine Clinical Research Ethics Committee (Approval Number: 2022-914, Date: 26.07.2022).

Informed Consent: Written informed consent was obtained from all participants prior to inclusion.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: The authors declared that this study received no financial support.

Use of AI for Writing Assistance: Authors declare that artificial intelligence tools were used solely for English language editing and grammatical refinement.

Author Contributions: Concept – ZU; Design – ZU; Supervision – GG, ÜD; Resource – GG, ÜD; Materials – ZU; Data Collection and/or Processing – ZU; Analysis and/or Interpretation – ZU, GG, ÜD; Literature Review – ZU; Writing – ZU; Critical Review – ZU, GG, ÜD.

Peer-review: Externally peer-reviewed.

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Appendix 1. Complement Gene Panel used for genetic analysis in adult aHUS patients

Gene symbol	Full gene name
CFH	Complement Factor H
CD46 (MCP)	Membrane Cofactor Protein
CFHR1	Complement Factor H Related 1
CFI	Complement Factor I
C3	Complement Component 3
CFB	Complement Factor B
ADAMTS13	ADAM Metallopeptidase with Thrombospondin Type 1 Motif 13
PLG	Plasminogen
MASP2	Mannan-Binding Lectin Serine Protease 2
DGKE	Diacylglycerol Kinase Epsilon
THBD	Thrombomodulin
CFHR2	Complement Factor H Related 2
CFHR3	Complement Factor H Related 3
CFHR4	Complement Factor H Related 4
CFHR5	Complement Factor H Related 5

Genes included in the institutional next-generation sequencing-based atypical hemolytic uremic syndrome (aHUS) complement panel used for genetic evaluation of the study cohort.