Complement Factor H–Related Protein 1 Deficiency and Factor H Antibodies in Pediatric Patients with Atypical Hemolytic Uremic Syndrome

Johannes Hofer, Andreas R. Janecke,*† L.B. Zimmerhackl,*a Magdalena Riedl,* Alejandra Rosales,* Thomas Giner,* Gerard Cortina,* Carola J. Haindl,* Barbara Petzelberger,* Miriam Pawlik,* Verena Jeller,* Udo Vester,‡ Bettina Gadner,§ Michael van Husen,‖ Michael L. Moritz,¶ Reinhard Würzner,§ and Therese Jungraithmayr,* for the German-Austrian HUS Study Group*

*Department of Pediatrics I; †Division of Human Genetics; and §Department of Hygiene, Medical Microbiology, and Social Medicine, Innsbruck Medical University, Innsbruck, Austria; ‡Department of Pediatric Nephrology, Medical University of Essen, Essen, Germany; ‖Pediatric Nephrology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; and ¶Division of Pediatric Nephrology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Correspondence:
Dr. Reinhard Würzner, Innsbruck Medical University, Fritz Pregl Straße 3, 6020 Innsbruck, Austria. Email: reinhard.wuerzner@i-med.ac.at

Summary

Background and objectives This study evaluated the relevance of complement factor H (CFH)–related protein (CFHR) 1 deficiency in pediatric patients with atypical hemolytic uremic syndrome (aHUS) by evaluating both the frequency of deletions in *CFHR1* and the presence of complement factor H (CFH) antibodies.

Design, setting, participants, & measurements A total of 116 patients (mainly from central Europe) and 118 healthy blood donors were included from 2001 to 2012. The presence of *CFHR1* gene deletions was determined in 90 pediatric patients with aHUS and 118 controls by an easy, fast, and cheap PCR assay; 100 patients with aHUS and 42 controls were tested for CFH antibodies by ELISA. Questionnaires were administered to evaluate the clinical and laboratory data.

Results Homozygous deletion in *CFHR1* was detected in 32% of the patients with aHUS tested, compared with 2.5% of controls ($P<0.001$). CFH antibodies were present in 25% of the patients and none of the controls. CFH antibodies were detected in 82% of patients with homozygous *CFHR1* gene deletion and in 6% of patients without. CFH antibody–positive patients with aHUS showed a significantly lower platelet nadir at disease onset and significantly less frequent involvement of the central nervous system than did antibody-negative patients. Antibody-positive patients also received plasma therapy more often.

Conclusion Homozygous deletion in *CFHR1* is strongly associated with occurrence of CFH antibodies in pediatric patients with aHUS. However, despite this apparent genetic disease predisposition, it cannot be considered an exclusive cause for aHUS. Initial presentation of Shiga toxin–negative HUS with severe thrombocytopenia and no central nervous system complications in pediatric patients is especially suspicious for CFH antibody aHUS.

Clin J Am Soc Nephrol 8: 407–415, 2013. doi: 10.2215/CJN.01260212

Introduction

Hemolytic uremic syndrome (HUS) is a systemic disease characterized by hemolytic anemia, thrombocytopenia, and acute renal failure. Typical HUS is mainly associated with gastrointestinal infections by Shiga toxin–producing bacteria, foremost *Escherichia coli* O157:H7, and patients usually present with preceding diarrhea (1,2). Typical HUS constitutes approximately 90% of all HUS cases in children and occurs mainly in children 0.5–3 years of age (3).

The less frequent atypical HUS (aHUS) form represents a heterogeneous group of disorders associated with dysregulation of the complement alternative pathway. Prognosis is poor, with high risk of recurrence; about 50% of cases progress to ESRD (4,5).

Atypical HUS can occur in all age groups, with sporadic and familial presentations (6). Among the reported cases, approximately 50% had mutations of the complement regulatory proteins factor H (CFH) (7–12), membrane cofactor protein (13–15), or factor I (16–18); mutations occurred less frequently in factor B (19), C3 (20), and thrombomodulin (21).

Recently, factor H–related protein 1 and 3 (*CFHR1/3*) gene deletions were implicated in the pathogenesis of aHUS (22). The *CFH* gene and the genes encoding the five CFHR proteins reside in the centromeric 355-kb segment on chromosome 1, known as the "regulator of complement activation" cluster (23,24). *CFHR1–5* show high degrees of sequence identity with *CFH*, and the secreted protein products of these genes are related in structure (24).

Antibodies against CFH have been reported in patients with aHUS (25–27). These antibodies were shown to induce functional CFH deficiency (25–28) by binding to its C-terminal region and thereby reducing its regulatory function. CFHR1 was shown to neutralize CFH antibodies in patients with aHUS (28). Most recent studies (29–34) established a specific

relationship between *CFHR1* deficiency and the generation of antibodies against CFH.

Few descriptive reports on clinical and biologic data and treatments for CFH antibody–positive aHUS have been published (25,26,29–33,35–37). This study sought to evaluate the frequency of deletions in the *CFHR1–5* genes and the presence of CFH antibodies in pediatric patients with aHUS and in healthy blood donors. One of the principal questions to be answered was whether a homozygous deletion of the *CFHR1* gene is a prerequisite for CFH antibody–positive aHUS. In addition, characteristic clinical and laboratory data were assessed in pediatric patients with CFH antibody–associated aHUS.

Materials and Methods

Study Design, Participants, and Inclusion Criteria

The study was performed according to the Declaration of Helsinki (2000) and was approved by the local ethics committee (Innsbruck Medical University). All participants gave informed consent.

From 2001 to 2012, 116 pediatric patients with aHUS (age <18 years at disease onset) were investigated in our retrospective and prospective multicenter study. Blood specimens and questionnaires from the patients were sent to us by different centers that were seeking help with diagnostic work-up, treatment strategies, and scientific cooperation. Thus, EDTA blood and serum samples from patients and 118 healthy blood donors (recruited from a routine blood drive) were analyzed. Because this was a prospective and retrospective study, some of the data are missing. We could not obtain all information or samples from every patient.

All patients presented with the criteria for diagnosis of HUS: acute anemia, thrombocytopenia, and renal dysfunction. Renal dysfunction was defined by one or both of the following criteria: serum creatinine levels greater than normal values according to age and urine protein-to-creatinine ratio >0.2 g/g. Shiga toxin–associated HUS was excluded by PCR for Shiga toxin or ELISA for serum antibodies against lipopolysaccharides.

