



TO THE EDITOR:

Familial risk of developing atypical hemolytic-uremic syndrome

Emilia Arjona,¹ Ana Huerta,² Elena Goicoechea de Jorge,³ and Santiago Rodríguez de Córdoba¹

¹Center for Biological Research and Rare Disease Networking, Biomedical Research Center, Madrid, Spain; ²University Hospital Puerta de Hierro, Madrid, Spain; and ³Department of Immunology, Complutense University and Research Institute Hospital 12 de Octubre (imas12), Madrid, Spain

Atypical hemolytic-uremic syndrome (aHUS) is a life-threatening disease that in most cases progresses to end-stage kidney disease.¹ Rare pathogenic mutations in complement components or regulatory proteins play a crucial role in the pathogenesis of aHUS in 50% to 60% of patients.²⁻⁴ These rare variants cause defective regulation of the complement alternative pathway in the endothelial cell surface, which triggers episodes of thrombotic microangiopathy in the renal microvasculature and other vital organs.⁵⁻¹¹ Finding complement pathogenic mutations in genetic analyses, or not finding them, improves diagnosis and facilitates a personalized treatment with eculizumab, a humanized monoclonal antibody that avoids the pathological consequences of cell surface complement dysregulation.¹ Genetic analyses are widely used to help clinically diagnose aHUS, and they can help identify numerous asymptomatic relatives who carry different loads of aHUS genetic risk factors. A recurring question from both clinicians and patients is, How much risk of developing aHUS do these individuals have? There has been awareness of incomplete penetrance of aHUS in mutation carriers for a long time.^{12,13} However, our current estimates of disease penetrance in mutation carriers are based on very small cohorts and may be biased by uncertainties about the pathogenicity of the genetic variants found.^{12,14,15} To provide reliable estimates of the aHUS penetrance in mutation carriers, we searched the Spanish aHUS registry for patients with family data, in whom a comprehensive genetic analysis resulted in the identification of true pathogenic complement variants.

The Spanish aHUS registry includes 640 entries with a clinical diagnosis of aHUS based on established criteria¹ (supplemental Materials and Methods, available on the *Blood* Web site). Genetic analysis of complement in the DNA from these patients was performed as previously described⁷ (supplemental Materials and Methods). Ninety-six patients fulfilled the criteria of having family data and carrying complement pathogenic variants that have been demonstrated to cause reduced protein levels or altered function. Seven additional patients carrying mutations most likely pathogenic were also included in these studies. Supplemental Tables 1 and 2 provide a detailed description of the mutations found in each of the 103 aHUS patients selected for this study. All available relatives (n = 372) were analyzed for the mutations that had been identified in the respective index patient and genotyped for the aHUS risk haplotypes *CFH-H3* and *MCPggaac*. Overall, the relatives had a balanced sex distribution, with only 21.3% corresponding to descendants of the probands (supplemental Table 3). The study was approved by

the Ethics Committee of the Spanish Consejo Superior de Investigaciones Científicas (ref: SAF2015-66287-R), and all patients included in this study provided written consent.

Eighty of the selected aHUS pedigrees carry a single complement pathogenic variant. These pedigrees include 286 relatives of whom 134 were carriers of the pathogenic variant. Notably, 10 of these 134 mutation carriers developed the disease, whereas there were none among the non-carrier relatives (n = 152) ($P < .0004$) (Table 1). As expected, these data confirm that the main driver of aHUS in these pedigrees is the pathogenic mutation. Affected relatives carry pathogenic mutations in *C3* (2), *CFH* (3), *CFB* (3), or *MCP* (2) genes. A detailed age-adjusted analysis of the relatives who carry mutations showed a relatively low aHUS penetrance of 6.8% by age 35 years, which raises the aHUS penetrance to a maximum of 9.6% at age 48 years (Figure 1B).

