The familial risk of developing atypical Hemolytic Uremic Syndrome.

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Running title: The familial risk of developing aHUS

Keywords: aHUS. Genetics. Risk assesement. Polymorphisms.

Text word count: 1185

Atypical hemolytic uremic syndrome (aHUS) is a life-threatening disease that in most cases progresses to end-stage kidney disease¹. Rare pathogenic mutations in complement components or regulatory proteins play a crucial role in the pathogenesis of aHUS in 50-60% of patients²⁻⁴. 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 organs⁵⁻¹¹. Finding complement pathogenic mutations, or not, in genetic analyses improves diagnosis and facilitates a personalized treatment with eculizumab, a humanized monoclonal antibody that avoids the pathological consequences of cell surface complement dysregulation¹. Genetic analyses are widely used to assist aHUS clinical diagnosis and result in the identification of numerous asymptomatic relatives carrying different loads of aHUS genetic risk factors. How much risk of developing aHUS these individuals have, is a permanent request by clinicians and patients. Incomplete penetrance of aHUS in mutation-carriers is known for a long-time^{12,13}. However, our current estimates of disease penetrance in mutation-carriers are based in 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 search the Spanish aHUS registry for cases with family data, in whom a comprehensive genetic analysis has 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 (See Suppl. Materials and Methods). Complement genetic analysis in the DNA from these patients was done as

previously described⁷ (See Suppl. Materials and Methods). Ninety-six cases fulfill 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. Supplementary Tables 1 and 2 provide a detailed description of the mutations found in each of the 103 aHUS cases 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 have a balanced gender distribution with only 21,3% corresponding to descendants of the probands (Suppl. Table 3).

Eighty of the selected aHUS pedigrees carry a single complement pathogenic variant. These pedigrees include 286 relatives of whom 134 are 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) ($p<0.0004$) (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* (2), *CFB* (3) or *MCP* (2) genes. A detailed age-adjusted analysis in the mutation-carrier relatives showed a relatively low aHUS penetrance of 6.8% by 35y of age, which raises the aHUS penetrance to a maximum of 9.6% the age of 48y (Figure 1).

There are several single nucleotide polymorphisms (SNPs) 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 SNP haplotype blocks spanning these genes. The haplotype *CFH-H3*, encoding

slightly reduced plasma levels of FH, strongly associates with risk for aHUS^{16,17}. Similarly, the *MCPggaac* haplotype, showing 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 is used to explain the incomplete penetrance of aHUS among carriers of complement pathogenic variants, but it is currently unknown how much these polymorphisms contribute to the 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 in four groups, carriers of only *CFH-H3*; carriers of only *MCPggaac*; carriers of both and no-carriers (Table 1). Notably, the frequency of carriers of both risk haplotypes is significantly increased in the affected group compared to the non-affected [48.3 vs 28.5; $p=0.0056$; OR (0.95 CI)=2.32 (1.29-4.10)], and correspondingly, the frequency of individuals lacking both risk haplotypes is significantly increased in non-affected mutation-carriers compared to affected individuals [31 vs 8.98; $p=0.00012$; OR (0.95 CI)= 0.21 (0.1-0.5)] (Table 1). Consistent with these data, we found that carrying the *MCPggaac* haplotype or both the *MCPggaac* and the *CFH-H3* haplotypes, in addition to the complement pathogenic mutation, raises the aHUS penetrance by the age of 35y to 9.4% and 14.5%, and the maximum aHUS penetrance to 15% (48y) and 18.8% (37y), respectively (Figure 1).

Twenty-three additional families from our registry, in which the proband presents two variants demonstrated pathogenic provided similar data: only

relatives carrying the two mutations developed aHUS (Table 1). The overall risk of developing aHUS in two-mutations carriers was approximately 3-fold 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 two mutation-carrier relatives showed an aHUS penetrance of 18.6% by the age of 35y and a maximum aHUS penetrance of 36% at 44y (Figure 1).

Our data also show an additional contribution of the *MCPggaac* and *CFH-H3* risk haplotypes to aHUS development when concurring with two pathogenic mutations, increasing the aHUS penetrance to 56% by the age of 35y and to a complete penetrance (100%) at 44y (Figure 1). Notably, no single-mutation carriers (n=46) developed aHUS in these 23 double-mutation pedigrees, when 2-3 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 (Table 1).

In conclusion, the analysis of 372 relatives of aHUS patients carrying one or two 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 *MCPggaac* risk haplotypes to the aHUS penetrance in mutation-carriers. Accordingly, the presence of both risk haplotypes in single-

mutation and double-mutation carriers increases 2-3-fold the aHUS penetrance, 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 triggering 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 characterizes ultra-rare diseases, but should help clinicians to improve tailored management of aHUS patients and facilitate genetic counseling to their family members.

Author contribution statement

SRdeC and EA designed research. EA, AH, EGdeJ and SRdeC, collected and analyzed the data. SRdeC wrote the paper.

Conflict-of-interest disclosure

Authors declare no conflicts of interest

Acknowledgments

We are in debt to all patients and relatives participating in this study. This work was developed under the supervision of the Spanish Registry of atypical Hemolytic Uremic Syndrome and C3-Glomerulopathy (aHUS/C3G) and was supported by the Spanish Ministerio de Economía y Competitividad/FEDER grant SAF2015-66287-R and Autonomous Region of Madrid grant S2017/BMD-3673.