Clinical and laboratory data, including results of genetic analysis, were retrospectively acquired by a standardized questionnaire completed by 19 CFH antibody–positive and 54 CFH antibody–negative patients. Questionnaires elicited the following information: description of acute phase with renal impairment; hematologic data; BP development; central nervous system (CNS) and further organ involvement; treatment and investigations toward the cause, including stool and serum investigations for enterohemorrhagic *E. coli* and Shiga toxin.

CFH antibody titer follow-up was performed prospectively. We had recommended this follow-up for all antibody-positive patients. However, because exact preanalytic treatment and shipment on dry ice are absolutely necessary, only some centers sent us suitable samples for follow-up.

Data were compared with those of a control group of 118 healthy blood donors. Although the control group is significantly older than the pediatric aHUS cohort, this age difference does not influence the frequency of genetic deletions because inborn genetic defects are not generated over the years. Nevertheless, there is indeed an influence on possible CFH-antibody positivity because CFH antibodies are thought to occur as a result of an adequate triggering event. To our knowledge, no healthy individuals have been described with CFH antibodies, and CFH antibodies are thought to be the pathogenic hallmark for aHUS.

CFH Antibody Assessment

CFH antibody titers were determined using an ELISA (25). The antibody titer cutoff was defined as 100 AU/ml. Briefly, ELISA plates were coated with purified human factor H (Calbiochem, Meudon, France). Serum was added at a dilution of 1:50, and detection was performed using goat antihuman IgG, labeled with horseradish peroxidase (both from Sigma-Aldrich).

Genetic Analyses

DNA was extracted from peripheral blood samples using an automated extractor according to the manufacturer's protocols (GenoM 48; Qiagen, Vienna, Austria). The genomic DNA was analyzed for copy number variation of *CFHR1–5* genes by comparative genomic hybridization (CGH) using a customized oligoarray and CGHPRO software and by our PCR technique and was compared with that of healthy controls. CGH analysis has been described in detail elsewhere (38). A homozygous deletion of *CFHR1* was additionally confirmed by PCR amplification of part of *CFHR1* exon 2 using allele-specific primers, CFHR1 2af,5′-GTTTTGTGTTATTTTCCCAGCAACA and CFHR1 2br,5′-GAATGACATCCATTTAATGAACAGA, revealing a 252-bp fragment on agarose-gel electrophoresis.

A 199-bp *CFHR2* exon 2 fragment was co–PCR-amplified using oligonucleotide primers CFHR2 2af,5′-GTTTTGTGTTATTTTCCCAGCAAT and 2ar,5′-TGGTGACCATCCTTCTTCTGC as a control for PCR success and to show that our PCR setting can distinguish between different high homologous sequences within the cluster of sequence related *CFHR* genes.

We amplified 15–25 ng of genomic DNA in a 25-μl reaction volume that included 1× GoTaq PCR buffer (Promega, Mannheim, Germany), 1.5 mM MgCl2, primers at 0.8 mM, deoxyribonucleotide triphosphates at 200 mM (final concentrations), and 0.5 U of GoTaq polymerase (Promega). The following PCR conditions were used for all amplifications: initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation at 94°C for 20 seconds, annealing at 62°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 7 minutes.

Statistical Analyses

Data were calculated using SPSS software, version 15.0, for Windows (SPSS, Inc., Chicago, IL). Chi-squared tests, odds ratios, and 95% confidence intervals (CIs) were used for comparisons of categorical data. When expected values in any of the cells of a contingency table were <5, a Fisher exact test was used. A *t* test (for normally distributed values) and Mann-Whitney U test (for nonnormally distributed values) were used to compare control and study groups according to independent metric variables. *P* values <0.05 were considered to represent statistically significant differences.

Results

Patients Characteristics

Overall, 116 patients were included in this study and compared with 118 healthy blood donors. Patients had a mean age ± SD of 5.6±4.8 years at time of disease onset; the mean age in the control group was 40.5±3.5 years at time of blood withdrawal (Supplementary Table 1). However, as detailed in the Materials and Methods section, this difference was not considered essential for the key findings.

PCR and CGH Array Analyses

The presence/absence of *CFHR1* exon 2 was tested by PCR to detect homozygous *CFHR1* deletion in 90/116 patients (patients in whom DNA was available) and 118 controls (Figure 1). Among the patients and controls, 32.2% (n=29) and 2.5% (n=3), respectively, showed a homozygous deletion ($P<0.001$).

Twenty-eight of 90 patients and 37/118 controls were analyzed using CGH analysis (Table 1). We selected patients and controls irrespective of their sex and age on the basis of the amount of sufficient DNA available.

In 61% of the patients (17/28), CGH analysis detected *CFHR* deletions: Forty-three percent (n=12) showed a homozygous deletion (3 of whom had an additional heterozygous *CFHR3* gene deletion) and 18% (n=5) showed a heterozygous deletion of *CFHR1*.

No copy number variation of the *CFHR2* and *CFHR5* genes were detected. One patient who was homozygous for *CFHR1* and heterozygous for *CFHR3* gene deletions showed an additional heterozygous deletion of *CFHR4*.

CFHR gene deletions were detected in 35% (13/37) of the controls using CGH: Eight percent (n=3) showed a homozygous deletion and 27% (n=10) a heterozygous deletion of *CFHR1*, 8 of these together with a heterozygous *CFHR3* deletion.

For the 28 of 90 selected patients and the 37 of 118 controls, PCR results showed a 100% match with the CGH analysis for homozygous *CFHR1* deletions (Table 1). This aHUS population is out of Hardy-Weinberg equilibrium (Table 2) for analysis of the frequency of *CFHR1* deletions.

CFH Antibody Analyses

Serum samples for CFH antibody analysis were available from 100 patients with aHUS and 42 controls (Figure 1). Twenty-five patients with aHUS (25%) and none of the controls were positive for CFH antibodies ($P<0.001$).

Of the 74 patients in whom DNA and serum samples were available, 30% (n=22) had a homozygous *CFHR1* gene deletion. In 82% (18/22) of these patients, CFH antibodies were detected. One antibody-positive patient with a homozygous *CFHR1* deletion was the one with the additional heterozygous deletion of both *CFHR3* and *CFHR4*. Of the remaining 52 patients without homozygous *CFHR1* deletion, we identified 3 patients (6%) with CFH antibodies.