There are several single nucleotide polymorphisms in the human *CFH* and *MCP* gene regions, but the very strong linkage disequilibrium between them reduces the genetic variability within these regions to a few single nucleotide polymorphisms haplotype blocks that span these genes. The *CFH-H3* haplotype, which encodes for slightly reduced plasma levels of factor H, is strongly associated with a risk for aHUS.^{16,17} Similarly, the *MCPggaac* haplotype, which shows reduced *MCP* transcriptional activity, is also an important risk factor for aHUS.^{6,13,18} Identification of the *CFH-H3* and *MCPggaac* haplotypes has diagnostic value and explains the incomplete penetrance of aHUS among carriers of complement pathogenic variants, but it is currently unknown how much these polymorphisms contribute to aHUS penetrance.

As expected, the allele frequencies of the *CFH-H3* and *MCPggaac* risk haplotypes are increased in the affected mutation carriers in the single-mutation aHUS pedigrees (Table 1). To determine the contribution of *CFH-H3* and *MCPggaac* to the aHUS penetrance, we divided the single-mutation carriers into 4 groups, carriers of only *CFH-H3*, carriers of only *MCPggaac*, carriers of both, and non-carriers (Table 1). Notably, the frequency of carriers of both risk haplotypes is significantly increased in the affected group compared with the non-affected group (48.3 vs 28.5; odds ratio, 2.32; 95% confidence interval, 1.29-4.10; $P = .0056$), and correspondingly, the frequency of individuals who lacked both risk haplotypes is significantly increased in non-affected mutation carriers compared with

Table 2. Pathogenic variants and risk haplotypes in aHUS pedigrees with 2 pathogenic variants (n = 23)

	Index cases (%) (n = 23)	Non-carrier relatives (n = 23)		Carrier relatives with 1 mutation (n = 46)		Carrier relatives with 2 mutations (n = 17)		Total affected carriers (%) (n = 28)	P	OR (95%CI)
		Affected (n = 0)	Non-affected (%) (n = 23)	Affected (n = 0)	Non-affected (%) (n = 46)	Affected (%) (n = 5)	Non-affected (%) (n = 12)			
<i>CFH-H3</i> allele frequency	13/46 (28.26)	0	9/46 (19.57)	0	20/92 (21.74)	5/10 (50)	8/24 (33.33)	18/56 (32.14)	1	—
<i>MCP_{ggaac}</i> allele frequency	24/46 (52.17)	0	6/46 (13.04)	0	35/92 (38.04)	6/10 (60)	4/24 (16.67)	30/56 (53.57)	.0028	5.76 (1.74-19)
<i>CFH-H3</i> only	3/23 (13.04)	0	5/23 (21.74)	0	8/46 (17.39)	0	5/12 (41.67)	3/28 (10.71)	.038	0.16 (0.03-0.9)
<i>MCP_{ggaac}</i> only	7/23 (30.43)	0	1/23 (4.35)	0	15/46 (32.61)	0	3/12 (25)	7/28 (25)	1	—
<i>CFH-H3</i> + <i>MCP_{ggaac}</i>	9/23 (39.13)	0	3/23 (13.04)	0	11/46 (23.91)	5/5 (100)	1/12 (8.33)	14/28 (50)	.029	11 (1.24-97)
None	4/23 (17.39)	0	14/23 (60.87)	0	12/46 (26.09)	0	3/12 (25)	4/28 (14.29)	.65	—

15% (age 48 years) and 18.8% (age 37 years), respectively (Figure 1). Interestingly, the 4 affected relatives without the *CFH-H3* haplotype carry C3 (2) and FB (2) gain-of-function mutations.

Twenty-three additional families from our registry, in which the proband presents 2 variants that were demonstrated to be pathogenic, provided similar data: only relatives carrying the 2 mutations developed aHUS (Table 2). The overall risk of developing aHUS in double-mutation carriers was ~threefold that of single-mutation carriers (Figure 1), suggesting that increasing the load of genetic risk factors reduces the dependence of environmental triggers and elevates aHUS penetrance. Consistently, the age-adjusted analysis in double-mutation carrier relatives showed an aHUS penetrance of 18.6% by age 35 years and a maximum aHUS penetrance of 36% at age 44 years (Figure 1).