Ethical approval

The study was approved by the Ethics Committee of the Spanish Consejo Superior de Investigaciones Científicas (CSIC) (ref: SAF2015-66287-R).

Informed consent

All patients included in this report provided written consent.

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Figure Legend

Figure 1. Disease penetrance in relatives of aHUS patients

A) Kaplan-Meier estimations of the aHUS penetrance for different loads of genetic risk factors. B) aHUS penetrance at 35y-old and maximum aHUS penetrance in relatives of probands carrying one and two pathogenic variants and contribution of the *CFH-H3* and *MCPggaac* risk haplotypes.

TABLE 1. Pathogenic variants and risk haplotypes in aHUS pedigrees

Pedigrees with a single pathogenic variant (n=80)

	Index cases (n=80)	Non-carrier relatives (n=152)		Carrier relatives (n=134)		Total affected carriers (n=90)	p-value OR (0.95%CI)	
		Affected (n=0)	Non-affected (n=142)	Affected (n=10)	Non-affected (n=116)			
Allele Freq. <i>CFH-H3</i>	76/160 (47.50%)	-	80/284 (28.17%)	6/20 (30%)	63/232 (27.16%)	82/180 (45.56%)	0.00011	2.24 (1.48-3.39)
Allele Freq. <i>MCP_{ggaac}</i>	70/160 (44.38%)	-	93/284 (32.75%)	11/20 (55%)	76/232 (32.76%)	82/180 (45.56%)	0.010	1.71 (1.14-2.57)
Only <i>CFH-H3</i>	22/80 (27.50%)	-	30/142 (21.13%)	0 (0%)	19/116 (16.38%)	22/90 (24.72%)	0.162	-
Only <i>MCP_{ggaac}</i>	13/80 (16.25%)	-	47/142 (33.10%)	4/10 (40%)	28/116 (24.14%)	17/90 (17.97%)	0.399	-
<i>CFH-H3</i> + <i>MCP_{ggaac}</i>	37/80 (46.25%)	-	36/142 (25.35%)	6/10 (60%)	33/116 (28.45%)	43/90 (48.31%)	0.0056	2.3 (1.29-4.10)
None	8/80 (10%)	-	29/142 (20.42%)	0 (0%)	36/116 (31.03%)	8/90 (8.98%)	0.00012	0.21 (0.1-0.50)

Pedigrees with two pathogenic variants (n=23)

Index cases (n=23)	Relatives non-carriers (n=23)		Relatives carriers one mutation (n=46)		Relatives carriers two mutations (n=17)		Total affected carriers (n=28)	p-value OR (0.95%CI)	
	Affected (n=0)	Non-affected (n=23)	Affected (n=0)	Non-affected (n=46)	Affected (n=5)	Non-affected (n=12)			
13/46 (28.26%)	-	9/46 (19.57%)	-	20/92 (21.74%)	5/10 (50%)	8/24 (33.33%)	18/56 (32.14%)	1	-
24/46 (52.17%)	-	6/46 (13.04%)	-	35/92 (38.04%)	6/10 (60%)	4/24 (16.67%)	30/56 (53.57%)	0.0028	5.76 (1.74-19)
3/23 (13.04%)	-	5/23 (21,74%)	-	8/46 (17.39%)	0	5/12 (41.67%)	3/28 (10.71%)	0.038	0.16 (0.03-0.9)
7/23 (30.43%)	-	1/23 (4.35%)	-	15/46 (32.61%)	0	3/12 (25%)	7/28 (25%)	1	-
9/23 (39.13%)	-	3/23 (13.04%)	-	11/46 (23.91%)	5/5 (100%)	1/12 (8.33%)	14/28 (50%)	0.029	11 (1.24-97)
4/23 (17,39%)	-	14/23 (60,87%)	-	12/46 (26.09%)	0	3/12 (25%)	4/28 (14.29%)	0.65	-

Figure 1

A)

6	6	4	4	4	3	3	2	2	1	0	0	0	0
17	17	15	15	15	13	13	11	10	8	7	3	1	0
39	34	32	30	27	23	21	19	15	12	8	7	6	2
32	29	27	25	22	19	17	14	10	7	4	1	0	0
126	116	108	101	97	90	82	72	60	50	39	28	20	10

Healthy Relatives

B)

aHUS penetrance in relatives

	Carriers	Affected / Total	Penetrance (35y)	Max. Penetrance (y)
Pedigrees 1 mutation	No mutation	0/152	-	-
	Mutation	10/126	6.8%	9.6% (48y)
	Mutation only	0/36	-	-
	Mutation + <i>CFH-H3</i>	0/19	-	-
	Mutation + <i>MCPggaac</i>	4/32	9.4%	15% (48y)
	Mutation + <i>CFH-H3</i> + <i>MCPggaac</i>	6/39	14.5%	18.8% (37y)
Pedigrees 2 mutations	No mutation	0/23	-	-
	1 mutation	0/46	-	-
	2 mutations	5/17	18.6%	36% (44y)
	2 mutations only	0/3	-	-
	2 mutations + <i>CFH-H3</i>	0/5	-	-
	2 mutations + <i>MCPggaac</i>	0/3	-	-
	2 mutations + <i>CFH-H3</i> + <i>MCPggaac</i>	5/6	56%	100% (44y)