Statistical evaluation disclosed a significant association between a given homozygous *CFHR1* deletion and CFH antibody positivity (OR, 73; 95% CI, 15–361). Heterozygous *CFHR1* gene deletion was not significantly associated with CFH antibody production.

For 8 of 25 CFH antibody–positive patients, genetic screening data were available. One patient showed a heterozygous complement factor I mutation; no mutations were found for any other patient (screening for CFH, membrane cofactor protein, C3, and complement factor I).

Twelve patient samples obtained at disease onset or recurrence showed a significantly higher mean CFH antibody titer than the 13 samples obtained during periods of clinical remission (1342±1458 AU/ml versus 405±116 AU/ml; P=0.03). Likewise, in five patients in whom a titer follow-up was available, the highest CFH antibody titers were always found at disease onset or recurrence (Figure 2).

Clinical and Laboratory Characteristics of Patients Positive and Negative for CFH Antibody

Age at disease onset was significantly greater in the CFH antibody–positive group (mean, 7.9±3.4) than the antibody-negative group (median, 2.2 years [interquartile range, 1.0–5.7 years]) ($P<0.001$). No differences were found for prodromal gastrointestinal symptoms and respiratory tract infections between the groups (Table 3).

CFH antibody–positive patients had significantly less CNS involvement at disease onset compared with patients without CFH antibodies (P=0.02). No differences were found for oliguria/anuria, arterial hypertension, pancreatic involvement, cardiac involvement, hepatopathia, gastrointestinal tract involvement, or need for dialysis or erythrocyte or platelet infusions.

A higher percentage of CFH antibody-positive patients received plasma therapy in the acute phase compared with CFH antibody–negative patients (P=0.006). This is reflected by a significant difference in patients undergoing plasma exchange but not in patients undergoing plasma infusion (Table 3).

CFH antibody–positive patients show a significantly lower platelet count nadir than CFH antibody–negative patients (P=0.008). No differences were found in peak creatinine, hemoglobin, lactate dehydrogenase, C4, C3, or CFH levels (Table 4).

Discussion

A homozygous *CFHR1* gene deletion, accurately assessed by an easy, fast, and cheap PCR assay that reliably replaces CGH analysis for detection of homozygous *CFHR1* deletions, was common in our pediatric patients with aHUS and was frequently associated with CFH antibodies. This finding suggests that a homozygous *CFHR1* gene deletion is a predisposing factor for CFH antibody production.

The frequency of CFH antibody positivity among patients with aHUS in our cohort (25/100 [25%]) was considerably higher than that in published cohorts (25,29,31) from Dragon-Durey *et al.* (3/48 [6.3%]), Józsi *et al.* (5/50 [8.3%]), and Moore *et al.* (13/142 [9.2%]). This difference in antibody prevalence may correspond to the differences in age distribution within these cohorts because our cohort is the only one focusing on pediatric patients with aHUS.

This aHUS population is out of Hardy-Weinberg equilibrium (Table 2) for analysis of the frequency of *CFHR1* deletions. Together with similar observations (31), this finding might hint at an unknown advantage for heterozygotes.