Our data also show an additional contribution of the *MCP_{ggaac}* and *CFH-H3* risk haplotypes to aHUS development when concurring with 2 pathogenic mutations, increasing the aHUS penetrance to 56% by age 35 years and to a complete penetrance (100%) at age 44 years (Figure 1). Notably, no single-mutation carriers (n = 46) developed aHUS in these 23 double-mutation pedigrees, when 2 to 3 affected relatives were expected. This may be because the pathogenicity associated with these mutations is lower than that of mutations found in the single-mutation pedigrees. Alternatively, the lack of affected individuals in this group could be related to the fact that they have a reduced load of aHUS risk haplotypes, resembling that of non-affected carriers in single-mutation pedigrees (Tables 1 and 2).

In conclusion, the analysis of 372 relatives of aHUS patients carrying 1 or 2 complement pathogenic variants provided us with reliable estimates of the disease penetrance in individuals carrying different loads of true pathogenic genetic risk factors. As expected, none of the non-carrier relatives developed aHUS. An important finding of our study has been to determine the contribution of the *CFH-H3* and *MCP_{ggaac}* risk haplotypes to the aHUS penetrance in mutation carriers. Accordingly, the presence

of both risk haplotypes in single-mutation and double-mutation carriers increases the aHUS penetrance twofold to threefold, whereas the absence of both risk polymorphisms reduces their risk of developing aHUS significantly. aHUS is a complex disease, with additive genetic risk factors delineating the predisposition to aHUS and environmental risk factors that trigger disease development. We have shown how different genetic risk factors contribute to aHUS penetrance and would like to suggest that these penetrance values have an inverse correlation with the intensity of environmental triggers required for disease development. Data presented in this report have the limitations associated with low numbers that characterize ultra-rare diseases but should help clinicians improve tailored management of aHUS patients and facilitate genetic counseling to their family members.

Acknowledgments

The authors thank all patients and their relatives who participated in this study.

This work was supported by the Spanish Ministerio de Economía y Competitividad/FEDER grants SAF2015-66287-R, RTI2018-095955-B-100, and RYC-2013-13395 and Autonomous Region of Madrid grant S2017/BMD-3673. This work was developed under the supervision of the Spanish Registry of Atypical Hemolytic Uremic Syndrome and C3-Glomerulopathy.

Authorship

Contribution: S.R.d.C. and E.A. designed the research; E.A., A.H., E.G.d.J., and S.R.d.C. collected and analyzed the data; and S.R.d.C. wrote the letter.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: A.H., 0000-0003-3342-7628; E.G.d.J., 0000-0002-4978-2483; S.R., 0000-0001-6401-1874.

Correspondence: Santiago Rodríguez de Córdoba, Centro de Investigaciones Biológicas, Ramiro de Maeztu 9, 28040 Madrid, Spain; e-mail: srdecordoba@cib.csic.es.

Footnotes

Submitted 8 May 2020; accepted 11 May 2020; prepublished online on *Blood* First Edition 2 June 2020.

Please send requests for data to Santiago Rodríguez de Córdoba at srdecordoba@cib.csic.es.

The online version of this article contains a data supplement.