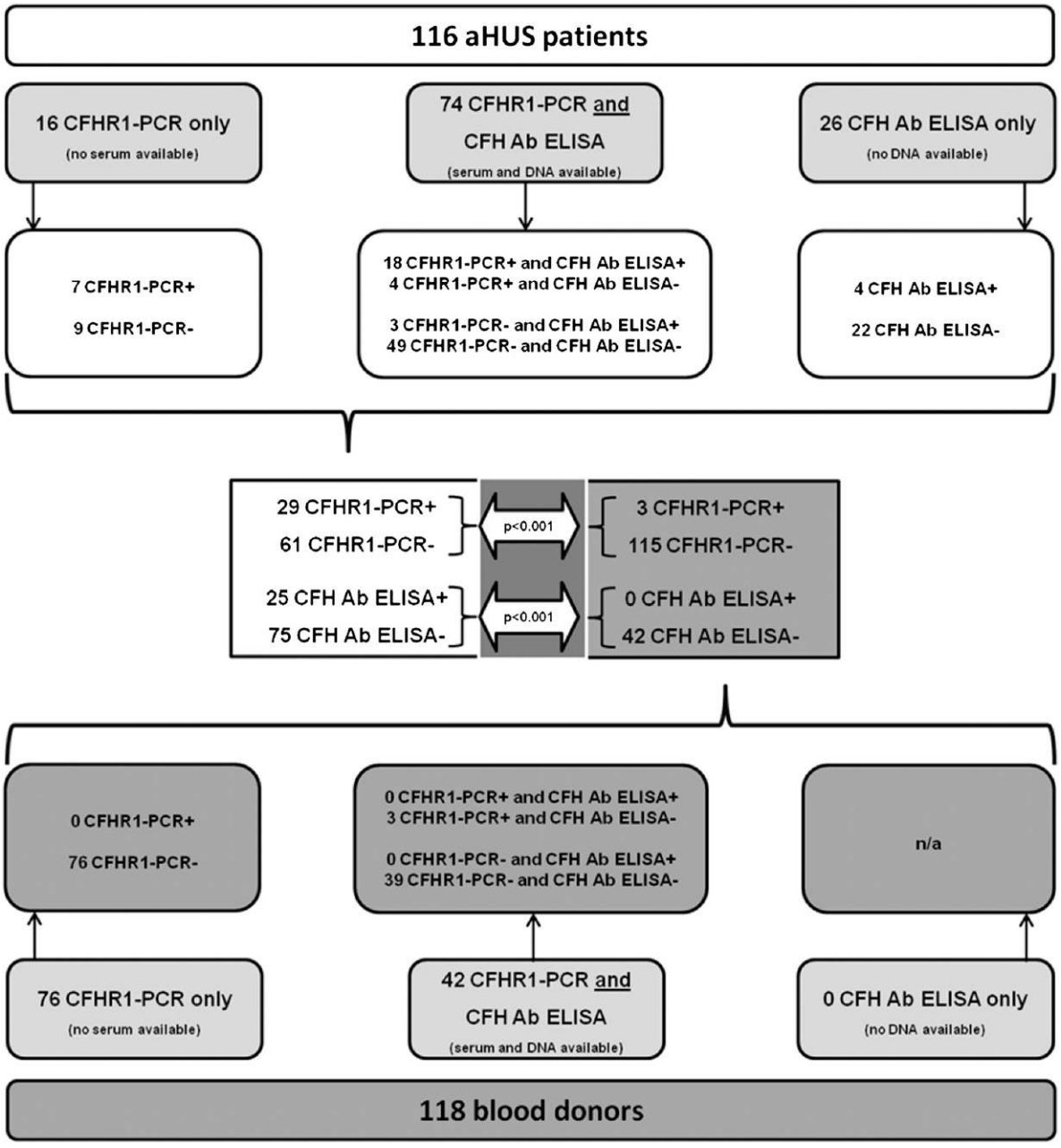

Figure 1. | **Comparison between patients with atypical hemolytic uremic syndrome (aHUS) and healthy blood donors concerning presence of complement factor H (CFH) antibodies (by ELISA) and absence of *CFHR1* gene (by PCR).** CFHR1 PCR+/PCR− means homozygous *CFHR1* deletion detected/not detected, respectively. The comparison of patients and controls shows a significantly greater percentage of *CFHR1* homozygous deletions and CFH antibody within the patient group (*P* value was calculated using a chi-squared test). Ab, antibody; n/a, not available.

Our study revealed a strong association between the generation of CFH antibodies and the presence of homozygous *CFHR1* deletion. How this genetic background is able to increase the risk for CFH antibody production is unclear. Moore *et al.* (31) have suggested that deficiency of *CFHR1* may result in a failure of immune tolerance to the homologous region in CFH. Other possibilities, such as cross-reactivity with microbial antigens, have been discussed by Rodríguez de Córdoba (39).

Other investigators have also demonstrated that homozygous *CFHR1* deletions are not universally associated with antibodies against CFH. In our cohort, 86% (18/21) of the analyzed CFH antibody–positive patients had a homozygous deletion of *CFHR1*. This finding corresponds with the cohorts of Józsi *et al.* (29) (14/18 patients [88%]) and the cohort of Moore *et al.* (31) (10/13 patients [77%]). On the other hand, a significant autoantibody response to CFH can develop in the presence of normal *CFHR1* (31). In

Table 1. Comparison of PCR and comparative genomic hybridization data from patients and controls in whom both methods were performed

CGH Results	PCR Results	
	Homozygous Deletion of *CFHR1*(*n*)	No Homozygous Deletion of *CFHR1*(*n*)
Patients with aHUS (*n*=28)		
CFHR1 homozygous deletion	12	0
CFHR1 heterozygous deletion	0	5[a]
CFHR1 no deletion	0	11[a]
Controls (*n*=37)		
CFHR1 homozygous deletion	3	0
CFHR1 heterozygous deletion	0	10[b]
CFHR1 no deletion	0	24

CGH, comparative genomic hybridization; aHUS, atypical hemolytic uremic syndrome.
[a]Our PCR analysis cannot distinguish between the absence of a deletion and heterozygous deletion of *CFHR1*.
[b]Eight of the controls had a heterozygous deletion of *CFHR1* and *CFHR3*, and two had a heterozygous deletion of *CFHR1* only.

our cohort we found three patients positive for CFH antibodies without deletion in *CFHR1*.

The presence of CFH antibodies has not been reported in healthy individuals to date, a result confirmed in this study. Homozygous *CFHR1* gene deletions, however, were found in 2.5% of healthy individuals in this study, and may be related to the development of CFH antibodies. Thus, one may speculate that the action of an eventual triggering event in individuals with homozygous *CFHR1* gene deletion could play a role in setting off the production of CFH antibodies, leading to aHUS. The initial triggering event leading to the development of aHUS or CFH antibodies is still a matter of speculation, as in other autoantibody-mediated diseases (37). It is not clear whether an infectious agent, some other inflammatory or immunologic stimulus, or another unknown factor leads to complement activation.

Atypical HUS is diagnosed in 1 of 10 patients with HUS (40). Although the incidence of complement associated aHUS is not known, one can estimate that the incidence of aHUS is about 1–2:10^6 (41). The prevalence of homozygous *CFHR1* deletion in our population of healthy controls was 2.5%. According to our data, one can calculate the relation between patients with aHUS patients who have a homozygous *CFHR1* deletion and healthy individuals with the same deletion to be 1:10^5 (Figure 3). Thus, from the frequency of homozygous *CFHR1* deletion in the normal population, a dominant causative link is unlikely. However, a homozygous *CFHR1* deletion in aHUS may be a marker for a different genetic background predisposing to aHUS or may be a cofactor.

Knowledge of an asymptomatic individual with a *CFHR1* homozygous gene deletion could have important clinical implications, especially when in the context of potential living donors for renal transplantation. Should the potential risk of developing aHUS from CFH antibodies, possibly generated in the future in a potential donor with a *CFHR1* homozygous gene deletion, preclude him or her from being a donor? The low absolute risk for individuals with this deletion to develop aHUS would make their exclusion from being a donor questionable. Nevertheless, it could be that the low *a priori* risk of an individual with a homozygous deletion increases as a consequence of a potential complement activation associated with the transplant procedure. These factors may have to be taken into account when an individual with a homozygous *CFHR1* gene deletion is being considered as a kidney donor. So far, we are

Table 2. Results of comparative genomic hybridization analysis in comparison with expected frequencies calculated according to Hardy-Weinberg equilibrium

Variable	Normal Population (%)	Patients with aHUS (%)	*P* Value[a]
No *CFHR1* deletion	64.8	39.3	0.04
Expected heterozygous *CFHR1* deletions calculated according to HW equilibrium	31.4	46.8	0.25
Observed heterozygous *CFHR1* deletions	27.0	17.8	0.11
Expected homozygous *CFHR1* deletions calculated according to HW equilibrium	3.8	13.9	0.08
Observed homozygous *CFHR1* deletions	8.1	42.8	<0.001

aHUS, atypical hemolytic uremic syndrome; HW, Hardy-Weinberg.
[a]*P* values are calculated with chi-squared test for the normal population compared with patients with aHUS. Calculations were done on the basis of the observed frequency of individuals without *CFHR1* deletions in the normal population and the aHUS population. The expected frequencies are calculated using the HW equilibrium on the basis of the given frequency of "no CFHR1 deletion" for patients and controls.

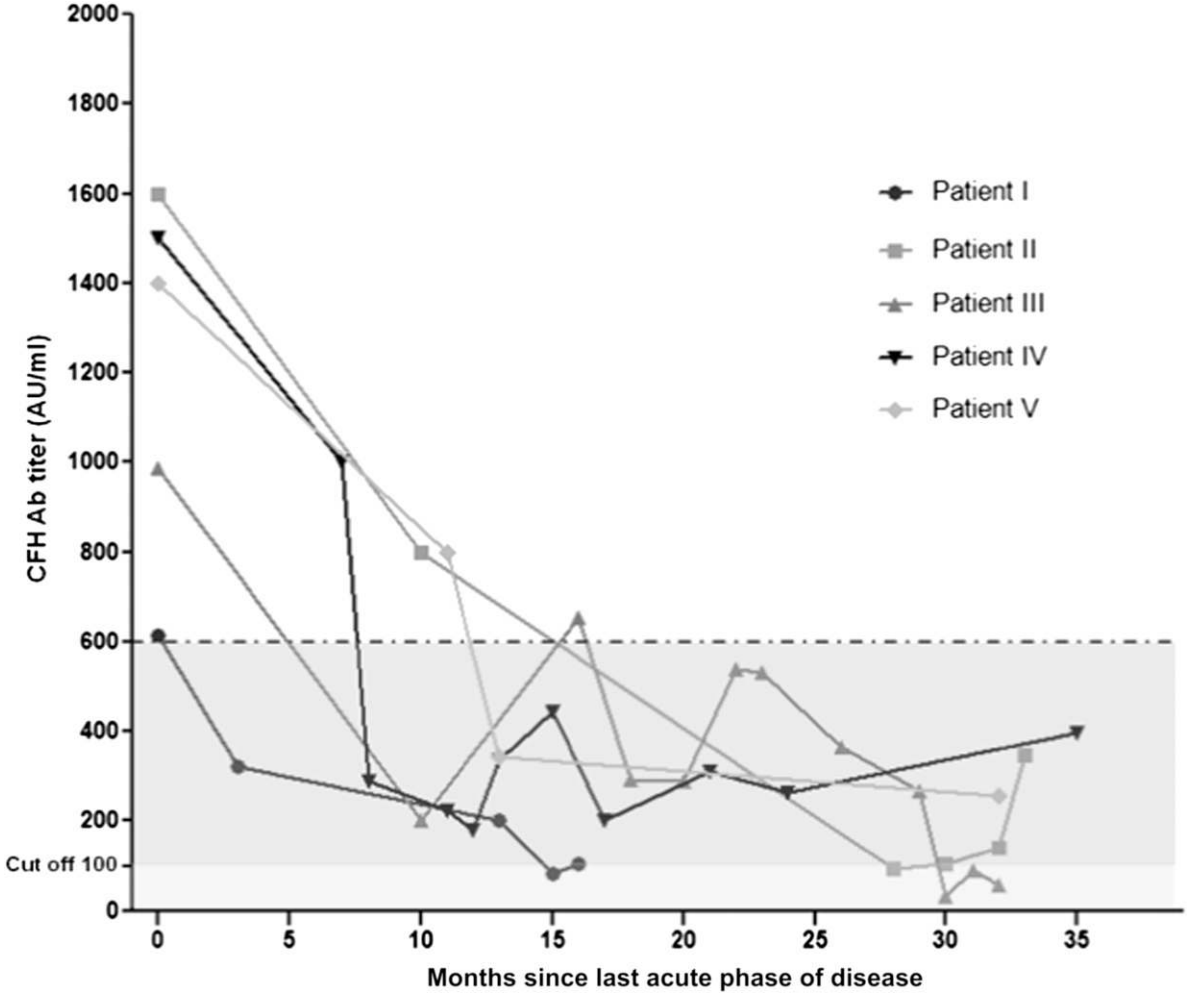

Figure 2. | Complement factor H (CFH) antibody (Ab) titer of five patients with atypical hemolytic uremic syndrome who were positive for CFH antibody and had homozygous *CFHR1* deletion at acute phase of the disease and during remission. CFH antibody titer follow-up was recommended for all antibody-positive patients. However, because exact preanalytic treatment and shipment on dry ice are absolutely necessary, only some centers sent suitable samples for follow-up. Data for those patients are presented in this graph. Acute phase is defined as disease onset or disease recurrence. The area under the dashed line (<600 AU/ml) marks the area of suspected low recurrence risk according to the authors' observations. Nevertheless, it is remarkable that patient I showed a CFH antibody titer of only 600 AU/ml at disease onset. In contrast to the other patients, serum for CFH antibody analysis in this patient was taken after plasmapheresis, suggesting a higher CFH antibody titer directly at disease onset. The cutoff level for CFH antibody positivity for ELISA in this study is 100 AU/ml.

not aware of any case in the literature with *de novo* aHUS due to CFH antibodies in either donor with a homozygous *CFHR1* deletion or transplant recipient.

We report clinical and laboratory data of 19 CFH-antibody positive pediatric HUS patients. The age at disease onset in our cohort (mean age, 7.9 years) was similar to the published age range (37). Age at onset was significantly greater than that in patients with CFH-antibody negative cases (31,37).

Extrarenal complications in CFH antibody–positive aHUS during the first flare of disease were common, although CNS involvement was found in a significantly lower percentage of patients than in CFH antibody–negative patients (11% versus 38%) and than in non–exclusively pediatric CFH antibody–positive population (37).

The presence of CFH antibodies was associated with a significantly lower platelet nadir at disease onset compared with CFH antibody–negative patients, and even lower than described for the nonexclusively pediatric cohort (37). The platelet nadir in our cohort is quite close to the mean platelet nadir in ADAMTS 13 activity–deficient patients with thrombotic thrombocytopenia (42), which may lead to diagnostic difficulties.

In contrast to dialysis, plasma therapy was used significantly more often in patients with CFH antibodies, but antibody positivity was known for only 5 of 18 antibody-positive patients receiving plasma therapy during the first 4 weeks after disease onset. This fact could reflect the high response rate of this disease entity to plasma treatment at disease onset (43) or a more pronounced plasma dependency during first disease flare.