REFERENCES

1. Goodship TH, Cook HT, Fakhouri F, et al; Conference Participants. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int*. 2017;91(3):539-551.
2. Rodríguez de Córdoba S, Hidalgo MS, Pinto S, Tortajada A. Genetics of atypical hemolytic uremic syndrome (aHUS). *Semin Thromb Hemost*. 2014;40(4):422-430.
3. Afshar-Kharghan V. Atypical hemolytic uremic syndrome. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):217-225.
4. Feitz WJC, van de Kar NCAJ, Orth-Höller D, van den Heuvel LPJW, Licht C. The genetics of atypical hemolytic uremic syndrome. *Med Genet*. 2018;30(4):400-409.
5. Fidan K, Gökner N, Gülhan B, et al. Extra-renal manifestations of atypical hemolytic uremic syndrome in children. *Pediatr Nephrol*. 2018;33(8):1395-1403.
6. Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J, et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci U S A*. 2007;104(1):240-245.
7. Goicoechea de Jorge E, Tortajada A, García SP, et al. Factor H competitor generated by gene conversion events associates with atypical hemolytic uremic syndrome. *J Am Soc Nephrol*. 2018;29(1):240-249.
8. Kavanagh D, Richards A, Noris M, et al. Characterization of mutations in complement factor I (CFI) associated with hemolytic uremic syndrome. *Mol Immunol*. 2008;45(1):95-105.
9. Liszewski MK, Atkinson JP. Complement regulator CD46: genetic variants and disease associations. *Hum Genomics*. 2015;9(1):7.
10. Sánchez-Corral P, Pérez-Caballero D, Huarte O, et al. Structural and functional characterization of factor H mutations associated with atypical hemolytic uremic syndrome. *Am J Hum Genet*. 2002;71(6):1285-1295.
11. Schramm EC, Roumenina LT, Rybkine T, et al. Mapping interactions between complement C3 and regulators using mutations in atypical hemolytic uremic syndrome. *Blood*. 2015;125(15):2359-2369.
12. Caprioli J, Noris M, Brioschi S, et al; International Registry of Recurrent and Familial HUS/TTP. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood*. 2006;108(4):1267-1279.
13. Esparza-Gordillo J, Goicoechea de Jorge E, Buil A, et al. Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. *Hum Mol Genet*. 2005;14(5):703-712.
14. Sullivan M, Erlic Z, Hoffmann MM, et al. Epidemiological approach to identifying genetic predispositions for atypical hemolytic uremic syndrome. *Ann Hum Genet*. 2010;74(1):17-26.
15. Sullivan M, Rybicki LA, Winter A, et al. Age-related penetrance of hereditary atypical hemolytic uremic syndrome. *Ann Hum Genet*. 2011;75(6):639-647.
16. Bernabéu-Herrero ME, Jiménez-Alcázar M, Anter J, et al. Complement factor H, FHR-3 and FHR-1 variants associate in an extended haplotype conferring increased risk of atypical hemolytic uremic syndrome. *Mol Immunol*. 2015;67(2 pt B):276-286.
17. Caprioli J, Castelletti F, Bucchioni S, et al; 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*. 2003;12(24):3385-3395.
18. Martínez-Barricarte R, Heurich M, López-Perrote A, et al. The molecular and structural bases for the association of complement C3 mutations with atypical hemolytic uremic syndrome. *Mol Immunol*. 2015;66(2):263-273.

DOI 10.1182/blood.2020006931

© 2020 by The American Society of Hematology

TO THE EDITOR:

Anticoagulant activity of edoxaban in patients with cirrhosis

Sarah Bos,^{1,2} Tim Schreuder,² Hans Blokzijl,² Jelle Adelmeijer,¹ Ton Lisman,^{1,*} and Pieter W. Kamphuisen^{3,4,*}

¹Surgical Research Laboratory and Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, and ²Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; ³Department of Internal Medicine, Tergooi Hospital, Hilversum, The Netherlands; and ⁴Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Patients with end-stage chronic liver disease (ie, cirrhosis) acquire multiple and complex alterations in their hemostatic system. Recent insights in the hemostatic changes in patients with cirrhosis have indicated a balanced, but unstable hemostatic system in these patients with a risk for both bleeding and thrombotic complications including venous thromboembolism and portal vein thrombosis.^{1,2} Prevention and treatment of thrombotic events are a challenge because of a frequently prolonged baseline international normalized ratio and substantially decreased levels of antithrombin, impeding correct dosing and monitoring of vitamin K antagonists and heparins, respectively.^{3,4} There is very limited clinical experience with the new-generation direct oral anticoagulants

(DOACs) in patients with cirrhosis because these patients were excluded from all clinical trials with these new agents. However, DOACs have potential advantages over vitamin K antagonists and heparins, such as the oral route of administration, the lack of requirement of laboratory monitoring, their mechanism of action, and the wider therapeutic window,⁴ which has resulted in increasing interest from the hepatology community.

DOACs are contraindicated in patients with advanced liver disease accompanied with coagulopathy and clinically relevant bleeding risk. Importantly, because all the DOACs are cleared by the liver and kidney, drug accumulation with a potentially