None of our antibody-positive patients with aHUS received eculizumab at the first disease flare; even today this decision may be justified because aggressive plasmapheresis, followed by maintenance therapy with immunosuppression,

Table 3. Prodromes, clinical presentation at disease onset, and therapeutic interventions during first disease flare in patients with atypical hemolytic uremic syndrome who were positive versus those who were negative for complement factor H antibody

Variable	CFH Antibody–Positive Patients (n=19), % (n/n)[a]	CFH Antibody–Negative–Patients (n=54), % (n/n)[a]	P Value
Prodromes			
Gastrointestinal symptoms	87 (13/15)	67 (32/48)	0.09
Respiratory tract infections	42 (5/12)	34 (16/47)	0.23
Clinical presentation			
Oliguria/anuria	50 (9/18)	63 (33/52)	0.32[b]
Arterial hypertension	59 (10/17)	75 (38/51)	0.22[b]
CNS involvement	11 (2/19)	38 (20/52)	0.02
Further organ involvement[c]	58 (11/19)	53 (28/53)	0.17
Therapy	74 (14/19)	57 (31/54)	0.21[b]
Dialysis			
Erythrocyte transfusion	95 (18/19)	87 (45/52)	0.24
Platelet transfusion	26 (5/19)	24 (12/51)	0.81[b]
Plasma therapy	95 (18/19)	63 (31/49)	0.006
Plasma exchange	74 (14/19)	35 (18/51)	0.004[b]
Plasma infusion	63 (12/19)	40 (19/48)	0.08[b]

The CFH antibody–positive group was compared with the CFH antibody–negative group using the Fisher exact test unless noted otherwise. CFH, complement factor H; CNS, central nervous system.
[a]Questionnaires were completed for 19 CFH antibody–positive and 54 CFH antibody–negative patients. The percentages were calculated according to the number of documented patients with the given feature. Prodromal gastrointestinal symptoms include abdominal pain (57% versus 21%), vomiting (80% versus 58%), nonbloody diarrhea (13% versus 35%), and bloody diarrhea (0% versus 11%). CNS involvement is defined by seizures, severe headache, or cerebral paresis. Further organ involvement includes pancreatitis (defined by elevated levels of amylasemia and/or lipasemia), cardiac involvement (includes cardial ischemia, dilatative cardiomyopathia, and cardiac insufficiency), hepatopathia (defined by elevated liver enzyme levels), and gastrointestinal tract involvement (vomiting, diarrhea, gastrointestinal bleeding, ileus).
[b]Compared using chi-squared test.
[c]Individual assessment showed no significant differences in the subgroups.

Table 4. Laboratory characteristics at disease onset for patients with atypical hemolytic uremic syndrome who were positive versus those who were negative for complement factor antibody

Variable	CFH Antibody–Positive Patients (n=19)[a]	CFH Antibody–Negative Patients (n=54)[a]	P Value
Platelet count ($10^3/mm^3$)	30±13 (17/25)	59±43 (49/75)	0.008
Creatinine level (mg/dl)	5.5±3.2 (13/25)	3.9±3.3 (48/75)	0.12
Hemoglobin level (g/dl)	5.8±1.5 (18/25)	6.3±1.5 (51/75)	0.28
Lactate dehydrogenase level (U/L)	3607±3775 (17/25)	2613±1996 (48/75)	0.18
C4 level (mg/dl)	21±8 (13/25)	21±9 (37/75)	0.76
Patients with decreased C4 level, % (n/n)	15 (2/13)	19 (7/37)	0.32[b]
C3 (mg/dl)	92±41 (17/25)	84±37 (26/48)	0.46
Patients with decreased C3 level, % (n/n)	41 (7/17)	54 (26/48)	0.36[c]
CFH level (μg/ml)	467±170 (12/25)	443±275 (39/75)	0.71

Metric laboratory values are shown as follows: mean ± SD (number of stated values/total number of patients with completed questionnaire). For C4 and C3, the percentage of patients with decreased values is given (number/total number). P values for normally distributed metric laboratory variables were calculated using a t test. CFH, complement factor H.
[a]Completed questionnaires were available for 19 CFH antibody–positive and 54 CFH antibody–negative patients.
[b]P value was calculated using Fisher exact test.
[c]P value was calculated using chi-squared test.

appears to be a good treatment option for patients with CFH antibody–associated aHUS (25,26,29–33,35–37).

CFH antibody titers were significantly higher during disease activity than during remission but may increase again when an adequate triggering event is present. Thus, repeated measurements in these patients are recommended to recognize a possible recurrence as early as possible. Because of a high variability among patients, CFH antibody titers can be interpreted only individually.

In conclusion, patients with aHUS and a homozygous *CFHR1* gene deletion frequently have CFH antibodies, and the latter are nearly exclusively found in these patients,

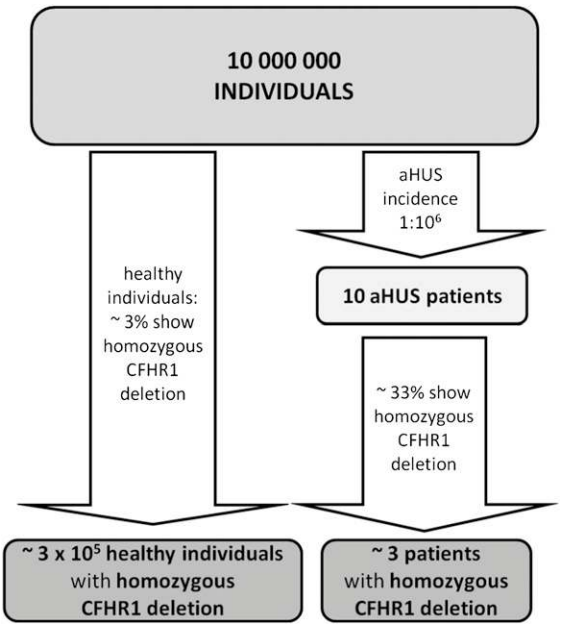

Figure 3. | Prevalence of atypical hemolytic uremic syndrome (aHUS) and homozygous *CFHR1* deletions. The calculation is based on 10^7 individuals. The incidence of aHUS is estimated to be $1:10^6$ (41). In the current study, 32.2% (29/90) of patients with aHUS showed homozygous *CFHR1* deletion compared with 2.5% (3/118) of healthy blood donors. On the basis of this frequency of homozygous *CFHR1* deletions in the healthy population, an exclusive causative link appears to be highly unlikely.

confirming that homozygous *CFHR1* gene deletion is related to antibody production. However, this gene deletion on its own is clearly not sufficient for the development of aHUS.

Initial presentation of Shiga toxin–negative HUS with severe thrombocytopenia and absent CNS complications in 6- to 10-year-old patients is especially suspicious for CFH antibody aHUS.

Acknowledgments

We are thankful to all collaborating medical centres and physicians (listed on www.hus-online.at).

Supported by the European Pediatric Research group for HUS, the Gesellschaft für Pädiatrische Nephroplogie, the Tiroler Wissenschaftsfond, and the Österreichische Nationalbank (Grant 12711).

Disclosures

None.

References

1. Rosales A, Hofer J, Zimmerhackl LB, Jungraithmayr TC, Riedl M, Giner T, Strasak A, Orth-Höller D, Würzner R, Karch H; German-Austrian HUS Study Group: Need for long-term follow-up in enterohemorrhagic Escherichia coli-associated hemolytic uremic syndrome due to late-emerging sequelae. *Clin Infect Dis* 54: 1413–1421, 2012
2. Scheiring J, Pruefer F, Martini S, Wygoda S, Knueppel T, Toenshoff B, Konrad M, Drube J, Offner G, Zipfel P, Heinen S, Kirschfink M, Zimmerhackl LB: Updated outcome in patients with a typical/recurrent haemolytic uraemic syndrome. *Pediatr Transplant* 11: 82, 2007
3. 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* 70: 423–431, 2006
4. 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, Nivet H, Alberti C, Loirat C; French Society of Pediatric Nephrology: Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 18: 2392–2400, 2007
5. Zimmerhackl LB, Scheiring J, Prüfer F, Taylor CM, Loirat C: Renal transplantation in HUS patients with disorders of complement regulation. *Pediatr Nephrol* 22: 10–16, 2007
6. Constantinescu AR, Bitzan M, Weiss LS, Christen E, Kaplan BS, Cnaan A, Trachtman H: Non-enteropathic hemolytic uremic syndrome: Causes and short-term course. *Am J Kidney Dis* 43: 976–982, 2004
7. Caprioli J, Bettinaglio P, Zipfel PF, Amadei B, Daina E, Gamba S, Skerka C, Marziliano N, Remuzzi G, Noris M; Itaslian Registry of Familial and Recurrent HUS/TTP: The molecular basis of familial hemolytic uremic syndrome: Mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. *J Am Soc Nephrol* 12: 297–307, 2001
8. Caprioli J, Castelletti F, Bucchioni S, Bettinaglio P, Bresin E, Pianetti G, Gamba S, Brioschi S, Daina E, Remuzzi G, Noris M; International Registry of Recurrent and Familial HUS/TTP: Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: The C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. *Hum Mol Genet* 12: 3385–3395, 2003
9. Dragon-Durey MA, Frémeaux-Bacchi V, Loirat C, Blouin J, Niaudet P, Deschenes G, Coppo P, Herman Fridman W, Weiss L: Heterozygous and homozygous factor H deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: Report and genetic analysis of 16 cases. *J Am Soc Nephrol* 15: 787–795, 2004
10. Pérez-Caballero D, González-Rubio C, Gallardo ME, Vera M, López-Trascasa M, Rodríguez de Córdoba S, Sánchez-Corral P: Clustering of missense mutations in the C-terminal region of factor H in atypical hemolytic uremic syndrome. *Am J Hum Genet* 68: 478–484, 2001
11. Richards A, Buddles MR, Donne RL, Kaplan BS, Kirk E, Venning MC, Tielemans CL, Goodship JA, Goodship THJ: Factor H mutations in hemolytic uremic syndrome cluster in exons 18-20, a domain important for host cell recognition. *Am J Hum Genet* 68: 485–490, 2001
12. Warwicker P, Goodship THJ, Donne RL, Pirson Y, Nicholls A, Ward RM, Turnpenny P, Goodship JA: Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int* 53: 836–844, 1998
13. 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* 17: 2017–2025, 2006
14. Noris M, Brioschi S, Caprioli J, Todeschini M, Bresin E, Porrati F, Gamba S, Remuzzi G; International Registry of Recurrent and Familial HUS/TTP: Familial haemolytic uraemic syndrome and an MCP mutation. *Lancet* 362: 1542–1547, 2003
15. Richards A, Kemp EJ, Liszewski MK, Goodship JA, Lampe AK, Decorte R, Müslümanoğlu MH, Kavukcu S, Filler G, Pirson Y, Wen LS, Atkinson JP, Goodship THJ: Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. *Proc Natl Acad Sci U S A* 100: 12966–12971, 2003
16. Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J, Vigneau C, Kuypers D, Boudailliez B, Loirat C, Rondeau E, Fridman WH: Complement factor I: A susceptibility gene for atypical haemolytic uraemic syndrome. *J Med Genet* 41: e84, 2004
17. Kavanagh D, Kemp EJ, Mayland E, Winney RJ, Duffield JS, Warwick G, Richards A, Ward R, Goodship JA, Goodship THJ: Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 16: 2150–2155, 2005

18. Kavanagh D, Richards A, Noris M, Hauhart R, Liszewski MK, Karpman D, Goodship JA, Fremeaux-Bacchi V, Remuzzi G, Goodship THJ, Atkinson JP: Characterization of mutations in complement factor I (CFI) associated with hemolytic uremic syndrome. *Mol Immunol* 45: 95–105, 2008
19. Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J, Carreras L, Arranz EA, Garrido CA, López-Trascasa M, Sánchez-Corral P, Morgan BP, Rodríguez de Córdoba S: Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci U S A* 104: 240–245, 2007
20. Frémeaux-Bacchi V, Miller EC, Liszewski MK, Strain L, Blouin J, Brown AL, Moghal N, Kaplan BS, Weiss RA, Lhotta K, Kapur G, Mattoo T, Nivet H, Wong W, Gie S, Hurault de Ligny B, Fischbach M, Gupta R, Hauhart R, Meunier V, Loirat C, Dragon-Durey MA, Fridman WH, Janssen BJ, Goodship TH, Atkinson JP: Mutations in complement C3 predispose to development of atypical haemolytic uraemic syndrome. *Blood* 112: 4948–4952, 2008
21. Delvaeye M, Noris M, De Vriese A, Esmon CT, Esmon NL, Ferrell G, Del-Favero J, Plaisance S, Claes B, Lambrechts D, Zoja C, Remuzzi G, Conway EM: Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 361: 345–357, 2009
22. Zipfel PF, Edey M, Heinen S, Jozsi M, Richter H, Misselwitz J, Hoppe B, Routledge D, Strain L, Hughes AE, Goodship JA, Licht C, Goodship THJ, Skerka C: Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. *Plos Genet* 3: e41, 2007
23. Weis JH, Morton CC: Bruns, Weis JJ, Klickstein LB, Wong WW, Fearon D: A complement receptor locus: Genes encoding C3b/C4b receptorand C3d/Epstein Barr virus receptor map to 1q32. *J Immunol* 138: 312–315, 1987
24. Heine-Suñer D, Díaz-Guillén MA, de Villena FP, Robledo M, Benítez J, Rodríguez de Córdoba S: A high-resolution map of the regulator of the complement activation gene cluster on 1q32 that integrates new genes and markers. *Immunogenetics* 45: 422–427, 1997
25. Dragon-Durey MA, Loirat C, Cloarec S, Macher MA, Blouin J, Nivet H, Weiss L, Fridman WH, Frémeaux-Bacchi V: Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 16: 555–563, 2005
26. Józsi M, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, Zipfel PF, Skerka C: Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency and affect recognition functions. *Blood* 111: 1512–1514, 2007
27. Józsi M, Strobel S, Dahse HM, Liu WS, Hoyer PF, Oppermann M, Skerka C, Zipfel PF: Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. *Blood* 110: 1516–1518, 2007
28. Strobel S, Abarrategui-Garrido C, Fariza-Requejo E, Seeberger H, Sánchez-Corral P, Józsi M: Factor H-related protein 1 neutralizes anti-factor H autoantibodies in autoimmune hemolytic uremic syndrome. *Kidney Int* 80: 397–404, 2011
29. Józsi M, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, Zipfel PF, Skerka C: Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 111: 1512–1514, 2008
30. Abarrategui-Garrido C, Martínez-Barricarte R, López-Trascasa M, de Córdoba SR, Sánchez-Corral P: Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood* 114: 4261–4271, 2009
31. Moore I, Strain L, Pappworth I, Kavanagh D, Barlow PN, Herbert AP, Schmidt CQ, Staniforth SJ, Holmes LV, Ward R, Morgan L, Goodship TH, Marchbank KJ: Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. *Blood* 115: 379–387, 2010
32. Lee BH, Kwak SH, Shin JI, Lee SH, Choi HJ, Kang HG, Ha IS, Lee JS, Dragon-Durey MA, Choi Y, Cheong HI: Atypical hemolytic uremic syndrome associated with complement factor H autoantibodies and CFHR1/CFHR3 deficiency. *Pediatr Res* 66: 336–340, 2009
33. Strobel S, Hoyer PF, Mache CJ, Sulyok E, Liu WS, Richter H, Oppermann M, Zipfel PF, Józsi M: Functional analyses indicate a pathogenic role of factor H autoantibodies in atypical haemolytic uraemic syndrome. *Nephrol Dial Transplant* 25: 136–144, 2010
34. Dragon-Durey MA, Blanc C, Marliot F, Loirat C, Blouin J, Sautes-Fridman C, Fridman WH, Frémeaux-Bacchi V: The high frequency of complement factor H related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. *J Med Genet* 46: 447–450, 2009
35. Kwon T, Dragon-Durey MA, Macher MA, Baudouin V, Maisin A, Peuchmaur M, Fremeaux-Bacchi V, Loirat C: Successful pre-transplant management of a patient with anti-factor H autoantibodies-associated haemolytic uraemic syndrome. *Nephrol Dial Transplant* 23: 2088–2090, 2008
36. Le Quintrec M, Zuber J, Noel LH, Thervet E, Frémeaux-Bacchi V, Niaudet P, Fridman WH, Legendre C, Dragon-Durey MA: Anti-Factor H autoantibodies in a fifth renal transplant recipient with atypical hemolytic and uremic syndrome. *Am J Transplant* 9: 1223–1229, 2009
37. Dragon-Durey MA, Sethi SK, Bagga A, Blanc C, Blouin J, Ranchin B, André JL, Takagi N, Cheong HI, Hari P, Le Quintrec M, Niaudet P, Loirat C, Fridman WH, Frémeaux-Bacchi V: Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol* 21: 2180–2187, 2010
38. Ullmann R, Turner G, Kirchhoff M, Chen W, Tonge B, Rosenberg C, Field M, Vianna-Morgante AM, Christie L, Krepischi-Santos AC, Banna L, Brereton AV, Hill A, Bisgaard AM, Müller I, Hultschig C, Erdogan F, Wieczorek G, Ropers HH: Array CGH identifies reciprocal 16p13.1 duplications and deletions that predispose to autism and/or mental retardation. *Hum Mutat* 28: 674–682, 2007
39. Rodríguez de Córdoba S: aHUS: a disorder with many risk factors. *Blood* 115: 158–160, 2010
40. Gerber A, Karch H, Allerberger F, Verweyen HM, Zimmerhackl LB: Clinical course and the role of shiga toxin-producing Escherichia coli infection in the hemolytic-uremic syndrome in pediatric patients, 1997-2000, in Germany and Austria: A prospective study. *J Infect Dis* 186: 493–500, 2002
41. Loirat C, Frémeaux-Bacchi V: Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis* 6: 60, 2011
42. Coppo P, Schwarzinger M, Buffet M, Wynckel A, Clabault K, Presne C, Poullin P, Malot S, Vanhille P, Azoulay E, Galicier L, Lemiale V, Mira JP, Ridel C, Rondeau E, Pourrat J, Girault S, Bordessoule D, Saheb S, Ramakers M, Hamidou M, Vernant JP, Guidet B, Wolf M, Veyradier A; French Reference Center for Thrombotic Microangiopathies: Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. *PLoS One* 5: e10208, 2010
43. 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* 5: 1844–1859, 2010

Received: February 3, 2012 **Accepted:** November 8, 2012

[a]Deceased.

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.01260212/-/DCSupplemental.